

Comparison of Small Volumes of Blood for the Preparation of Platelet-Rich Plasma

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Authors' Contributions

Muddasir Hassan Abbasi supervised the research project and approved the final draft (Conception & Study Design, Data Collection, Data Analysis, Drafting, Critical Review). Tanzeem Anjum and Tehseen Zahra contributed equally to the study (Data Collection, Data Analysis, Drafting).

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ABSTRACT

Platelet-rich plasma (PRP) is used in variety of surgeries from oral to cosmetic and cardiac to orthopedic for therapeutic purposes. It releases the growth factors upon activation which are thought to accelerate the regenerative and healing process. We aimed to investigate the concentration of PRP obtained by using three different volumes of whole blood keeping centrifugation time and speed constant. Briefly, after taking 3 ml, 5 ml and 7 ml volumes of blood from healthy males (n=5) the samples were centrifuged at 500 RPM for 15 min. The supernatant containing platelet poor and rich plasma was obtained from the actual sample for each volume used followed by platelet count. The number of platelets in the supernatant obtained from GI (3 ml) decreased while from GII (5 ml) and GIII (7 ml) increased. In all the studied groups, no linear relationship has been noted. From the results we can conclude that small volumes of peripheral blood used for the preparation of PRP can be equally significant in platelet concentration in contrast to the large volumes in practice.

Keywords: Blood, centrifugation, platelet-rich plasma (PRP), platelet count, platelet-rich plasma (PRP) vs. volume.

INTRODUCTION

Platelet-rich plasma (PRP) is a proportion of plasma with an enriched concentration of platelets above baseline ($150 \times 10^9/L$ to $350 \times 10^9/L$) [1]. It works as a therapeutic tool since it contains growth factors which are released upon its activation. PRP has both mitogenic and chemotactic properties [2]. It also contains complement of clotting and growth factors which play role by attracting undifferentiating cells and thereby triggering cell division [3]. It has been documented that PRP reduces inflammation by suppressing cytokine release and boost up tissue regeneration and angiogenesis in chronic wounds [2]. It produces signaling proteins which attract macrophages at the wound site accelerating host-defense mechanism. Many studies have shown that PRP also self-possess anti-microbial activity. Currently this therapy is being used for various

surgeries including; periodontal, oral, maxillofacial, orthopedic, cosmetic, plastic, spinal, and heart bypass surgery and chronic skin and soft tissue ulceration as well [4-6].

The routine preparation methods involved for PRP are independent protocols keeping in view the optimal centrifugation conditions but there have been marked variables in accordance with the treatment procedure. These variables include volume of blood sample, centrifugation time, and range of centrifugal acceleration. Due to the intricacy of PRP as subjected to the efficacy of an autologous product specifically for clinical use, it is requisite to explicitly illustrate features of the protocol to achieve consistent results [7]. Generally, PRP is prepared employing large volumes of blood and double centrifugation. An initial centrifugation is done to separate red blood cells (RBCs) which is followed by a second centrifugation

to get the concentration of platelets suspended in plasma [8, 9].

It is substantial that the documentation on PRP is noteworthy but still there is no consensus on a standard preparation method due to many reasons. Either because the published results are not unanimous or due to variability of numerous volumes of blood sampling and/or centrifugation speed/time. Our study focused only on figuring out the concentration of PRP and platelet poor plasma (PPP) obtained by taking small volumes of blood, keeping centrifugation speed and time constant on lab scale.

MATERIALS AND METHODS

Collection and Processing of Blood Samples

Blood samples of three different volumes (3 ml, 5 ml and 7 ml) were drawn from randomly selected 15 healthy male volunteers from Centre for Applied Molecular Biology (CAMB), Punjab University, Lahore, Pakistan. Samples were taken in sodium citrate vacutainers following standard protocols maintaining aseptic conditions.

Platelet Count of Whole Blood

The subjects' blood was categorized as GI, GII and GIII on the basis of volume of blood collected (3 ml, 5 ml and 7 ml), respectively. All the samples were then examined for complete blood count (CBC) within 1-2 hours by using RT-7600 Auto Hematology Analyzer (Rayto). Platelet count in samples was then noted.

