Effect of Centrifugation Speed and Duration of the Quantity of *Platelet Rich Plasma* (PRP)

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Abstract

Platelet rich plasma (PRP) is a promising alternate to surgery with a safe and natural healing. The standard protocol for PRP preparation must be determined from the matrix of fibrin, leukocytes, platelets, and growth factors. The use of PRP in the success of periodontal treatment would not be separated from methods to obtain it. The effect of centrifugation speed and centrifugation duration of the quantity of PRP.There are 41 subjects studied by taking 21 ml of venous blood in each of the seven tubes. Centrifugation performed twice with different speed and duration. ANOVA statistical test results obtained value of P=0.000 (P<0.05), which means the massive difference in the quantity of PRP in the three groups with the lowest score in the control group and the lowest at 3.8% citrate acid group. The outcome of this research provides the effect of speed centrifugation to the quantity of PRP. There is no effect of duration of centrifugation to the quantity of PRP, while there is the effect of the use of anticoagulants EDTA 3.8% citrate acid compared with in the quantity of PRP.

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Introduction

Periodontal disease is defined as a complex, multifactorial disease and is characterized by loss of attachment of connective tissue with accompanying periodontal tissue destruction. The goal of periodontal therapy is to eliminate the inflammatory process, prevent regression from periodontal disease and also regenerate loss of periodontal tissue.[1]

Periodontal regeneration is a complex multifactorial process that involves biological events such as cell adhesion, migration, proliferation, and differentiation in a regular order. Periodontal regenerative procedures include soft tissue graft, bone graft, root biomodification, guided tissue regeneration, and a combination of such procedures.[1]

Periodontal healing requires a sequence of interactions between epithelial cells, gingival fibroblasts, periodontal ligament cells, and osteoblasts. Disruption of vascularization during wound healing results in the formation of fibrin, platelet aggregation, and the release of several growth factors into tissue from platelets through molecular signals primarily mediated by cytokines and growth factors. There is evidence that growth factor and cytokine content in platelet plays an important role in inflammation and wound healing. Platelet also secretes fibrin, fibronectin, and vitronectin, which acts as a matrix for connective tissue and molecular adhesion for more efficient cell migration. This led to the idea of using platelets as a therapeutic tool to improve tissue repair, especially on periodontal wound healing.[1]

In Europe, and more recently in the United States, there has been increased use of autologous blood products in an effort to provide healing in a variety of uses. In recent years, scientific research and technology has provided a new perspective on understanding the wound healing process. Initially platelets function exclusively in clotting processes. However, platelets also release many bioactive proteins have a role to attract macrophages, mesenchymal stem cells, and osteoblasts that not only enhance the removal of necrotic tissue, but also enhance tissue regeneration and healing. Based on this principle platelets are introduced to

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stimulate physiologically growth factors in an effort to accelerate the healing that begins in chronic wounds.[2]

Platelet rich plasma (PRP) is a blood concentrate that has a platelet concentration several times higher than normal thrombocyte concentrations in human blood. PRP is used because platelet concentrations are at least four times the normal platelet concentration, or 1,000,000 platelets/μL is said to support bones and tendons healing. This is because, thrombocyte is a blood cell that has more than thirty bioactive proteins that play a role in the tissue healing process. The various proteins will be removed from platelets when the platelets undergo activation, ie when the platelet cells are exposed to damaged blood vessels or with injured tissue.[2]

The use of PRP was first proposed by M. Ferrari in 1987 in post-operative patients with open heart surgery in order to minimize the amount of blood that must be transfused. Since then the use of PRP in various areas of health has continued to evolve. And in recent times, PRP has been increasingly used in treatment of various sports injury cases related to ligament or tendon injury. There are currently more than 5200 NCBI articles on PRP in orthopedics, sports medicine, dentistry, otolaryngology, neuro surgery, ophthalmology, urology, wound healing, cosmetics, cardiothoracic and maxillofacial surgery.[2,4]

