Comparison of Single Centrifugation, Double Centrifugation and Turn down-Turn up Techniques for Platelet-Rich Plasma Quality

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Abstract

Background: Platelet-rich plasma (PRP) is a new concept used in the medical world, especially for wound healing. The main process that affects the PRP quality is the centrifugation process. This study aimed to assess the PRP separation process and determine the best technique for various centrifugation processes.

Methods: This experimental study used acid citrate dextrose (ACD) blood taken from 11 healthy respondents and compared three techniques including the single centrifugation (SC), the double centrifugation (DC), and the double centrifugation turn down - turn up (DC-TDTU) techniques. The quality of PRP was measured based on blood cell count (platelet, leukocyte, erythrocyte count, and Ht value) at each stage of centrifugation. The examination was carried out in 2021 at the Hematology Laboratory, Poltekkes Jakarta 3.

Results: The mean values of platelets, leukocytes, and Ht were increased in PRP compared to plasma supernatant both using the DC and DC-TDTU techniques, whereas the SC technique decreased in plasma compared with whole blood. When the procedures using DC and DC-TDTU are carried out properly, platelets would be concentrated in the second centrifugation. However, some erythrocyte and leukocyte contamination occurred with the DC-TDTU technique compared to the DC technique.

Conclusion: The double centrifugation technique is the best platelet-rich plasma separation technique compared to the DC-TDTU and SC techniques.

Keywords: Blood cells count, centrifugation, PRP

Introduction

Platelet-rich plasma (PRP) is a new concept used in the medical world, especially for wound healing or skin rejuvenating effects.[1,2] The PRP has been used for the treatment of acne, burns, and baldness, especially in women. Platelets contain several growth factors that play a role in the formation or regeneration of blood vessels from the existing network of blood vessels. Furthermore, platelets can improve oxygen supply to tissues when blood flow is reduced.[3]

Although PRP is a promising therapy, however, the standardization of the PRP procedure has not been determined, and the quality examination of PRP is still limited.[3,4] In general, PRP is obtained through a centrifugation process. There are various techniques in obtaining PRP, ranging from the double centrifugation (DC) techniques at various temperatures, the buffy coat separation techniques, to the technique of adding a platelet activator.[5] Currently, there are also commercial PRP kits, which are more expensive than conventional and manual methods.[6]

The main process that determines the quality of PRP is the centrifugation process, which includes the number of spins of centrifugation, centrifugation time, centrifugal acceleration, and the volume of the blood.[7,8] The PRP procedure commonly used is the double centrifugation (DC) technique, however, there are also modifications, among others the turn down-turn up technique (DC-TDTU) which is claimed to produce a maximum number of platelets.[9] To measure the optimization of the centrifugation process and the separation of blood cells from plasma,

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it is necessary to count platelets, leukocytes, erythrocytes, and hematocrit (Ht) at each step of the centrifugation procedure.[5] In this study, we compared the quality of PRP obtained by various techniques which were single centrifugation (SC), double centrifugation (DC) and double centrifugation turn down-turn up technique (DC-TDTU). The blood cell counts (platelet, leukocyte, erythrocyte count, and Ht) were compared in order to assess the PRP separation process and to determine the best quality among the three techniques.

Methods

This research was an experimental study using acid citrate dextrose (ACD) blood taken from 11 healthy respondents by routine blood tests within the normal range. The procedure has been approved by the Ethics Committee of Poltekkes Kemenkes Jakarta 3 no KEPK-PKJ3/043/VI/2021. The examination was carried out at the Hematology Laboratory, Poltekkes Kemenkes Jakarta 3. Blood samples for the three techniques were drawn in a vacuum tube (BD vacutainer ACD solution A REF 364606) under strict aseptic precautions. The centrifuge type used was a swing centrifuge, conducted by the same person.

In the single centrifugation (SC) technique, the blood was centrifuged at 3,200 rpm for 15 minutes, and the plasma supernatant was separated. Furthermore, the cell counts, including platelet, leukocyte, erythrocyte count, and hematocrit (Ht) were measured (Sysmex Xs500i hematology analyzer).

In the double centrifugation (DC) technique,

the first spin was performed at 400 g for 10 minutes. The plasma supernatant formed was separated into another tube without anticoagulant and then centrifuged at 1200 g for 10 minutes. The supernatant formed (the upper 2/3rd tube) was further separated from the precipitate (PRP) before resuspending by a gently shaking tube.

