Original Article

Platelet Rich Plasma: Cost-effective & simple technique with high yield platelet count

Ambreen Kashif, Sidra Ghazanfer, Saira Moin, Sadia Taj, Fatima Zahraa

Departments of Hematology, Shaikh Zayed Hospital and Fatima Memorial Hospital, Lahore and Khawaja M. Safdar Medical College, Sialkot, Pakistan

Objective: To find a cost effective, simple and safe technique with high platelet concentration factor.
Methodology: This retrospective cross-sectional study was done in Shaikh Zayed Hospital, Lahore for 6 months. Thirty-two volunteers both gender were selected and were divided into four groups with eight individuals in each group. After informed consent, socio-demographic data like age and gender was recorded. Four different techniques for platelet rich plasma (PRP) preparation were applied and tested on each group. Baseline platelet count in blood was checked before, during and after technique by Cell Dyne Ruby Haematology analyser.
Results: The technique II and IV were found quite effective with up to 2.5 to 6 fold increases in platelet count in the final product.
Conclusion: We found a simple, cost-effective and beneficial technique for the preparation of PRP. (Rawal Med J 202;46:266-269).
Keywords: PRP (platelet rich plasma), growth factors, thrombocytopenia.

INTRODUCTION

Platelets are derived from megakaryocytes in the bone marrow. The average platelet count ranges from 150 to 450 × 10^9/L.[1,2] These are anucleate fragments of megakaryocytes and their life span is 7-10 days in the blood stream.[3] The proteins are present inside secretory granules which are dense granules, α-granules and lysosomes.[4] In early days, platelet rich plasma (PRP) was only used for transfusion in thrombocytopenic patients. Ten years later, it was used as platelet rich fibrin (PRF), which is a source of fibrinogen in maxillofacial surgery. Afterwards, use of PRP was extended to many other fields including cardiac surgery, pediatric surgery, musculo-skeletal field for sports injury, ophthalmology, urology, gynaecology and plastic surgery. The chance of hypertrophic keloids and scars is also decreased by its use.[5]

PRP is an autologous concentration of human platelets to supra-physiologic levels. Platelets are a reservoir for the important growth factors which include platelet-derived growth factor (PDGF), transforming growth factor-beta 1 (TGF-β1), epidermal growth factor (EGF), basic fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF-I) and hepatocyte growth factor (HGF).[6] A specific volume of whole blood is withdrawn from a patient's peripheral vein, placed in vacutainers with either EDTA or Sodium Citrate anticoagulants and centrifuged at specific speed for specific time and eventually plasma with high concentration of platelet is achieved. This PRP is then re-infused in the patient at the site of injury or inserted as a gel or other biomaterials during surgery.

PRP therapy has grown in popularity over the past few years.[7] There is no major side effect of PRP therapy except for minimal pain and redness at the time of injections.[5] In the knee, PRP is found very useful in patient with articular cartilage pathology, ligamentous and meniscal injuries.[8] It is also very safe and efficient in cosmetic interventions,[9,10] in skin rejuvenation, hair regrowth, wound healing, and fat graft take, healing of diabetic foot as well as in reproductive medicine and gynaecological disorders.[11-13] It is used in sports medicine for enhancing the tendon healing.[14,15] In dentistry, its use for the growth factors at the sites requiring osseous grafting is also effective.[16] The current classification of autologous platelet concentrates comprises mainly of 4 types based on their cell content and fibrin architecture. Pure PRP (P-PRP) or leukocyte-poor PRP, Leukocyte- and PRP (L-PRP), pure platelet-rich fibrin (P-PRF) or

leukocyte-poor PRF and Leukocyte- and PRF (L-PRF).[17] The aim of this study was to find a cost effective, simple and safe technique with high platelet concentration factor.