For twenty years, the application of PRP autologous has been used safely and documented in a variety of areas including: orthopedics, sports medicine, dentistry, ENT, neurosurgery, ophthalmology, urology, wound healing, cosmetics, cardiothorax, and maxillofacial oral surgery. PRP is a promising alternative to surgery with a safe and natural healing.[2]

The standard protocol for PRP preparation should be determined to obtain the proper quantity and quality of the fibrinmatrices, leucocyte, platelet, and growth factor. This is not separated from the number of PRPs produced. The use of PRP to support the success of periodontal treatment is determined by the method used to obtain the PRP. This way of determining the quality and quantity of PRPs. The PRP creation protocols in different literary sources vary from centrifugation rate, centrifugation duration, addition of anticoagulant as well as separation method (one spin method or two spin method).

Based on the description above, the authors are interested in conducting research to determine the effect of centrifugation rate, centrifugation duration, and anticoagulant use (EDTA and 3.8%Citrate acid) on the quantity ofPlatelet rich plasma (PRP).

Materials and methods

Patients were screening to meet both inclusion and exclusion criteria. A total of 41 patients willing to follow the research, asked to fill informed concent . Prepare seven tubes (1 tube containing EDTA anticoagulants, 5 tubes containing 3.8% anticoagulant citrate acid , 1 tube BD contains no anticoagulants). Sampling of venous blood of patients as much as 3 ml on each tube.

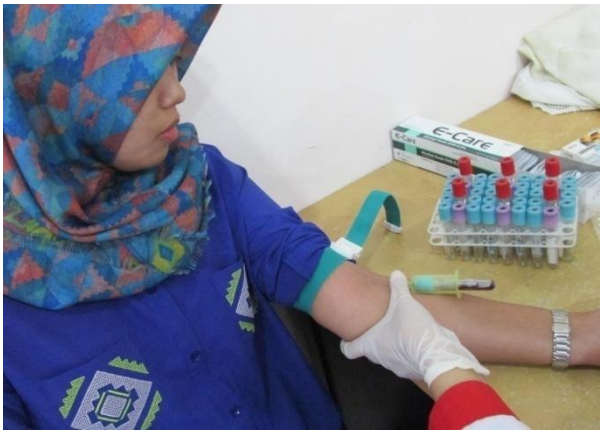

Figure 1. Samples undergoing Venous Blood taking using BD Vacutainer® flashback Blood Collection Needle 22GA x 1"(0.7mm x 25mm).

Then, at first EDTA tube is used for routine blood examination. The first treatment (with and without anticoagulation): tube 1 (EDTA) containing 3 ml of blood, tube 2 (3.8% citrate acid) containing 3 ml of blood , tube 3 (no anticoagulant) containing 3 ml of blood was done twice centrifugation at a rate of 800 rpm for 15 minutes using a blood centrifuge machine Hettich® Zentrifugen EBA21, then played back at 2000 rpm for 10 minutes. The resulting PRP is taken using an automatic measuring micropipette to view the resulting PRP volume. The resulting PRP is then stored in a sterile sample cup. The results of each tube were then recorded.

The second treatment (different centrifugation speed): tube 4 (3.8% citrate acid) containing 3 ml of blood was performed

twice centrifugation at 1500 rpm for 10 min, then replayed at 3000 rpm for 15 min. The resulting PRP is taken using an automatic measuring micropipette to view the resulting PRP volume. The resulting PRP is then stored in a sterile sample cup. The tube 5 (3.8% citrate acid) containing 3 ml of blood was centrifuged at 2700 rpm for 12 min, then turned back at 2900 rpm for 10 min. The resulting PRP is taken using an automatic measuring micropipette to view the resulting PRP volume. The resulting PRP is then stored in a sterile sample cup. The results of each tube were then recorded.

