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Original Research Article

A Hospital-Based Assessment of the Effects of Various Centrifugation Speeds and Inclusion of the Buffy Coat in Platelet-Rich Plasma Preparation: A Comparative Study

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Abstract

Aim: The aim of the present study was to analyze the centrifuge spin rates at which to attain an ideal platelet-rich plasma yield and also to study the effect of inclusion of the buffy coat after the first spin on the final platelet concentration in platelet-rich plasma.

Methods: A cross-sectional observational study was conducted at Department of Dermatology, over a period of 12 months.

Results: Among the three centrifugation methods, the highest rise in platelet concentration was seen with 100g/400 g speeds which were higher than the output of the other two methods. The difference was statistically significant. Among the three spin variations with buffy coat included, the 350 g/1350 g spin showed a higher percentage rise in platelet concentration from whole blood. This increase was more consistent (with less standard deviation) as compared to the other two variations. The 100 g/400 g variant also showed a mean rise in platelet counts and the difference between the two variations was not statistically significant. The difference between the 800/1600 g spin (lower mean increase in platelet concentration) versus the 100/400 g and 350/1350 g variations (higher values) was statistically significant. When compared to pure platelet-rich plasma (leukocyte-poor platelet-rich plasma), inclusion of the buffy coat layer for the second spin (leukocyte-rich platelet-rich plasma) resulted in 175%, 1220% and 512% higher platelet concentrations in the 100 g/400 g, 350 g/1350 g and 800 g/1600 g spin variations, respectively.

Conclusion: An ideal platelet yield in platelet-rich plasma can be achieved with both the 100 g/400 g as well as the 350 g/1350 g spins while using the buffy coat (leukocyte-rich-platelet-rich plasma), and the 100 g/400 g spins method for pure platelet-rich plasma (leukocyte-poor- platelet-rich plasma) accomplishes a near-ideal platelet count along with low contamination with other cells.

Keywords: Buffy coat, centrifugation speed, platelet count, platelet-rich plasma

Introduction

The Platelet Rich Plasma (PRP) is plasma rich in platelets, obtained from venous blood. In order to get a therapeutic aimed PRP, the concentration must be between $200x10^3$ and $1000x10^3$ platelets/μL. A higher concentration would be unfavourable. [1] Many studies showed that, during contact with collagen, PRP released growth factors such as Platelet Derived Growth Factor (PDGF), Vascular Endothelial Growth Factor (VEGF), Epidermal Growth Factor (EGF), Platelet Factor 4 (PF-4), Insulin like Growth Factor-1 (IGF- 1) and, Transforming Growth Factor Beta (TGF β) by their α-granules. [2]

These growth factors play a key role in the early stage of wound healing by allowing a stem cell proliferation and, angiogenesis. Many medical specialities such as orthopedic, ophthalmic and maxillofacial surgery are looking for this tissue engineering effect. In our day-to-day practice in maxillofacial surgery, we use PRP in preimplant surgery during bone grafts, in order to get a good mucosal healing: an important and essential step to avoid the main complication linked to this surgery: infection. Indeed, a bad mucosal healing is a source for higher infection probability, due to the fact that the graft is exposed in a so-called dirty environment. [3] Moreover, PRP use allows a quicker haemostasis and a better bone regeneration. [4]

The application of platelet-rich plasma (PRP) has emerged as a safe and viable alternative to surgical procedures in orthopedics. PRP is defined as a volume of autologous plasma that has a platelet concentration above baseline. [5] The term PRP was created in the 1970s by hematologists and was

initially used to treat patients with thrombocytopenia. PRP is currently used in different medical fields [6], like maxillofacial surgery, orthopedics, and dermatology. There are now several high-quality studies about the use of PRP in these specialties, specifically in the field of treatments for musculoskeletal diseases. In the last decades, PRP has gained increased attention in orthopedic and sports medicine [7-9] and has been advocated by several researchers as a potentially effective therapy to control degenerative disease in joints like the knee, spine, and hip [10-12] and has been subject to great interest in the current research agenda. [13]

The therapeutic effects of PRP are attributed to the high concentration of growth factors present in platelets, like vascular endothelial growth factor (VEGF), platelet-derived growth factor AB (PDGF-AB), and transforming growth factor beta 1 (TGF-β1), which are responsible for providing regenerative stimulus that promotes tissue repair [14] by cell proliferation, angiogenesis, and cell migration. [15]

The aim of the present study was to analyze the centrifuge spin rates at which to attain an ideal platelet-rich plasma yield and also to study the effect of inclusion of the buffy coat after the first spin on the final platelet concentration in platelet-rich plasma.