In the double centrifugation turn down-turn up (DC-TDTU) technique, the first spin to collect plasma was performed at 200 g for 15 minutes with the vacuum tube facing down (inverted position) in the centrifuge. Blood sediment was separated with a syringe through the rubber tube cap at the bottom of the tube for 3.5 mL (Figure). The second centrifugation was carried out on the tube at 1600 g for 10 minutes with the position of the tube facing up (normal position). The supernatant formed was separated for 3.5 mL volume, and the precipitate (PRP) was also separated for 1–2 mL in a different tube without anticoagulant.

The data on the blood cell count were analyzed in each centrifugation process. Statistical differences between whole blood, plasma from first centrifugation, and PRP after double centrifugation techniques were tested using t-test and Wilcoxon, based on the Shapiro Wilk distribution data test. P-value <0.05 was considered statistically significant.

Results

The mean platelet, leukocyte, erythrocyte count, and Ht in whole blood and PRP in each technique and centrifugation step were shown in Table 1. The platelet count on PRP using DC
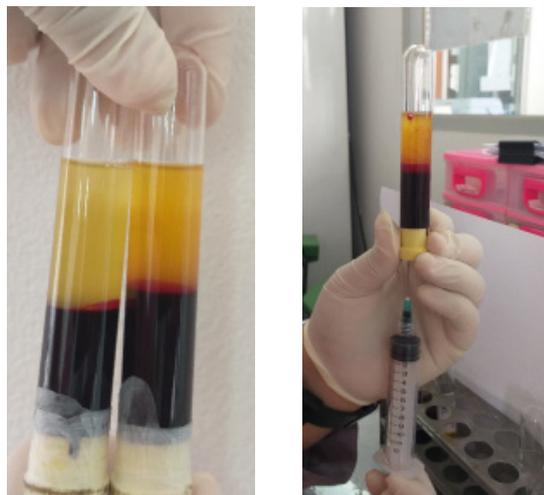

Figure Preparations of PRP using DC-TDTU Technique after the first Centrifugation

and DC-TDTU techniques showed an increase between the first and second centrifugation, whereas using the SC technique there was a decrease. Interestingly, there was a significant difference (p<0.05) in the number of platelets between forms of whole blood, plasma, and PRP.

The leukocyte count showed an increase in the PRP compared to plasma. The decrease in the leukocyte count from whole blood to plasma in the three techniques showed a significant difference (p<0.05).

The mean erythrocytes count in the DC technique was lower than in the DC-TDTU and SC techniques. This result was also related to the Ht value. Our results showed that the Ht value in the DC technique was smaller compared to the two techniques.

Discussion

Examination of blood cell count in the PRP procedure is used to check the process of separating plasma from blood cells, where a large concentration of platelets is produced.[5] Every PRP procedure and technique must be checked for quality because the composition of PRP is different for every person, device, and method.[10] Centrifugation in the same RPM will exert different centrifugal forces if centrifuge rotors have different radius sizes, bucket types, or bucket sizes.[11] In the DC and DC-TDTU techniques, the mean platelet count is 3 times higher than in the whole blood. According to the principle of PRP centrifugation, the first centrifuge is to separate erythrocytes (bottom), buffy coats (middle), and plasma (top). The second centrifugation is to concentrate the platelet, which is suspended in the smallest volume of plasma. To ensure that the platelets are suspended and do not form a clot, PRP should be prepared from anticoagulated blood, ACD is the best anticoagulant for maintaining platelet viability compared with heparin and sodium citrate.[12] The platelets have begun to be concentrated in the 1/3 volume of the tube bottom to form a white and slightly cloudy layer.[5] In this procedure, each PRP is incubated for 30 to 60 minutes at room temperature, to inhibit platelet aggregation, which can appear as clots that interfere with the blood cell count. The incubation aim also facilitates the settling of platelets onto the buffy coat.[13] The number of platelets in plasma is less than platelets in whole blood. In the SC technique, the number of platelets in plasma is lower

Table 1 Platelet, Leukocyte, Erythrocyte Count, and Ht Value in Whole Blood, Plasma, and PRP

