

Comparison of different methods of centrifugation for preparation of platelet- rich plasma (PRP)

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Abstract

Introduction: Platelet- rich plasma (PRP) has wide clinical applications which have been proved by various studies. There are various methods available now-a-days to prepare PRP but no standard protocol determining the time and centrifugation speed is available. This variation in the reported methods may produce samples with different platelet concentrations which may induce different clinical responses. Hence, the aim of the present study was to compare the different methods of centrifugation for preparation of platelet- rich plasma by estimating the platelet concentration in them.

Methods: Whole blood was drawn from 50 healthy volunteers into two citrate tubes which were subjected to different centrifugation speed and time. The first tube underwent double spin at 100g (soft spin) and 400g (hard spin) respectively, for 10 minutes; whereas the second tube underwent single spin at 100g for 10 minutes. The platelet counts before and after centrifugations were then compared.

Results: Single spin method yielded a platelet concentration factor of 2.19 as compared with the double spin method where, actually the platelet counts reduced (platelet concentration factor= 0.83).

Conclusion: From the above findings we conclude that single centrifugation method is better than double centrifugation method in preparation of platelet rich plasma as evidenced by the high platelet concentration factor (2.19).

Keywords: PRP, Single spin, Platelet concentration factor

Introduction

Platelet- rich plasma (PRP) is a preparation of plasma wherein the concentration of platelets is increased. Platelets play a major role in hemostasis and they are also a natural source of various growth factors. Growth factors are stored within the α -granules of platelets, such as the platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin like growth factor (IGF), platelet derived angiogenic factor (PDAF) and transforming growth factor beta (TGF- β). The release of these growth factors depends upon the activation of platelets which can be achieved by a variety of substances like calcium chloride, thrombin and collagen.¹ These above mentioned growth factors help in wound healing and are also involved in regenerative processes like chemotaxis, proliferation, differentiation, and angiogenesis.²

Various studies have proved the clinical applications and results of PRP in the fields of dermatology, dentistry, oral maxillo-facial surgery, plastic surgery, orthopedics, rheumatology, and also in the treatment of different injuries including chronic wounds and muscle injuries.³ PRP is also used in platelet aggregation studies in cases of suspected platelet dysfunction.⁴

There are many protocols in the current literature which have described the optimal conditions for centrifugation. However, these protocols have been optimized according to numerous variables like the volume and sampling of processed whole blood,

number of spins of centrifugation, time period of centrifugation and range of the centrifugal acceleration.

There are various methods available now-a-days to prepare PRP but no standard protocol determining the time and speed of centrifugation is available. This variation in the reported methods may produce samples with different platelet concentrations which may induce different clinical responses.

Hence, the aim of the present study was to compare the different methods of centrifugation for preparation of platelet- rich plasma (PRP) by estimating the platelet concentration in them.

Materials and Methods

The study was a prospective study conducted at the Department of Pathology, Bangalore Medical College and Research Institute after obtaining the approval by the Ethics Committee. Whole blood was collected from 50 healthy volunteers, after taking written informed consent. All whole blood samples having normal platelet concentration (1.5-4.5 lakh/cumm) were included in the study. Samples which were clotted or hemolysed, all cases of thrombocytopenia or thrombocytosis, and samples with low hematocrit values were excluded from the study.

Methodology: Complete medical details were collected from the volunteer, including past history and history of intake of any drugs. Using aseptic precautions, 10 mL of whole blood was collected from 50 volunteers. After collection of blood, 2 mL of blood was taken in EDTA

(ethylene diamine tetra acetic acid) tube; 3.6 mL of the whole blood was taken in two tubes containing 0.4 mL of 3.8% trisodium citrate each. The two tubes were labeled as first and second tubes. The EDTA sample was run in Coulter LH 780 hematology analyzer to check for hemoglobin, hematocrit and platelet count.