Materials and Methods

A cross-sectional observational study was conducted at Department of Dermatology, Netaji Subhas Medical College and Hospital, Bihta, Bihar, India over a period of 12 months. A total of 90 samples from 45 apparently healthy individuals/patients above 18 years of age, of either gender, attending the inpatient and outpatient clinic over that period, who were receiving platelet-rich plasma injections as a treatment for androgenic alopecia, alopecia areata, or chronic ulcers were randomly collected for the study. Among participants who were analyzed more than one time, care was taken to allot different spin rate during every monthly visit. Informed consent was taken from the participants. Those who refused consent or patients on antiplatelet agents were excluded from the study.

Procedure

Twenty milliliters of whole blood were obtained by venipuncture, out of which 10 ml was transferred into two ethylenediaminetetraacetic acid tubes (BD Vacutainer ethylenediaminetetraacetic acid 6 ml 13 × 100 mm). One milliliter was sent for complete blood count and the rest (9 ml) used for platelet-rich plasma preparation. All the tests were done from the same lab. The remaining 10 ml was used for treatment purpose. The collected sample was centrifuged by two spins in a non-refrigerated centrifuge machine (Remi- 8c) – a "soft" spin (at lower revolutions per minute [rpm]) initially, followed by a "hard" spin (at higher rpm). Various centrifugation speeds were considered based on the previous studies on determining optimum centrifugation speed in platelet-rich plasma preparation. [16-21]

Following the first spin, three layers (red blood cells (RBC), buffy coat and plasma) were formed from whole blood. For the second spin, the sample was separated and transferred into sterile plain tubes (6 ml 13 × 100 mm) by either one of these two methods:

1. Pure platelet-rich plasma method (leukocyte-poor platelet-rich plasma): only the supernatant plasma layer was separated from the soft spin tube without disturbing the buffy coat or RBC layers, using a 2ml disposable syringe with 24G needle and subjected to a second spin. The duration of spin was ten minutes. [22,23]

2. Buffy coat method (leukocyte-rich platelet-rich plasma): in this method, the supernatant plasma was collected along with the whole buffy coat layer using a 2 ml disposable syringe with 24G needle and subjected to second spin. The duration of spin was ten minutes.

After the second spin, the upper two-third of the sample (platelet-poor plasma) was removed and the lower one-third was homogenized, by gentle shaking, to obtain platelet-rich plasma, which was then analyzed for the platelet count. The whole procedure was measured at room temperature (24–25°C by the same investigator throughout the study).

The size and radius of the rotor vary with the centrifuge machine. Hence, relative centrifugal force values are used instead of rpm (revolutions per minute) for easy comparison. Relative centrifugal force is expressed as "g" and which is derived from the formula:

g = (1.118×10-5) RS2 where R is the radius of the rotor (in centimeters) and S is the speed of the centrifuge in revolutions per minute (rpm) In our study centrifuge R was 11.5 cm.

The g values were calculated for the respective RPM and approximated to the nearest whole number (the nearest fifty or hundred):

Variation 1 - 900/1800 rpm =104g/417g

Variation 2 - 1600/3200 rpm = 330g/1329g

Variation 3- 2500/3500 rpm = 805g/1578g.

Results were analyzed using SPSS (statistical package for social sciences) version 16.0.