Parameter*	SC	DC	DC TD-TU
Platelet count			
Whole blood	240.5±29.4	240.5±29.4	240.5±29.4
Plasma	148.3±94	507.5±89.8	427.8±48.5
PRP	-	1,123.3±441.5**	1,088.9±277.6**
p-test	p < 0.05	p < 0.05	p < 0.05
Leukocyte count			
Whole blood	6.9±1.7	6.9±1.7	6.9±1.7
Plasma	2.8±3.4	1.2±0.7	5.2±2.8
PRP	-	3.8±2.5	13.3±5.5**
p-test	p < 0.05	p < 0.05	p < 0.05
Erythrocyte count			
Whole blood	4.1±0.5	4.1±0.5	4.1±0.5
Plasma	0.2±0.3	0.0	1.6 ±0.6
PRP	-	0.06±0.1**	3.0±0.8
p-test	p < 0.05	p < 0.05	p < 0.05
Ht			
Whole blood	3.8±5.2	3.8±5.2	3.8±5.2
Plasma	2.2±2.8	0.1±0.1	15 ±6.2
PRP	-	0.4±0.2	27.9 ±8.1**
p-test	p < 0.05	p < 0.05	p < 0.05

Note: * number is mean + standard deviation; p-value < 0.05 statistically significant, Ht, hematocrit; PRP, platelet-rich plasma; SD, standard deviation; SC, the single centrifugation; DC, the double centrifugation; and DC-TDTU, the double centrifugation turn down - turn up techniques

than the number of platelets in the whole blood, although some authors recommend the single-spin technique. Another study proved that centrifugation of 541 g for 61 mL of whole blood at 5 minutes was optimal for obtaining high platelet in the sample.[14] In this study, we also included a single centrifugation technique that is commonly used.

The platelet counts in PRP can affect and positively correlate with the concentration of growth factors and better clinical results.[15,16] Several studies have shown that PRP therapy for hip disorders, shows maximum improvement with platelet concentrations 2–7 times higher than the number of platelets in the whole blood.[17]

The leukocyte count in PRP should be counted because it is an important factor in tissue healing, although several studies have produced different hypotheses.[18] The number of leukocytes in PRP can have positive or negative effects. Leukocytes can cause an inflammatory reaction, but some experts also find that leukocyte cells can act as anti-bacterial in PRP and protect against infection. In addition, leukocyte cells correlated with an increased release of growth factors.[4] A study showed that PDGF and TGF-β1 released from leukocytes play a role in the therapeutic process of fracture healing. However, another study has shown that PRP rich in leukocytes can inhibit wound healing due to the release of reactive oxygen species (ROS) by neutrophils in the wound area. The negative effect produced by leukocytes may not occur in all tissues, considering that there is a positive effect produced by leukocytes on PRP.[19] The results of this study have shown that the DC and DC-TDTU techniques have a higher average number of leukocytes in PRP than plasma. PRP has been filled with a buffy coat due to the first centrifugation. However, the leukocyte count in the DC-TDTU technique is higher than in the DC technique, indicating the large number of blood cell volumes remaining in plasma.

The erythrocyte count and the Ht value in PRP indicate the presence or absence of erythrocyte contamination at each stage of the PRP procedure.[5] This study has shown that the erythrocyte count is minimal or even absent in the DC technique, although a small number of erythrocytes has been obtained in PRP due to the process of concentration and resuspension with a buffy coat. In the DC-TDTU technique, there are still more blood cells than in the DC and SC techniques. In the DC-TDTU technique, red blood cells are separated from the plasma by taking a volume of 3.5 mL of erythrocytes

from the bottom of the tube, but still leaving more erythrocyte volumes in the second centrifuge. This affects the higher Ht value compared to the DC and SC techniques that separate plasma from the red blood cells. Compared to the SC technique, the DC is being the preferred technique for PRP preparation, as shown in a study for dermatologic use, due to its low cost, maximal platelet count, and adequate platelet volume.[20]

The recovery efficiencies data measures the effect of the volume of plasma after the centrifugation process.[18] This measurement is part of the limitation of this study. The recovery efficiencies in plasma, platelet, and leukocyte have not been calculated due to the unavailability of plasma or buffy coat volume data after centrifugation. Furthermore, specific analysis like growth factor measurement is needed to enhance the technique quality.

To conclude, the DC technique is the best PRP separation technique compared to the DC-TDTU and SC techniques.

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