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Comparative Study of Four Platelet-Rich Plasma Methods for Preparing Platelet Concentrates

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Abstract

Background: Platelet preparations have been used for a wide variety of clinical applications such as hemorrhage, tissue engineering and cellular therapy. Platelet concentrates can be prepared by the apheresis method or from the whole blood using the Buffy-coat or Platelet-rich plasma methods. The purpose of this study was to compare four variations of platelet-rich plasma method based on double centrifugation protocol to identify the optimal centrifugation conditions with greatest platelet recovery and highest enrichment capacity for preparing platelet concentrates.

Materials and Methods: Blood samples were obtained from 145 donors, chosen randomly from the donation department at the Tehran Blood Transfusion Center, Tehran, Iran. Four variations of platelet-rich plasma methods were selected for preparation of platelet concentrates. Platelet counting analysis was performed on samples and platelet enrichment and platelet recovery were calculated by investigating the correlation between the number of platelets in the whole blood, platelet-rich plasma and platelet concentrates.

Results: Platelet count analysis revealed that the method performed with 2100 ×g for 2.30 min in the first centrifugation step and 4150 ×g for 6 min in the second centrifugation step had the highest platelet enrichment (5.59fold) and greatest platelet recovery (78.63%).

Conclusion: Within the limits of this study, it can be concluded that 2100 ×g for 2.30 min in the first centrifugation step and 4150 ×g for 6 min in the second centrifugation step yielded the greatest platelet recovery and highest enrichment capacity and is a good choice for preparing platelet concentrates.

Keywords: Platelet, concentrates, plasma, centrifugation, recovery, enrichment.

Introduction

Platelets play an important role in the haemostatic process by sealing damaged blood vessels, forming a platelet plug and preventing the blood loss. In transfusion medicine, platelet concentrates were originally used for treatment and prevention of hemorrhage due to severe thrombocytopenia, which is often caused by medullary aplasia, acute leukemia or significant blood loss during long-lasting surgery. However platelets contain high quantities of key growth factors and other bioactive molecules and the expansion of platelet-derived growth factor (PDGF) through applications of platelet-rich plasma (PRP) or platelet gels is thought to stimulate angiogenesis and promote more rapid tissue repair. Therefore Platelet preparations have been used for a wide variety of clinical applications such as oral and maxillofacial surgery, plastic surgery, ophthalmology, orthopedics, treatment of chronic wounds, sports-related injuries, tissue engineering and cellular therapy [1-3].

Platelet concentrates (PCs) can be prepared using apheresis method (AP) or from the whole blood using the Buffy-coat (BC) or platelet-rich plasma (PRP) methods. When using the PRP method to prepare PCs, whole blood is centrifuged by soft spin to prepare PRP followed by a high-speed centrifugation to obtain a platelet pellet.

Then most of the plasma is removed, and the platelets are stored in a reduced volume of the remaining plasma [4-6].

While the normal range of platelets in the whole blood of healthy individuals is 150,000 to 350,000 platelets/µl of whole blood, the working definition of PC is a concentration of 1,000,000 platelets/µl of platelet preparation. In other words, the concentration of platelets should be developed to have a 3 to 5 fold increase over the baseline [7-8].

In fact the natural variations in platelet concentrations among individuals as well as the daily variation in platelet parameters observed within individuals can further affect the consistency, efficacy and clinical outcomes of the final product [9]. In addition, the final platelet concentration of any PRP product is based on the initial volume of the whole blood taken, the platelet recovery efficiency of the technique used, and the final volume of plasma used to suspend the concentrated platelets and changing any of the aforementioned variables will proportionally change the final platelet concentration [10].

The three major variables that affect the recovery of cells from the whole blood by double-centrifugation protocol are rotor size, speed and duration of centrifugation. More than one combination of these parameters can provide the optimal yield of platelets in the preparation. For a given centrifuge, the rotor size is generally not variable. Therefore the concentration can be developed over baseline by altering the other two variables (speed and duration of centrifugation) in a stepwise fashion [11].

Thus the purpose of the present study was to compare four different variations of PRP method based on double centrifugation protocol to identify the optimal centrifugation conditions for preparing platelet concentrates with greatest platelet recovery and highest enrichment capacity.

Materials and Methods:

Blood samples were obtained from 145 donors, chosen randomly from the donation department at Tehran Blood Transfusion Center (TBTC), Tehran, Iran. The whole blood was then subjected to two centrifugation steps within 8 hours of blood draw. The initial centrifugation step (Soft Spin) separated the red cells from the PRP. The resulting plasma supernatant which contained the suspended platelets was subjected to a second, longer centrifugation step (Hard Spin), further concentrating the platelets into pellet. The platelet poor plasma (PPP) was removed and the platelets were stored in 50 ml of remaining plasma.

Four variations of platelet-rich plasma method were selected for preparing platelet concentrates; Method A: 2100 ×g for 2.30 minutes in the first centrifugation step and 4150 ×g for 6 minutes in the second centrifugation step; method B: 2100 ×g for 4 minutes in the first centrifugation step and 4150×g for 9 minutes in the second centrifugation step; method C: 2100 ×g for 4 minutes in the first centrifugation step and 4100 ×g for 9 minutes in the second centrifugation step; method D: 2100 ×g for 4 minutes in the first centrifugation step and 4200 ×g for 9 minutes in the second centrifugation step.