The tube labeled as 'first tube' underwent two centrifugation steps under constant temperature conditions: first at 100g for 10 minutes (soft spin) to separate RBCs from remaining whole blood volume. After the first spin, the whole blood was separated into three layers: an upper layer containing platelets and WBC, an intermediate layer which is the buffy coat rich in WBCs and a bottom layer consisting mostly of RBCs. The upper layer plus buffy coat from the first tube were then transferred to an empty tube. The second spin step (hard spin) was then performed on it at 400g for 10 minutes. The upper portion of the volume composed mostly of PPP (platelet- poor plasma) was removed to create the PRP (Platelet- rich plasma).

The tube labeled as 'second tube' underwent only single centrifugation step at 100g for 10 minutes under similar temperature conditions and the upper layer composed of platelet rich plasma was separated in an empty tube.

Platelet concentrations were then analyzed in both the tubes using Coulter LH 780 hematology analyzer and were compared in both the methods.

Statistical analysis: Platelet concentrations before and after centrifugation, were entered in excel sheet and analyzed using T-test.

Results

Out of the 50 samples collected, 16(32%) were males and 34(68%) were females. The age group ranged from 20-45 years, with a mean of 26.38 years. The platelet count before centrifugation varied from 1.93 to 4.01 lakh/cumm, with a mean of 2.81 lakh/cumm.

After centrifugation the Platelet concentration factor (FC_{pt}) was calculated using the formula:

$$FC_{pt} = \frac{C_{pt}(\text{after single spin or double spin})}{C_{pt}(\text{Whole blood})}$$

Where, C_{pt} = platelet count

After single spin, the mean platelet count was 6.05 lakh/cumm, with a mean platelet concentration factor of 2.19. The maximum platelet count which was obtained after single spin was 8.06 lakh/cumm and maximum platelet concentration factor was 3.15.

However, after double spin the mean platelet count dropped to 2.34 lakh/cumm with a mean platelet concentration factor of 0.83. The maximum platelet count which was obtained after double spin was 8.97 lakh/cumm and maximum platelet concentration factor was 2.88.

Thus, single spin method yielded higher platelet counts and platelet concentration factor as compared to double spin method ($P < 0.05$).

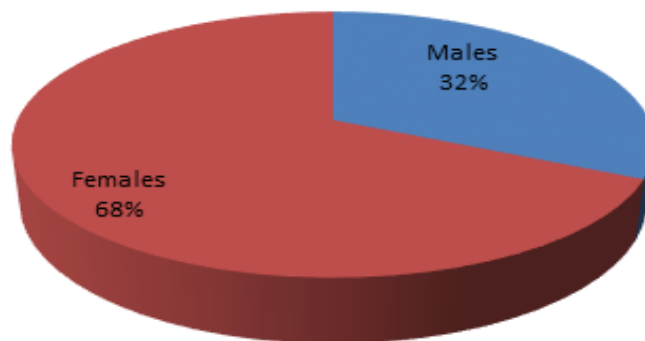
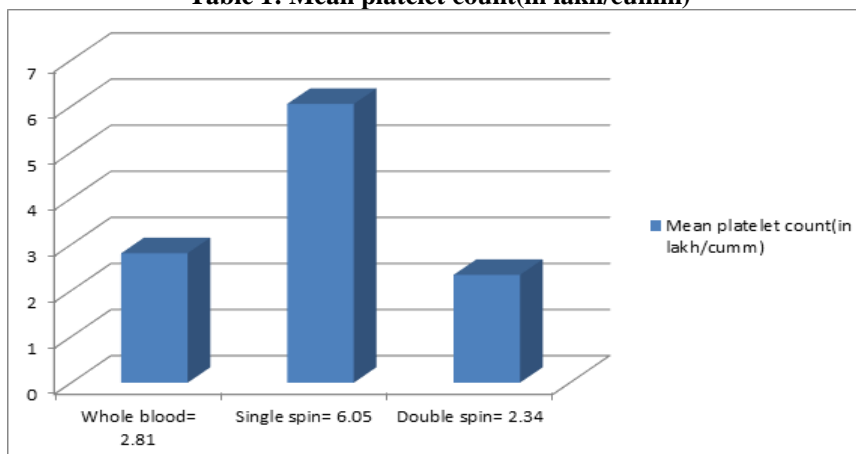
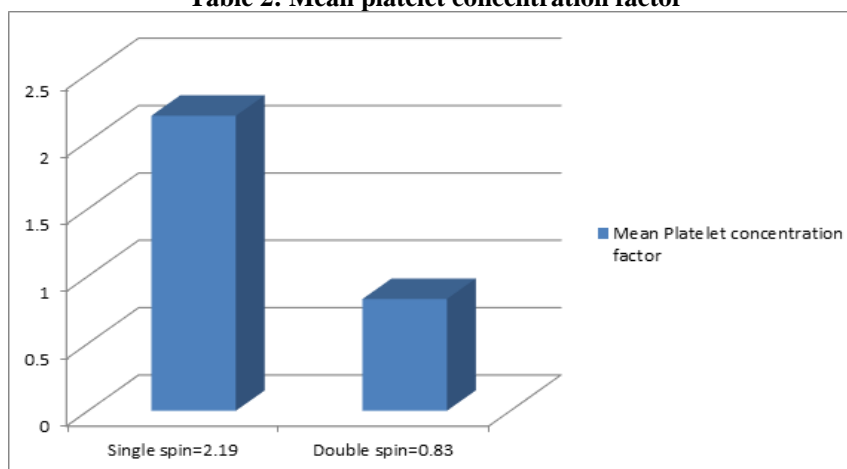