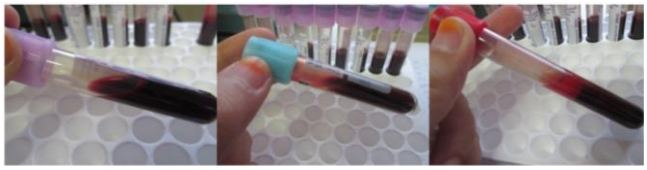

Figure 2. BD Vacutainer® Purple Tube (containing EDTA anticoagulants), Vacutest® blue tube (containing 3.8% Citrate Acid Anticoagulant, BD Vacutainer® Red Tube (contains no anticoagulants).

Figure 3. Platelet Rich Plasma (PRP) sampling from each Tube using a Automatic measuring micropipette 10-100 mL F3 ThermoScientific Finnpipette®

The third treatment (different centrifugation duration): tube 6 (3.8% citrate acid) containing 3 ml of blood was performed twice centrifugation at 3000 rpm for 3 min, then replayed at 3000 rpm for 13 min. The resulting PRP is taken using an automatic measuring micropipette to view the resulting PRP volume. The resulting PRP is then stored in a sterile sample cup. Tubes 7 (3.8% citrate acid) containing 3 ml of blood was performed twice centrifugation at 3200 rpm for 15 min, then replayed at 3000 rpm for 10 min. The resulting PRP is taken using an automatic measuring micropipette to view the resulting PRP volume. The resulting PRP is then stored in a sterile sample cup. The results of each tube were then recorded.

Figure 4. Seventh Tube before and after Separation of PRP with PPP and RBC

Results

Table 1 shows that the average PRP quantity in the EDTA group was 1193.4 ml with SD 181,85, the average PRP quantity in the 3.8% Citrate acid group was 871.2 ml with SD 89.62, the average quantity of PRP in the control group was 1200.68 ml with SD 161.27. The result of statistical test withanova was obtained P=0.000 (P<0.05), which means that there was a difference of PRP quantity in the three groups with the highest score in the control group and the lowest in the 3.8% citrate acid group. Among these two groups the PRP varies significantly between EDTAs higher than the 3.8% citrate acid of 322.2 ml, then the control is higher than the 3.8% citrate acid of 329.5 ml whereas EDTA with the controls is no different. This can be seen in Table 1 below:

Coagulant	N	Mean	SD	P
EDTA	41	1193.41	181.85	0.000
Citrate acid3.8%	41	871.22	89.62	
Control	41	1200.68	161.27	
Total	123	1088.44	214.00	

Table 1. Difference in PRP Quantity Based on Anticoagulant Usage (Treatment I)

Table 2 shows that the average of PRP quantity in 3.8% citrate acid at 4500 rpm

was 949.63 with SD 87.55, mean of PRP quantity in 3.8% citrate acid at 5600 rpm was 925.8 with SD 96,69, the average of PRP quantity in 3.8% citrate acid at 6000 rpm was 932,44 with SD 77.81, whereas the average of PRP quantity in 3.8% citrate acidat 6200 rpm was 935,61 with SD 100.35. The result of statistical test with anova was obtained *P*=0.685 (*P*>0.05) which mean there was no centrifugation rate difference in four groups on the PRP quantity on the use of 3.8% citrate acidanticoagulant (Figure 2).

Table 3 shows the regression test results obtained value *P*=0.353 (*P*>0.05), which means there is no effect centrifugation speeds on the PRP quantity with the use of anticoagulant 3.8%Citrate Acid.

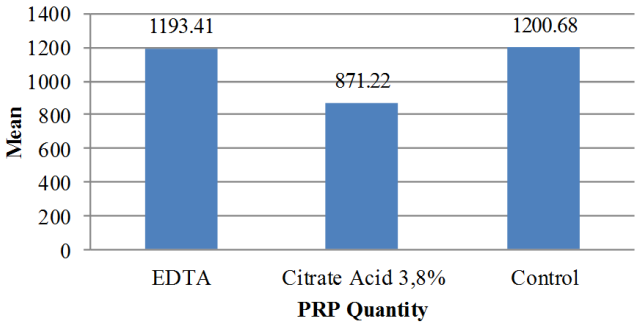

Figure 1. Differences in the PRP quantity based on Anticoagulant Used.