Platelet counting analysis was performed in samples using a cell counter (Sysmex, KX-21N, Japan) in the Quality Control Department of TBTC. Statistical analysis was performed using SPSS and the correlation between the number of platelets in the whole blood, PRP and platelet concentrates were investigated. The best method for preparing platelet concentrates with the greatest platelet recovery and highest enrichment capacity was specified using the following equations [12-13]:

$$\text{First platelet enrichment} = \frac{\text{Platelet concentration of PRP}}{\text{Platelet concentration of WB}}$$

$$\text{Second platelet enrichment} = \frac{\text{Platelet concentration of PC}}{\text{Platelet concentration of PRP}}$$

$$\text{Total platelet enrichment} = \frac{\text{Platelet concentration of PC}}{\text{Platelet concentration of WB}}$$

$$\text{First platelet recovery} = \frac{\text{Platelet count of PRP}}{\text{Platelet count of WB}} \times 100$$

$$\text{Second platelet recovery} = \frac{\text{Platelet count of PC}}{\text{Platelet count of PRP}} \times 100$$

$$\text{Total platelet recovery} = \frac{\text{Platelet count of PC}}{\text{Platelet count of WB}} \times 100$$

Results:

Platelet enrichment is expressed as the fold increase in platelet concentration over the whole blood sample from which the platelet concentrate was prepared [11]. Thus the baseline whole blood platelet concentrations and the average platelet concentrations of samples were compared. The values for platelet enrichment and platelet recovery in four experimental methods are given in table 1 and table 2 respectively.

Based on the Platelet count analysis total platelet enrichment and total platelet recovery in method A (5.59fold, 78.63%) was higher than those in methods B (5.23 fold, 65.37%), C (5.05fold, 60.78%) and D (4.68fold, 57.31%).

Discussion:

Platelet concentrate preparations which have been used for a variety of clinical applications can vary widely in the amount of platelets they contain[10]. According to some experimental in vivo studeis, the therapeutic level of PCs is a concentration of 1,000,000 platelets/µL and lower platelet concentrations were suboptimal. Since the normal range of platelets in the whole blood of healthy individuals is 150,000 to 350,000 platelets/µL of whole blood, therefore working definition of platelet concentrate preparations has evolved to mean a 3 to 5 fold increase in the concentration of platelets over baseline [3,7-8]. Although different individuals may require different platelet concentrations to achieve comparable biological effect, the volume of platelet concentrate preparations should be minimal to decrease the total transfusion volume and intratendinous pressure and to minimize pain. These preparations should also have a raised platelet count [14].

Processing technique and platelet concentration ratio are two important variables that affect the quality and clinical effectiveness of platelet concentrate preparations [15]. Among processing techniques, centrifugation (either the single- or double-centrifugation protocol) forms the basis of the current methods for producing platelet concentrate preparations. Within the limits of different studies, it can be concluded that the double-centrifugation protocol using the correct g-forces and spin times results in higher platelet concentrations than the single centrifugation protocol [1-2, 16].

However, even when specific PRP protocols are used, the platelet concentration of the final preparation may vary greatly not only between different techniques but also within a given technique. For example a recent study has shown that platelet concentrations in the final preparation within a given technique can vary as much as 50%[17].

Although the final platelet concentration of any platelet preparation is based on the initial volume of the whole blood taken, the platelet recovery efficiency of the technique used and the final volume of plasma used to suspend the

Table1: Values for platelet enrichment in four different experimental methods.

Method	Enrichment (Fold)		
	First step	Second step	Total
A[a]	1.54	3.87	5.59
B[b]	1.44	3.75	5.23
C[c]	1.44	3.79	5.05
D[d]	1.44	3.76	4.68

a: 2100×g for 2.30 min in first step and 4150×g for 6 min in second step.
b: 2100×g for 4 min in first step and 4150×g for 9 min in second step.
c: 2100×g for 4 min in first step and 4100×g for 9 min in second step.
d: 2100×g for 4 min in first step and 4200×g for 9 min in second step.

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d: 2100×g for 4 min in first step and 4200×g for 9 min in second step.

concentrated platelets, but the natural variations in platelet concentration among individuals as well as the daily variation in platelet parameters observed within individuals can further affect the consistency and efficacy of the final product [4, 10].

Recent studies have confirmed that double-centrifugation techniques can yield concentration values equal or higher than those cited by Weibrich et al. [1, 12, 16, 18-20].

The present study was performed to confirm an effective platelet concentrate production method using the correct g-forces and spin times. We recovered 57.31 to 78.63% of total initial platelets and the procedures resulted in a 4.68 to 5.59 fold increase in platelet concentration. Besides, it was found that the greatest platelet recovery (78.63%) and the highest platelet enrichment (5.59 fold) in platelet concentrate preparation was achieved using 2100×g for 2.30 min and 4150×g for 6 min for the first and second centrifugation steps respectively. Thus in comparison with other studies with concentration ratios of less than 2-fold to 8.5 fold, this method provides acceptable platelet recoveries and enrichment capacities [21-26].

Conclusion:

Within the limits of this study, it can be concluded that 2100 ×g for 2.30 min in the first centrifugation step and 4150 ×g for 6 min in the second centrifugation step yielded the greatest platelet recovery and the highest enrichment capacity. This prevents associated circulatory overload by reducing the total transfusion volume of platelet concentrate preparation and is a good choice for preparing platelet concentrates.

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