Results

Table 1: Platelet yield by pure platelet rich plasma and buffy coat methods

100 g/400 g variant				350 g/1350 g variant					
Mean platelet count in WB (in lakh cells/mm 3)	Mean platelet count in PRP (in lakh cells/mm 3)	Mean platelet yield (%)	Mean platelet count in WB (in lakh cells/mm3)	Mean platelet count in PRP (in lakh cells/mm 3)	Mean platelet yield (%)	Mean platelet count in WB (in lakh cells/ mm3)	Mean platelet count in WB (in lakh cells/ mm3)	Mean platelet count in PRP (in lakh cells/mm 3)	Mean platelet yield (%)
Pure PRP method	2.4	8.42	392.48±112. 12	2.068	1.35	61.49±14 .84	2.14	1.58	72.98±24.68
Buffy coat method	2.06	12.48	690.42±316. 34	2.042	14.36	736.64±1 92.28	1.88	7.-4	378.72±223. 96

Among the three centrifugation methods, the highest rise in platelet concentration was seen with 100g/400 g speeds which were higher than the output of the other two methods. The difference was statistically significant. Among the three spin variations with buffy coat included, the 350 g/1350 g spin showed a higher percentage rise in platelet concentration from whole blood. This increase was more consistent (with less standard deviation) as compared to the other two variations. The 100 g/400 g variant also showed a mean rise in platelet counts and the difference between the two variations was not statistically significant. The difference between the 800/1600 g spin (lower mean increase in platelet concentration) versus the 100/400 g and 350/1350 g variations (higher values) was statistically significant.

Table 2: Comparison of platelet yield between pure PRP (leukocyte-poor platelet-rich plasma) and buffy coat (leukocyte-rich platelet-rich plasma) groups at various centrifugation speeds and percentage rise of leukocyte- rich platelet-rich plasma with respect to leukocyte-poor platelet-rich plasma

	Platelet yield in buffy coat method (%)
100g/400g	175%
350g/1350g	1220%
800g/1600g	512%

When compared to pure platelet-rich plasma (leukocyte-poor platelet-rich plasma), inclusion of the buffy coat layer for the second spin (leukocyte-rich platelet-rich plasma) resulted in 175%, 1220% and 512% higher platelet concentrations in the 100 g/400 g, 350 g/1350 g and 800 g/1600 g spin variations, respectively.

Table 3: Comparison of mean white blood cells in cells/mm3 between pure PRP (leukocyte-poor platelet-rich plasma) and buffy coat (leukocyte-rich platelet-rich plasma) groups at various centrifugation speeds

	Buffy coat	Pure PRP
100g/400g	40025	3883
350g/1350g	32460	512
800g/1600g	34512	860

Table 4: Comparison of mean red blood cell count (in million cells/mm3) between pure PRP (leukocyte-poor platelet-rich plasma) and buffy coat (leukocyte-rich platelet-rich plasma) groups at various centrifugation speeds

	Buffy coat	Pure PRP
100g/400g	2.048	0.094
350g/1350g	2.160	0.036
800g/1600g	2.024	0.072

Even though the buffy coat inclusion method offers higher platelet concentrations in platelet-rich plasma, it comes at the cost of higher contamination with RBCs and white blood cells (WBCs). The mean values in WBC and RBC counts with different spins, while including the buffy coat and in pure platelet-rich plasma, are given in Tables 3 and 4. An increase in the WBCs and RBCs was seen in the 100 g/400 g spin as compared to other spins in the pure platelet-rich plasma samples. A rise in platelet yield was also noted if the whole blood platelet count was <2 lakh/mm3 but the significance of this is unclear

Discussion

Platelet-rich plasma is an autologous preparation of platelets in concentrated plasma (with usually >1,000,000 platelets/μL or 2–7 times the concentration in whole blood). [24] Initial uses of

platelet-rich plasma included maintaining hemostasis during surgery and for platelet transfusions in thrombocytopenic disorders. [25] It has now attracted attention in several medical specialties such as orthopedics, maxillofacial surgery, regenerative medicine and dermatology, because of its ability to promote tissue regeneration and wound repair. [26,27] Since platelet-rich plasma is autologous in nature and its extraction is minimally invasive, affordable and without major side effects, its therapeutic profile has expanded to include many dermatologic indications such as chronic ulcers, scar treatment, alopecia and skin rejuvenation.