Rpm	n	Mean	SD	*P*
4500	41	949.63	87.55	
5600	41	925.85	96.69	
6000	41	932.44	77.81	0.685
6200	41	935.61	100.35	
Total	164	935.88	90.60	

Table 2. Differences in the PRP Quantity with the Use of Anticoagulant 3.8% citrate acid by Centrifugation Speed (Treatment 2 and Treatment 3).

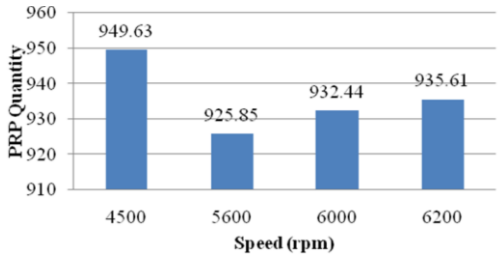

Figure 2. Difference in PRP Quantity with the Use of Anticoagulant 3.8% Citrate acid by Centrifugation Speed.

Variables	B	R	P
(Constant)	995.060		0.000
Round	-0.011	-0.073	0.353

Table 3. Effect of Centrifugation Rate on PRP Quantity with the Use of Anticoagulant 3.8% Citrate acid.

PRP citrate	N	Mean	Std, Deviation	*P*
25 minutes	82	942.62	93,852	
22 minutes	41	925.85	96,695	0.605
16 minutes	41	932.44	77,807	
Total	164	935.88	90,599	

Table 4. Difference in PRP Quantity with Use of 3.8% Citrate acid based on Centrifugation Duration.

Table 4 shows that the average of PRP quantity with the use of 3.8% citrate acidin total centrifugation duration for 25 min was 942.62 with SD 93.85; average of PRP quantity with 3.8% citrate acid use in total 22 min of centrifugation was 925.85 with SD 96.69; average of PRP quantity with 3.8% citrate acid use in total 16 min of centrifugation time was 932.44 with SD 77.81. The result of statistical test with anova obtained *P* value = 0.605 (*P*>0.05) which means there is no difference in the three groups total duration of centrifugation rotation on the PRP quantity 3.8% citrate acid.

Table 5 shows the regression test results obtained value *P*=0.558 (*P*>0.05), which means there is no effect on the quantity PRP centrifugation duration with the use of 3.8% citrate acid anticoagulant.

Variables	B	R	P
(Constant)	910,993		0,000
Round	1,131	0.046	0.558

Table 5. Effect of Centrifugation Duration on PRP Quantity with the Use of Anticoagulant 3.8% Citrate Acid.

Discussion

Platelet Rich Plasma (PRP) was a component of autologous plasma having a platelet concentration above baseline, rich in platelet derived growth factors (PDGF) and transforming growth factors (TGF-β). It was obtained by isolation and concentration of human

platelets by gradient density centrifugation forming platelet gel.[6]

There are various protocols in the literature for the preparation of PRP making. The centrifugation speed, the rotations number, the time and the space between particles and rotor on the blood volume processed is important for the PRP optimization. Each laboratory needs to standardize the protocols implemented. The PRP standard is prepared handmade and reliable and cost effective without using commercial kits. The platelet concentration factor can be changed by the centrifugation force applied to the PRP preparation.[7]

When PRP is taken into account for use in clinical practice, the clinician may face several problems, including centrifugation machine efficiency in obtaining proper platelets concentration, additional time and steps to prepare PRP coagulation for use. Hanna et al, the centrifugation process is considered to have an important role because the difference ofplatelets count is related to the difference in the centrifugation machine and the techniques used. The results also may have differed because of the individual divergence in platelet count and the time intervals between blood sample centrifugation, fibrin membrane collection, and cell culture treatment.[8,9]