Fig. 1: Distribution of cases according to gender

Table 1: Mean platelet count(in lakh/cumm)**Table 2: Mean platelet concentration factor**

Discussion

Platelet- rich plasma (PRP) was first described in 1997 by Whitman *et al* as a derivative of the fibrin glue.⁵ PRP is defined as a fraction of a volume of plasma having a higher platelet concentration than in the peripheral blood. Concentration of platelets and amount of growth factors in the PRP depend on the type of method used. On an average, PRP has about 3-5 times more growth factors than that of peripheral blood.

In 2009, Dohan Ehrenfest DM, Rasmusson L, Albrektsson T, proposed a classification wherein four main families of PRP preparation were defined depending upon their cell content and the fibrin architecture.⁶ These are:

1. Pure- PRP (P-PRP) or leucocyte-poor PRP: These are preparations without the presence of WBCs and having a low density fibrin network after activation.
2. Leucocyte- and PRP (L-PRP): These preparations have WBCs and have a low density fibrin network after activation. Most of the commercial systems belong to this family only.
3. Pure platelet- rich fibrin (P-PRF)/ leucocyte- poor platelet- rich fibrin preparations: These

preparations do not have WBCs but have a high density fibrin network. Such preparations exist only in a strongly activated gel form and cannot be used or injected like traditional fibrin glues.

4. Leucocyte- and platelet- rich fibrin (L-PRF)/ second generation PRP: These are preparations having WBCs along with a high density fibrin network.

The yield of platelets in PRP depends mainly on optimal centrifugation conditions. The use of a manual centrifuge which is available in most of the hospitals and laboratories can make it more easily available and ready to use. Therefore, PRP is a cheaper source of growth factors like TGF β , PDGF, VEGF, IGF-1, etc. and can also stimulate tissue healing.⁷

Centrifugation is the most commonly used processes in the separation of liquid-solid or liquid-liquid component. It's principle being based on the use of centrifugal force which is much greater than gravity. The separation depends upon the different sizes and density of the particles present in various phases of centrifugation. This separation is caused by the movement of the particles, which is a result of the centrifugal force acting in the radial (outward)

direction, the gravity acting in the downward direction and the frictional force in the opposite direction of the particle motion. This frictional force further depends upon the particle's velocity and the fluid viscosity, as per the Stokes law. All the forces are thus, quickly balanced. Hence, the acting centrifugal force is proportional to the apparent mass and the angular velocity of the particle; and the distance of the particle from the axis of the rotor of the centrifugation machine. The greater this distance, the greater will be the centrifugal force acting and hence, better separation of the components.^[3,8,9]