METHODOLOGY

PRP was prepared with four different manual techniques, which were applied on blood collected from 32 volunteers both male and female with median age of 30±15 years. These individuals were divided into four groups of eight. These four techniques were based on the effect of centrifugation speed and time on the final yield of platelet in the platelet rich plasma. Two methods were applied in these techniques, listed below:

Single spin method: Blood was collected in EDTA vacutainers and was centrifuged at 1000rpm for 15 minutes once at room temperature. Supernatant plasma was drawn by sterile syringe and shifted to sterile plain vacutainers. RBCs at bottom were discarded. Platelet count of plasma after first spin was checked by hematology analyzer and recorded.

Double spin method: Blood was collected in EDTA vacutainers and was centrifuged on different speed at room temperature. Same steps of single spin method were followed from step 2 to 4. The supernatant plasma was centrifuged again and upper 2/3rdof the plasma was discarded as platelet poor plasma and platelet were re-suspended in lower 1/3rd of plasma and platelet count was checked by hematology analyzer. All the tubes and syringes used were sterile and maximum precautions were taken to lessen the chances of contamination.

Procedure: Four groups were made having 8 volunteers in each group. Total four techniques were tested. One technique was applied on one group. The items required for each volunteer with their quantity are Butter fly (1), 10 CC syringe (3), EDTA filled Vacutainers (4) Plain Vacutainers (20). 10 ml blood was collected from each volunteer and was transferred to EDTA vacutainers making 3 sub-tubes of each volunteer. Platelet count of each volunteer was checked before, during and after centrifugation on Cell Dyne Ruby analyzer. All the techniques were performed at room temperature 22-28^{0}C. These techniques were applied individually on blood of each individual.

Statistical Analysis: All data analysis was performed using SPSS Statistics 22.

RESULTS

Platelet rich plasma was prepared by using 4 techniques. In technique I, the platelet count after double spin was 180 x 10^9/L and volume of the PRP obtained was 3ml. Technique II the final platelet count achieved was 825 x10^9/L and volume was 2.5 ml. In the Technique I and Technique II the Platelet concentration factor was 0.7 and 3.9, respectively (Table 1).

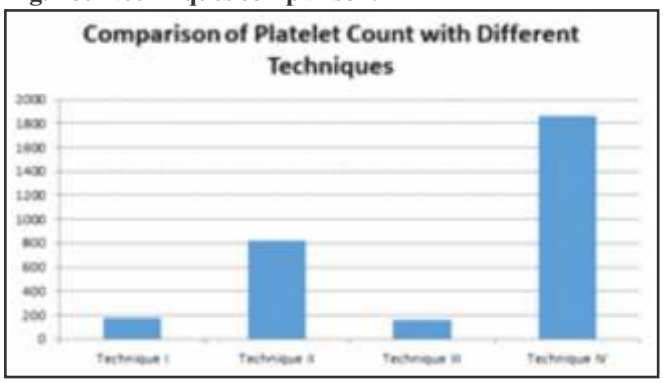
Table 1. Techniques and achieved platelet counts.

Technique	Volume Collected	Platelet count before centrifugation (Mean) X10^9/L	1st centrifugation		Platelet count after 1st spin (Mean) X10^9/L	2nd centrifugation		Platelet count after 2nd spin (Mean) X10^9/L	Volume of PRP prepared
			Speed (Rpm)	Time (min)		Speed (Rpm)	Time (min)		
Technique I	10ml	269x10^9	1500	10	130x10^9	6000	5	180 x10^9	3 ml
Technique II	10ml	210x10^9	1000	07	320x10^9	2500	10	825x10^9	2.5ml
Technique III	10ml	240x10^9	1000	15	160x10^9	No second spin was performed			4.0 ml
Technique IV	10ml	280x10^9	500	07	625x10^9	2500	10	1865x10^9	2.5 ml

Table 2. Time and platelet concentration factor for each technique.

Technique	Time in minutes	Platelet concentration factor
Technique-I	20 min	0.7
Technique-II	22 min	3.9
Technique-III	16 min	0.7
Technique-IV	22 min	6.7

Fig. Four techniques comparison.