Anitua et al uses only one centrifugation step and collects volume directly above the erythrocyte layer. This protocol obtains platelets with a concentration factor of 2.67 above the initial value. When all the volumes at the top layer are collected, regardless of the presence of a buffy coat layer taken in, additional rotation can be performed to obtain platelets with a higher concentration factor (> 3 times).[10]

Recent publications indicate that PRP prepared from an overall blood count of 8 ml to 10 ml is sufficient for periodontal regenerative therapy.However, in oral and maxillofacial reconstruction, it is necessary to take 8 ml to 500 ml of blood, thus achieving a large number of PRP for larger defective surgical procedures. For each 8 ml of blood, the supernatant volume obtained about 0.6-0.7 ml. The volume obtained is referred to as PRP and is used for surgical procedures.[11]

Further determination of the highest possible concentrations using various preparation methods, it is also important to determine the platelet concentration required for successful wound healing or other applications. Haynesworthy et al. showed that the proliferation of mature mesenchymal stem cells and their differentiation was directly related to platelet concentrations. Most individuals have a basic platelet count of 200,000±75,000 /µl, platelet count at PRP 1 million/µl as measured in standard 6 ml PRP as a therapeutic effect.[12]

PRP is obtained from the patient's blood sample taken at the time of treatment. A total of 30 cc of venous blood taken will produce 3-5 cc of PRP depending on the number of individual initial platelets, the tools used, and the technique used. Blood taken with the addition of anticoagulants, such as dextrose citrate A to prevent platelet activation before use.[7]

A total of 10 ml of blood was mixed with anticoagulant K2 potassium salt of ethylene-diaminetetraacetic acid (EDTA), PRP was isolated from the blood component as a whole at different centrifugal pressures at different time periods resulting in 0.5-1 ml of yellowish layer extract.[12]

Important issues associated with the selection of anticoagulants capable to maintaining platelets remain at the best functional potential of integrity and morphology. Actions in considering the type of anticoagulant used, most researchers agree not to use EDTA because this agent can damage the platelet membrane. Therefore, anticoagulants with citrate and dextrose from sodium citrate are recommended.[7]

In this study there is a difference PRP quantities count in all three groups with the highest score in the control group and the lowest in 3. 8%citrate acidgroup . This is probably due to the blood contained in the tube without anticoagulants rapidly separating the three layers fraction, resulting in a higher PRP quantity. In addition, earlier blood clots formed on tubes without anticoagulants, resulting in more serum after centrifugation twice.

After the whole blood centrifugation, the concentration gradient is formed in the tube for various blood components. So, to ensure reliable measurements, the previous sample needs to be well mixed. At the second centrifugation spin phase, the concentration gradient is more intense, because platelets are absorbed onto the remain eythrocytes surface. The presence of red blood cell content at volumes removed from the first centrifugation rotationphase is inevitable. Thus

efficient mechanical agitation with inversion is required to ensure platelet resuspension before concentration measurement. A number of protocols aim to optimize centrifugation procedures using various parameters and centrifugationtreatment.[10]

Based on the values obtained in this study, it was found that with the second treatment application using the weak first rotation to the strong second rotation that is 1500 rpm to 3000 rpm (4500 rpm) obtained higher PRP quantity results compared with constant rotation speed or strong rotation to weak rotation of 949.63 ml. This is in line with previous studies by Kavitha G et al. to see the highest average platelet count in PRP obtained with a weak rotation to strong rotation to be a better choice.[37] There are several articles that are propagated using strong rotation and a weak rotation on the second centrifuge indicating the success rate of obtaining platelets in large quantities in PRP. In line with the results in this study that the PRP quantity at the first rotation of 3200 rpm and the second rotation 3000 rpm (6200 rpm) was 935.61 ml.