PRP Preparation Method

Samples were then centrifuged by using 80-2 centrifuge (Shanghai) at 500 RPM for 15 min. Two layers were formed; the upper layer containing platelets, white blood cells (WBCs) and other growth factors and the lower layer containing RBCs. We collected the supernatant containing rich as well as poor platelet plasma (PRP and PPP) and carefully pipetted it into pre-sterile test tubes.

Platelet Count in PRP

The supernatant was again subjected to Hematology analyzer for second round of platelet count.

Statistical Analysis

The data was analyzed using Prism Graph pad 8 software (version 8.1.2, San Diego, CA). Statistically significance was calculated using one-way analysis of

variance (ANOVA). Significance was accepted at $P > 0.05$ while results are shown as mean \pm S.E.M. with $n=5$.

Ethics Approval and Consent to Participate

The study was approved by the Bioethics Committee of the University of the Punjab (Lahore, Pakistan). Consent was obtained from each participant.

RESULTS AND DISCUSSION

Platelet-rich plasma is a modern remedy which is commercially being used in cosmetic surgeries and in the treatment of various soft tissue ulceration. In the current study we tried small volumes of blood (3 ml, 5 ml, and 7 ml) to get maximum platelet count with just a single round of centrifugation at constant speed and time. The mean values were compared and found to be 150.800 ± 4.994 , 177.200 ± 3.734 and 178.200 ± 21.752 before and 146.200 ± 8.991 , 240.000 ± 17.584 and 526.600 ± 82.391 after centrifugation for GI, GII and GIII, respectively (Table 1, Figure 1). No significant difference was observed in GI and GII before and after centrifugation. However, significant difference was shown by GIII before and after centrifugation ($P < 0.0001$).

Routinely for the preparation of PRP, sodium citrate coated tubes containing blood samples are subjected to centrifugation at high speed of 3500rpm for 3 min. After centrifugation three layers are formed; the upper layer containing small number of WBCs and PPP, an intermediate layer referred to as buffy coat containing WBCs and the bottom layer containing RBCs. The upper two layers are transferred into another sterile tube and are subjected to centrifugation at slightly lower speed of 3000rpm for 22 min. After the second round of centrifugation, the supernatant ($2/3^{\text{rd}}$) containing PPP is discarded and the remaining ($1/3^{\text{rd}}$) volume is collected for PRP preparation [3]. In practice 20-60 ml volume of blood is taken for PRP preparation which usually varies depending upon the treatment [5] and achieving right platelet concentration *i.e.*, 4-5 times of baseline. This procedure is time taking and utilizes a large quantity of blood. Nonetheless, the required concentration of platelets can be achieved by single round of centrifugation and with a small volume of blood as well (not less than 7 ml as shown by the results).

It is noted that maximum mean value of platelet count after centrifugation is given by GIII when compared to all other study groups. Even though the mean value of platelets is almost same before centrifugation in GII and GIII but value after centrifugation is shown to be much higher in GIII ($P < 0.001$). It is important to mention here that even when individual values are

A linear correlation of platelet count has also been estimated before and after centrifugation in the whole blood and extracted supernatant (Figure 2a, b and c). In all the groups (GI, GII and GIII) no proper linear correlation has been noted as all the values are not in the line; just few are in close with the line.

Table 1. Platelet count in whole blood before (B) and after (A) centrifugation.

| S. No. | GI | | GII | | GIII | |
|---------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| | B ($10^3/\mu\text{l}$) | A ($10^3/\mu\text{l}$) | B ($10^3/\mu\text{l}$) | A ($10^3/\mu\text{l}$) | B ($10^3/\mu\text{l}$) | A ($10^3/\mu\text{l}$) |
| 1 | 168 | 140 | 163 | 284 | 232 | 801 |
| 2 | 137 | 132 | 177 | 226 | 207 | 292 |
| 3 | 152 | 148 | 180 | 204 | 111 | 533 |
| 4 | 148 | 131 | 184 | 206 | 147 | 457 |
| 5 | 149 | 180 | 182 | 280 | 194 | 550 |
| Mean ± SEM | 150.800 ± 4.994 | 146.200 ± 8.991 | 177.200 ± 3.734 | 240.000 ± 17.584 | 178.200 ± 21.752 | 526.600 ± 82.391 |

considered for the platelet count, it can be noted that when using volumes *i.e.*, 3 ml and 5 ml, the counts after centrifugation are not satisfactory *i.e.*, 168 vs. 140 in GI and 163 vs. 284 in GII. But when considering GIII all the individual counts are quite appraising except at sample 2 (Table 1). The results demonstrate that lower volumes of blood produce low concentration of PRP while as we keep on increasing volume the concentration of PRP also increases.

