Platelet Counts Analysis of Platelet-Poor Plasma (PPP) Produced by Several Centrifugation Techniques

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Abstract

Platelet-poor plasma (PPP) can be obtained by various centrifugation techniques. This study aims to analyze platelet count in PPP produced by three different centrifugation techniques. Samples came from a healthy adult who underwent medical check-up and had been given informed consent. A total of 2.7 mL of blood samples were collected using three citrated tubes. Samples were centrifuged by three different techniques to obtain PPP: 1,500 g for 15 minutes twice, 3,000 g for 15 minutes, and 3,260 g for 10 minutes. The platelet count of each PPP was examined using a hematology analyzer. All three centrifugation techniques produced <10,000/µL platelets in all PPP from 31 samples. The twice centrifugation of 1,500 g for 15 minutes produced a median number of platelets which was 1×10^3/µL (0-3). The 3,000 g centrifugation for 15 minutes produced a median number of platelets which was 1×10^3/µL (0-5). The 3,260 g centrifugation for 10 minutes produced a median number of platelets which was 2×10^3/µL (0-5). A comparison of platelet count showed a significant difference ($p < 0.05$) among the three centrifugation techniques. The three centrifugation techniques in this study were able to produce PPP. The centrifugation technique of 1,500 g for 15 minutes twice produced the lowest number of platelets.

Keywords: centrifugation, platelet-poor plasma, platelet count

Introduction

Clinical laboratory testing plays an essential part in the detection, diagnosis, and treatment of diseases. Laboratory results contribute around 60-70% in determining the decision for patients to undergo hospitalization and receive treatments[1]. Effective and efficient laboratory services are characterized by three factors: precision, accuracy, and timeliness which is assessed by turnaround time (TAT)[2,3]. TAT is often used by clinicians as a performance guide or key performance indicator in laboratory services. It was reported that the preanalytic phase contributes around 75% of the total TAT[2].

In addition, one of the pre-analytical processes commonly done is the centrifugation process. Centrifugation is the separation process of solid particles and their solvents. This process is often used in laboratories to separate blood cells from their plasmas to produce PPP. PPP is a plasma with a platelet count of less than 10,000/µL used for coagulation study[4,5].

The preparation of PPP recommended by the Clinical and Laboratory Standart Institute (CLSI) is to use a tube with sodium citrate anticoagulant and centrifugation processes at a speed of 1,500 g for at least 15 minutes[5]. Different results were obtained in a study conducted by Kristoffersen which stated that the number of platelets after a single 1,500 g centrifugation results in a platelet count <22,000/µL[6]. Magnette *et al.* recommend a repeat of centrifugation to ensure platelet residue is less than 10,000/µL. The remaining platelet in plasma has been known to affect phospholipid-dependent coagulation tests such as those on Lupus Anticoagulant (LA)[7]. This repeating centrifugation process must be prepared for all coagulation parameters if there is a possibility that the measurement is not carried out immediately after centrifugation, or if the sample will be frozen[6-10].

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A study conducted by Sicard showed that the amount of platelet residue in PPP produced at a speed of 3,260 g in 10 minutes was <10,000/μL[11]. PPP preparation in Dr. Soetomo Hospital used 3,000 g centrifugation for 15 minutes. There has been no prior research that confirmed this technique will produce less than 10,000/μL platelet residues. This centrifugation technique requires a long time in the preanalytic stage. An updated technique is needed to speed up this process without reducing the quality of the coagulation[4].

This study aimed to examine the differences in the number of platelets in PPP produced in the 1,500 g centrifugation technique for 15 minutes twice, 3,000 g for 15 minutes, and 3,260 g for 10 minutes. The results that are accurate, fast, and precise are hopeful to help improve the laboratory services quality in Dr. Soetomo Hospital.

Materials and Method

The study was an analytic study with a cross-sectional design. The sample was obtained by conducting consecutive sampling during April-May 2019. The inclusion criteria in this study were healthy adult patients aged >21 years who underwent a medical check-up, were in good health and were willing to take part in the study. The exclusion criteria in this study were the history of drug consumption in the last 10 days that interfere with platelet function.

Becton-Dickinson Vacutainer tubes (Rutherford, New Jersey, United States of America) with sodium citrate anticoagulant were used in this study. The concentration of sodium citrate used was 0.109 M (3.2%). Venous blood samples were taken and put in 3 tubes of 2.7 cc each with a ratio of 9:1. Slow inversion 3-6 times was done to make blood and anticoagulants homogeneous. Then, the samples were centrifuged with Sorvall ST 8R (Thermo Scientific small benchtop centrifuges) at 24 °C that was calibrated with a tachometer twice a year. Three samples were centrifuged with three different speeds and times: 1,500 g for 15 minutes twice (Technique 1), 3,000 g for 15 minutes (Technique 2), and 3,260 g for 10 minutes (Technique 3). After the initial centrifugation, plasma in technique 1 was carefully transferred to an inactive plastic centrifugation tube using an automatic pipette, and then centrifuged again for about 15 minutes. Centrifugation in technique 2 and technique 3 was only done once. The plasma was carefully transferred to the aliquot with a pipette, leaving 1 cm to remain above the buffy coat. This was done to avoid the removal of the remaining platelet in the area around the buffy coat. The plasma of each sample was assessed by platelet count using a Sysmex XN 1000 hematology analyzer.

Statistical analysis was done using SPSS version 17.0. The collected data was carried out by cleaning, coding, tabulating, and entry into the computer. The median was also calculated. A paired T-test was done for data with a normal distribution. For data that were not normally distributed, a Wilcoxon test was performed. p-value <0.05 was considered statistically significant.