Among the three centrifugation methods, the highest rise in platelet concentration was seen with 100g/400 g speeds which were higher than the output of the other two methods. The difference was statistically significant. Among the three spin variations with buffy coat included, the 350 g/1350 g spin showed a higher percentage rise in platelet concentration from whole blood. This increase was more consistent (with less standard deviation) as compared to the other two variations. The 100 g/400 g variant also showed a mean rise in platelet counts and the difference between the two variations was not statistically significant. The difference between the 800/1600 g spin (lower mean increase in platelet concentration) versus the 100/400 g and 350/1350 g variations (higher values) was statistically significant. Rughetti et al [28] found that the platelet count and functional activity were related in a bell-shaped manner, wherein optimal stimulation for proliferation of endothelial cells and angiogenesis peaked at 1.25 × 106 and 1.5 × 106 platelets/ mL, respectively, and further increased counts had an inverse effect on proliferation. A very high platelet count above the baseline value is also considered disadvantageous as it may have an inhibitory effect on the healing process. [29]

When compared to pure platelet-rich plasma (leukocyte-poor platelet-rich plasma), inclusion of the buffy coat layer for the second spin (leukocyte-rich platelet-rich plasma) resulted in 175%, 1220% and 512% higher platelet concentrations in the 100 g/400 g, 350 g/1350 g and 800 g/1600 g spin variations, respectively. Even though the buffy coat inclusion method offers higher platelet concentrations in platelet-rich plasma, it comes at the cost of higher contamination with RBCs and white blood cells (WBCs). Regarding the centrifugation process, there is a difference in the separation of the blood components in each spin of the double spin technique. The first spin (or soft spin), with low centrifugal force, is used to separate three layers from whole blood: red blood cells, buffy coat, and plasma. The second spin (or hard spin), with higher centrifugal force, is used to concentrate the platelets at the bottom part of the plasmatic phase. [30] In a review of different PRP protocols, Harrison et al [31] found that there is a difference in the white blood cells' concentration with one and two spin preparations. They found a depletion of granulocytes in single spin methods.

The mean values in WBC and RBC counts with different spins, while including the buffy coat and in pure platelet-rich plasma. An increase in the WBCs and RBCs was seen in the 100 g/400 g spin as compared to other spins in the pure platelet-rich plasma samples. A rise in platelet yield was also noted if the whole blood platelet count was <2 lakh/mm3 but the significance of this is unclear. Even though inclusion of the buffy coat in platelet-rich plasma preparation gives a high platelet yield, this comes along with a high number of other cells. The role of WBCs in platelet rich plasma is still unclear. It is hypothesized that platelet-rich plasma high in leukocyte concentration provides protection from infections, contributes to angiogenesis, increases growth factor release and hypercellularity. [32] Oudelaar et al [33] in their systematic review on blood components in platelet-rich plasma have mentioned that the concentration of vascular endothelial growth factors is significantly more in platelet rich plasma produced by systems with higher concentrations of platelets and leukocytes than pure platelet-rich plasma kits. A study conducted by Setta et al. on chronic diabetic ulcers of 12 weeks ulcer duration found that using leukocyte-rich-platelet-rich plasma gel led to a shorter mean healing time (11.5 weeks) as compared to leukocyte-poor-platelet-rich plasma (17 weeks). [34] Anandan et al [35] found that 92% of patients with leprosy ulcers showed complete re-epithelialization within six weeks after weekly treatment sessions with leukocyte-rich-platelet-rich plasma. Both leukocyte-rich-platelet-rich plasma and leukocyte poor- platelet-rich plasma are known to work in acne scars. [36,37] Addition of leukocyte-rich-platelet-rich plasma to laser ablation produced additional benefits in acne scarring and also improved symptoms such as erythema, edema and pain. [38]

Conclusion

An ideal platelet yield in platelet-rich plasma can be achieved with both the 100 g/400 g as well as the 350 g/1350 g spins while using the buffy coat (leukocyte-rich-platelet-rich plasma), and the 100 g/400 g spins method for pure platelet-rich plasma (leukocyte-poor- platelet-rich plasma) accomplishes a near-ideal platelet count along with low contamination with other cells.

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