In case of whole blood, the platelet concentration factor (FC_{pt}) is determined by the centrifugal force and time, which act by determining the volume of plasma at the upper layer, packing of RBCs at the bottom layer and recovery efficiency of the platelets.³

Today numerous methods of centrifugation are available.¹⁰ Some authors recommend the single spin method, wherein the whole blood is centrifuged only once. Kahn RA *et al* proved that a centrifugation of 3731g for 478 mL of whole blood for a time period of 4 minutes was optimal for obtaining the highest platelet concentration in the sample.¹¹ Marques *et al* demonstrated that a centrifugal force of 541g for 5 minutes on 60 mL of whole blood was optimal for producing platelet rich plasma (PRP).⁷ Anitua E proved that using only a single centrifugation spin and collecting the plasma immediately above the RBC layer, obtained a platelet concentration factor of 2.67.¹² This is in concordance with our study, wherein single centrifugation spin (100g for 10 minutes) showed a platelet concentration factor of 2.19 above the baseline value.

However, there have also been studies wherein double spin method of centrifugation has been proved to yield a higher platelet count. Perez AG *et al* concluded that the double spin centrifugation, that is, processing of 3.5 mL of whole blood at first 100g for 10 minutes, followed by 400g for 10 minutes and withdrawing 2/3rd of the remaining plasma, produced high platelet recovery of nearly 70-80% and also increased the platelet concentration by 5 times. Platelet integrity and viability were also maintained.³ Similarly, Landesberg R *et al* obtained a platelet concentration of approximately 3.2 in the PRP samples, by processing 5 mL of whole blood by double spin method; 200g for 10 minutes in each spin.¹³ Bausset O, Giraudo L, Veran J, *et al* found that a centrifugation speed of 130g or 250g for 15 minutes was optimal for double spin centrifugation, by processing 8.5 mL of whole blood in the first spin followed by processing 2.0 mL of plasma in the second spin. This gave a platelet concentration factor of 3.47. However, they also observed in their study that a centrifugation of 400g or 1000g did not increase the platelet concentration factor compared to a 250g centrifugation.¹⁴ They concluded that this could be due to platelet aggregation, which indicates that

centrifugation at higher speeds is not suitable for PRP preparation as it can be deleterious for the platelet function. This might be the reason, as to why in our study, the platelet concentration factor dropped significantly to 0.83 as compared to platelet concentration factor of 2.19 after single spin only.

Bausset *et al*¹⁴ also found on electron microscopy that high centrifugation speeds modified the morphology of the platelets considerably in the PRP preparation. At a centrifugation speed of 130g, the platelets displayed a discoid form with less number of pseudopodia. This is consistent with the morphology of a resting platelet.^[15,16] At 250g and 400g, the platelets showed greater number of pseudopodia and also show a trend towards centralization of its granules; thereby proving that lower speeds of centrifugation are better suited for preservation of the platelet morphology.¹⁴

Conclusion

The utility of platelet-rich plasma is increasing in day-to-day practice. Though there are commercially available systems, but the use of a manual centrifuge can provide a cost effective option. Today there are several techniques available depending upon the time and speed of centrifugation, which has led to confusion regarding its standardization. Our study concluded that a single centrifugation method of 100g for 10 minutes yielded a better platelet count (platelet concentration factor of 2.19 above the baseline value) as compared to a double centrifugation method of 100g and 400g for 10 minutes each (platelet concentration factor of 0.83). However, further research in this area still needs to be done and each laboratory should standardize its protocol for the use of PRP.

Acknowledgement

The authors deny any conflicts of interest related to this study.

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