The speed of centrifugation is relevant to the different volumes resulting from the first centrifugation rotation to the second rotation. In this case, thesame centrifugation parameter, the residual platelets in PPP vary due to centrifugal force changes acting on the platelets. As a result, cause changes in the platelet concentration factor.[10]

There was no significant effect on centrifuge rotation total duration of PRP quantity on the three 3.8 %citrate acid groups. However, the centrifugation duration for 25 minutes resulted in the highest PRP with an average of 942.62 ml. This result is in line with previous research that is faster duration is much better to produce optimal PRP.

In the case of blood centrifugation, the centrifugal force and time used in erythrocyte packing lie in the lower layer, the plasma volume in the upper layer, and platelets with the efficiency of healing platelets. For the same angular acceleration, the average centrifugal force applied to the erythrocytes decreases with a smaller mean distance of the rotor for the greater processed volume (8.5 ml). This factor explains the reduction of erythrocyte deposition in the lower layers as well as the platelets healing efficiency in the upper layers. Purpose restoring the same separation efficiency, erythrocyte packing must be returned with increasing time and centrifugal acceleration.[10]

However, before effectivelyusing PRP, several problems need to be solved including identifying methods to produce the most effective therapeutic concentrations. The type of anticoagulant, centrifugation rate, number and type of growth factor present in PRP, platelet count in the donor blood and the PRP itself as well as the clinical application of PRP play an important role in determining the maximum platelet concentrate produced and need to be considered before clinical application. In some previous studies, there were many methods for the preparation of PRP but no comparative studies have been reported previously.[12]

Growth factors (GF) abundantly exist in PRP. Successful results were reported from various animal and human studies provide that PRP is capable in enhancing the wound healing process and aids in the reconstruction of soft tissue.[6]

In the other research by Surijana Mappangara et al, correlation of blood quality (leucocyt, eritrocyt, hemoglobin, thrombocyt) with transforming growth factor (TGF-ß1) of PRP. This research found significant correlation was only observed in eritrocyt concentration variable higher than other dan only eritrocyt showed the significant relationship. This finding also showed the linear correlation between the among of eritrocyt in blood with (TGF-ß1) as the increased eritrocyt value was followed by the excalated (TGF-ß1) value. Even among individuals with similar platelet counts, it is possible for them to have different concentrations of TGF-β, this might interfere with the results. Incubation of platelet concentrate at 4 ^{o}C for 24 hours produces releases high concentration of TGF-β1. TGF-β1 are one of two main growth factors that encourages healing of soft tissue and bone through stimulation of collagen production, which to ameliorate promote wound strength and initiation of callus formation.[9,13,14]

Although growth factors and the mechanisms involved are poorly understood, the ease of PRP applications in dental practice and favorable outcomes, including rapid bleeding and rapid healing, can also be promising for further procedures. This autologous product eliminates worries about immunogenic reactions and

disease transfer. Recent animal studies and human trials show success. Research with good design and good control is required to provide strong evidence of PRP capacity and influence in wound healing, soft tissue reconstruction and (in combination with bone graft) augmentation procedures, especially in oral and periodontal therapy.

PRP contains high fibrin content that provides a sticky nature working as a haemostatic and stabilizing agent that might aid in blood clotting and bone graft immobilization in defect area, hence, plays an important role in periodontal regenerative procedures.[6]

The various factors that influence the PRP outcomes such as the amount of initial blood taken, the time and temperature of centrifugation and the use of anticoagulants. Therefore, all methods of producing PRP tend to be artistic rather than scientific and therefore an easy and standardized approach is needed for extracting the right platelet concentration layer needs to be developed if a more straightforward research is to be conducted.

Conclusions

Based on the data of research conducted on 41 samples, it can be concluded that: There is no effect of centrifugation velocity on the Platelet rich plasma (PRP) quantity; There is no effect of centrifugation duration on the Platelet rich plasma (PRP)quantity; there is an effect of EDTA anticoagulant usage compared with 3.8%citrate acid against Platelet Rich Plasmaquantity (PRP); and no effect of 3.8% anticoagulant Citrateacidon Plateletrich plasma (PRP) quantity.

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Declaration of Interest

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