Results

Participants who were willing to take part in this study were 31 people who came from Medical Check-Up (MCU). Samples consisted of 12 men and 19 women. All the 31 samples from the three centrifugation techniques have platelet count below 10,000/μL. The centrifugation technique of 1,500 g for 15 minutes twice produced a median number of PC which was 1×10^3/μL (0-3). The centrifugation technique of 3,000 g for 15 minutes obtained a median number of PC which was 1×10^3/μL (0-5). The centrifugation technique of 3,260 g for 10 minutes resulted in a median number of PC which was 2×10^3/μL (0-5) (Table 1).

Comparison analysis of the platelet count between groups of centrifugation techniques showed a significant difference between Technique 1 and Technique 2 ($p = 0.004$). Comparison analysis of platelet count from Technique 1 and Technique 3 showed a significant difference ($p = 0.0005$). Comparison analysis of platelet count from Technique 2 and Technique 3 also showed a significant difference ($p = 0.004$) (Table 2).

Table 1. Data of Platelet Count from the Three Centrifugation Techniques

Centrifugation Technique	n	Platelet Count (×103/ μL) Median (min-max)
1,500 g for 15 minutes twice	31	1 (0-3)
3,000 g for 15 minutes	31	1 (0-5)
3,260 g for 10 minutes	31	2 (0-5)

Table 2. Comparison Result of Platelet Count among Centrifugation Techniques

Variables	Centrifugation Techniques	p-Value
Platelet Count (×10³/µL)	Technique 1 compared with Technique 2	0.004
	Technique 1 compared with Technique 3	0.0005
	Technique 2 compared with Technique 3	0.004

Note: 1,500 g for 15 minutes twice (Technique 1)

3,000 g for 15 minutes (Technique 2)

3,260 g for 10 minutes (Technique 3)

Discussion

The centrifugation parameters for removing platelets depend on the duration, speed, and radius of the centrifuge arm. The speed and radius of the centrifuge arm determine the "g" value. Each centrifuge produces different values of g and varies the time of centrifugation to obtain the desired PPP[10,12,13].

However, CLSI recommends that tubes with anticoagulants should be centrifuged at a speed of 1,500 g no less than 15 minutes to obtain PPP[5]. A study conducted by Favaloro *et al.* suggested that using a centrifugal force relatively greater than 1,500 g was not recommended because it could cause platelet activation, hemolysis, or other undesirable effects[14]. Higher speed (greater than 1,500 g) and shorter time (less than 10 minutes) in an emergency could be used to prepare PPP for coagulation examination[4,7]. Several studies that evaluated the impact of higher speed centrifugation on platelet count concluded that fast centrifugation did not change results and contributions by reducing preanalytic duration[4,11,15].

This study compared the platelet count in the 1,500 g centrifugation technique for 15 minutes twice, 3,000 g for 15 minutes, and 3,260 g for 10 minutes to obtain PPP. The Sorvall ST 8R centrifuge uses a swinging bucket rotor for high-speed horizontal processing. This centrifuge accommodates a maximum speed of up to 3,260 g (4,500 rpm)[12]. A study conducted by Magnette *et al.* demonstrated that a centrifuge with a swinging bucket rotor is easier to separate plasma from cellular components and minimize re-mixing of plasma with red blood cells[7].

Samples with hemolysis cannot be processed because clotting factor activation can occur and interfere with the clot detection by optical devices that use the principle of changing plasma turbidity[16-18]. Those samples will also cause spectral interference in devices using photo-optical methods. It can affect the examination of the coagulation study[19]. Fortunately, there were no samples with hemolysis in this study. Plasma from each sample was taken to assess the platelet count with a Sysmex XN 1000 hematology analyzer.

The lowest platelet count ($<3\times10^3/\mu L$) was obtained from the 1,500 g centrifugation technique for 15 minutes twice. This result was similar to a previous study conducted by Kristoffersen and Sicard which showed that the number of platelets of PPP produced by doing 1,500 g of centrifugation for 15 minutes twice and 3,260 g of centrifugation in 10 minutes was <10,000/µL[6,11]. This study was the first study to examine the centrifugation of 3,000 g for 15 minutes.

A comparison of platelet count among centrifugation techniques showed significant differences ($p<0.05$). These results differ from a study conducted by Sultan in 2010 which showed no significant differences between the PPP platelet count produced at 2000 g centrifugation for 20 minutes and 3,000 g centrifugation for 5 minutes. This difference result is possibly due to the differences in the tools used and research procedures. Research conducted by Sultan used a Centurion-K40 centrifuge model with a fixed angle rotor[15].

In addition, Sultan showed no significant difference in the PT and APTT examination between PPP produced by 2,000 g centrifugation for 20 minutes and 3,000 g

centrifugation for 5 minutes[15]. The limitation of this study was no evaluation of the high acceleration impact of centrifugation conditions on routine coagulation study (including PT, APTT, and fibrinogen) in each PPP resulted from each centrifugation techniques.

Conclusion

In sum, the three centrifuge techniques in this study were able to produce PPP because platelet count obtained was less than 10,000/μL. The resulting PPP was compliant with the CLSI standard despite the statistically significant differences in platelet count among the three different centrifugation techniques. The twice 1,500 g centrifugation for 15 minutes each was the technique that produced the lowest number of platelets. The difference in results obtained from this study was due to the different types and techniques of the tools used.

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