The Technique III led to platelet count of 160 x10^9/L with volume prepared of 4ml, while in Technique IV the platelet count achieved after second spin was 1865x10/^{9}L with volume prepared of 2.5ml. The platelet concentration factor of Technique III & Technique IV was 0.7 and 6.7, respectively (Fig.). It was seen that Technique II & IV yielded highest platelet concentration factor. The total time taken for each technique and the platelet concentration factor is summarized in Table 2. Platelet concentration factor was calculated by dividing platelet count of PRP with the baseline line platelet count before centrifugation.

DISCUSSION

Growth factors are stored within the platelet α-granules. Since PRP contains several growth factors (e.g., PDGF, VEGF, etc) that are capable of stimulating angiogenesis and increase fibroblast cell differentiation.[18,19] A recent study by Gupta et al also concluded that the manual double spin method yielded higher platelet concentration as compared to automated methods.[20] Another study by Zhang et al also found that quality of PRP was affected by various factors, including centrifugal force, centrifugal time, temperature, and operation process.[21]

In 2018, a study by Hamid inferred that PRP can be prepared by centrifugation techniques. This method is not only simple but also cost effective and lead to a standard preparation of PRP.[22] In another study conducted in 2017 Shin et al it was also concluded that PRP can be made by using double centrifugation method and no special equipment or high technical abilities are required for its preparation.[23] The study by Nagata et al also concluded that the double-centrifugation protocol resulted in higher platelet concentrations as compared to single centrifugation protocol.[24]

It is inferred in our study that time and speed of centrifugation affect the platelet yield tremendously and a small change in any of them lead to a bigger effect in the final product. We worked on multiple techniques, in which 3 techniques were based on double spin method while one technique was based on single spin method. No special or expensive equipment is required. Use of sterile vacutainer, syringes and tubes in this technique also reduces the chances of cross contamination. Repeated sessions of PRP therapy are required for desired results, the cost for these repeated sessions is quite high all over the world whether it for cosmetic reasons or therapeutic reasons.

CONCLUSION

By opting our technique, a good, high yield PRP can be prepared rather than using commercial PRP kits, which more or less yields the same results but at a higher cost. Due to these new emerging and promising uses of PRP, introducing a cost-effective technique with high yield platelets is an important procedure for PRP preparation.

Author Contributions:
Conception and Design: Ambreen Kashif, Sidra Moin
Collection and Assembly of data: Ambreen Kashif, Sidra Moin, Fatima Zahra
Analysis and interpretation of data: Ambreen Kashif, Sidra Ghazenfer, Fatima Zahra
Drafting of the article: Ambreen Kashif, Fatima Zahra
Critical revision of the article for important intellectual content: Sidra Taj
Statistical Expertise: Sidra Ghazenfar, Ambreen Kashif
Final approval and guarantor of the article: Ambreen Kashif, Sidra Taj
Corresponding author email: Ambreen.kashif@fmhcmd.edu.pk: Ambreen Kashif
Conflict of Interest: None declared
Rec. Date: Dec 21, 2020 Revision Rec. Date: Feb 11, 2021 Accept Date: March 11, 2021