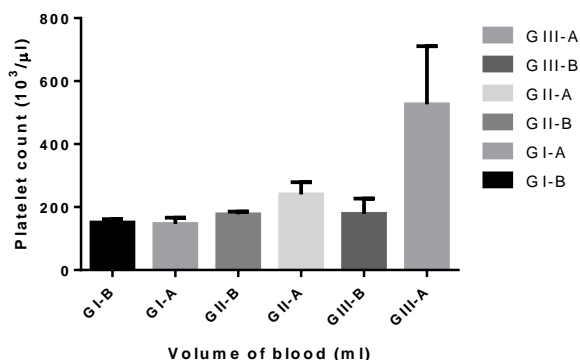
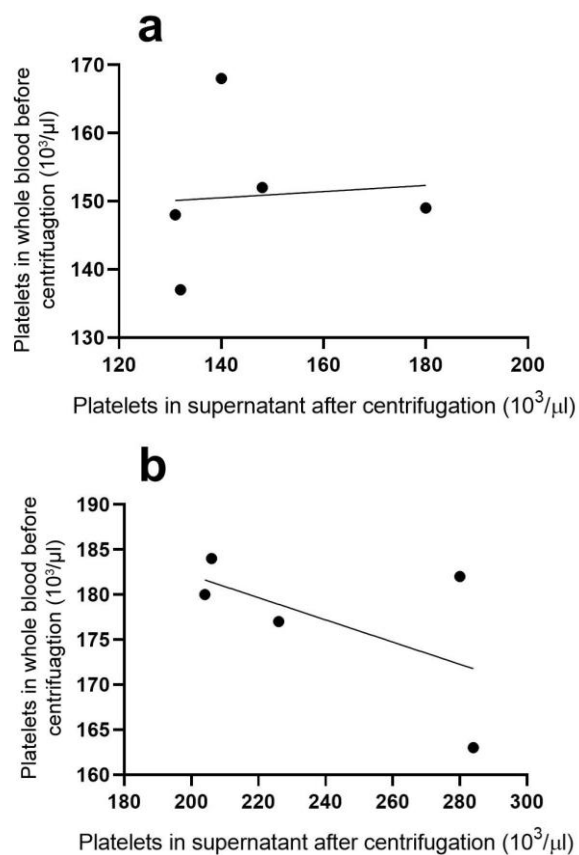


Figure 1. Comparison of Platelet count of GI, GII and GIII where B shows concentration of platelets in whole blood ($10^3/\mu\text{l}$) before centrifugation and A shows the value of platelets in supernatant ($10^3/\mu\text{l}$) after centrifugation against volume of blood.



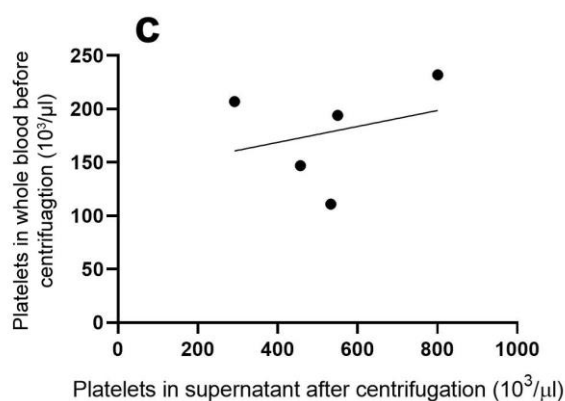


Figure 2. A linear correlation of platelet count before and after centrifugation in GI(a), GII(b) and GIII(c).

CONCLUSION

In view of the forgoing results we can conclude that it is the volume upon which the platelet concentration depends. To the best of our knowledge this small scale study sets a baseline as the results of the platelet concentration vs. volume used has not been reported yet. Though the study was just performed on a very small no. of blood samples but it gives obvious results. Hence such work can be extended on a large scale for ensuring its accuracy.

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