REFERENCES

1. Marx RE. Platelet-Rich Plasma (PRP): What Is PRP and What Is Not PRP? Implant Dent 2001;10:225-28.
2. Everts PA, Knape JT, Weibrich G, Schönberger JP, Hoffmann J, Overdevest EP, et al. Platelet-rich plasma and platelet gel: a review. J Extra Corpor Technol 2006;38:174-7.
3. Alves R, Grimalt R. A Review of Platelet-Rich Plasma: History, Biology, Mechanism of Action, and Classification. Skin Appendage Disord 2018;4:18-24.
4. Conde-Montero E, Dobao P de la C, González JMM. Platelet-rich plasma for the treatment of chronic wounds: evidence to date. Vol. 4, Chronic Wound Care Management and Research. Dove Press; 2017;p 107-20.
5. Khatu SS, More YE, Gokhale NR, Chavhan DC, Bendsure N. Platelet-Rich Plasma in Androgenic Alopecia: Myth or an Effective Tool. J Cutan Aesthetic Surg 2014;7:107-10.
6. Schmitz JP, Hollinger JO. The biology of platelet-rich plasma. J Oral Maxillofac Surg 2001;59:1119-20.
7. Middleton KK, Barro V, Muller B, Terada S, Fu FH. Evaluation of the Effects of Platelet-Rich Plasma (PRP) Therapy Involved in the Healing of Sports-Related Soft Tissue Injuries. Iowa Orthop J 2012;32:150-63.
8. Shahid M, Kundra R. Platelet-rich plasma (PRP) for knee disorders. EFORT Open Rev 2017;2:28-34.
9. Samadi P, Sheykhhasan M, Khoshinani HM. The use of platelet-rich plasma in aesthetic and regenerative medicine: a comprehensive review. Aesthet Surg J 2019;43:803-14.
10. Kim DH, Je YJ, Kim CD, Lee YH, Seo YJ, Lee JH, et al. Can Platelet-rich Plasma Be Used for Skin Rejuvenation? Evaluation of Effects of Platelet-rich Plasma on Human Dermal Fibroblast. Ann Dermatol 2011;23:424-31.
11. Chamata ES, Bartlett EL, Weir D, Rohrich RJ. Platelet-Rich Plasma: Evolving Role in Plastic Surgery. Plast Reconstr Surg 2021;147:219-30.
12. Ahmed M, Reffat SA, Hassan A, Eskander F. Platelet-rich plasma for the treatment of clean diabetic foot ulcers. Ann Vasc Surg 2017;38:206-11.
13. Dawood AS, Salem HA. Current clinical applications of platelet-rich plasma in various gynecological disorders: An appraisal of theory and practice. Clin Exp Reprod Med 2018;45:67-9.
14. Hall MP, Band PA, Meislin RJ, Jazrawi LM, Cardone DA. Platelet-rich plasma: current concepts and application in sports medicine. J Am Acad Orthop Surg 2009;17:602-8.
15. Mishra A, Harmon K, Woodall J, Vieira A. Sports medicine applications of platelet rich plasma. Curr Pharm Biotechnol 2012;13:1185-95.
16. Sánchez AR, Sheridan PJ, Kupp LI. Is platelet-rich plasma the perfect enhancement factor? A current review. Int J Oral Maxillofac Implants 2003;18-9.
17. Alser OH, Goutos I. The evidence behind the use of platelet-rich plasma (PRP) in scar management: a literature review. Scars Burns Heal 2018;4:77-9
18. Rutkowski JL, Thomas JM, Bering CL, Speicher JL, Radio NM, Smith DM, et al. An analysis of a rapid, simple, and inexpensive technique used to obtain platelet-rich plasma for use in clinical practice. J Oral Implantol 2008;34:25-33.
19. Wang H-L, Avila G. Platelet Rich Plasma: Myth or Reality? Eur J Dent 2007;1:192–4.
20. Gupta V, Parihar AS, Pathak M, Sharma VK. Comparison of platelet-rich plasma prepared using two methods: Manual double spin method versus a commercially available automated device. Indian J Dermatol 2020;11:575-7.
21. Zhang Z, Wei Y, Zhang C, Yuan T. Optimized preparation method of leukocytes-rich platelet-rich plasma by varying conditions during centrifugation. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi 2020;34:1025-30.
22. Hamid MSA. Cost Effectiveness of a Platelet-rich Plasma Preparation Technique for Clinical Use. Wounds 2018;30:186-90.
23. Shin HS, Woo HM, Kang BJ. Optimisation of a double-centrifugation method for preparation of canine platelet-rich plasma. BMC Vet Res 2017;13:198-201.
24. Nagata MJ, Messora MR, Furlaneto FA, Fucini SE, Bosco AF, Garcia VG, et al. Effectiveness of two methods for preparation of autologous platelet-rich plasma: an experimental study in rabbits. Eur J Dent 2010;4:395-8.