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RESEARCH ARTICLE

QUALITY ASSESSMENT OF PLATELET CONCENTRATES PREPARED BY PLATELET RICH PLASMA-PLATELET CONCENTRATE AND BUFFY COAT -PLATELET CONCENTRATE

¹Latha, B., ^{2,*}Chitra, M., ³Ravishankar, J.

¹Associate Professor, Department of Transfusion Medicine, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai, India

²Assistant Professor, Department of Transfusion Medicine, Government Kilpauk Medical College and Hospital, Chennai, India

³Assistant Professor, Department of Transfusion Medicine, Government Tirunelveli Medical College and Hospital, Tirunelveli, India

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ABSTRACT

Background: Two types of platelet concentrates (PC) are available, Random donor platelets (RDP) which is collected as a co-product of whole blood donation while Single donor platelets (SDP or Apheresis-PC) is collected from voluntary donors with the help of automated cell separator. RDPs can be separated by two methods, Platelet rich plasma method (PRP-PC) and Buffy coat poor method (BC-PC). In our hospital, Platelet concentrates were usually prepared by PRP method and recently Buffy coat method has been introduced. This study focuses on in-vitro quality assessment between PRP-PCs and BC-PCs so that if BC-PCs are superior, the Buffy coat method can be fully implemented for the benefit of patients. **Materials and Methods:** This was a prospective study done from March 2016 to August 2016 at Government Kilpauk Medical College Hospital, Chennai, Tamil Nadu, India. A total of 48 platelet concentrates (24 units of PRP-PC and 24 units of BC-PC) were selected randomly and tested for quality at the end of expiry. 2-3 mL of samples were collected from the selected platelet bags in plain test tubes using aseptic precautions and tested for the following parameters: 1. Volume of PC. 2. Swirling. 3. Platelet count per bag. 4. Leukocyte count per bag. 5. pH changes. **Results:** A total of 48 platelet concentrates (24 of PRP-PC, 24 of BC-PC) were enrolled in this study. The mean volume of PRP-PC and BC-PC was 65.1±3.0 ml and 79.5±5.3 ml respectively. The mean platelet count of PRP-PC and BC-PC was $5.5 \times 10^{10} \pm 0.2$ and $8.2 \times 10^{10} \pm 0.7$ respectively. The mean WBC count in PRP-PC and BC-PC was $14.7 \times 10^7 \pm 12.6$ and $0.7 \times 10^7 \pm 0.1$ respectively. All units were analyzed for pH changes. The mean pH was 6.9 ± 0.1 (PRP-PC) and 6.5 ± 0.1 (BC-PC). **Conclusion:** Our study, in concordance with similar studies, proved that even though BC-PC and PRP-PC fulfilled the desired quality control parameters, Buffy coat platelets were superior to PRP platelets in terms of increased platelet count, PC volume and decreased leukocyte count. There was reduced production related damage and improved platelet quality during storage, with BC-PC. It would be prudent to shift from PRP-PC to BC-PC method in whole blood component separation as the benefits like increased platelet yield, better quality platelets, reduced bacterial contamination and cost-effectiveness outweigh the reduction in packed red cell volume.

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INTRODUCTION

Since platelets were first identified in 1881, there has been a continuous and accelerating progress in our basic understanding of platelet function and its utilization in various bleeding disorders. The first successful attempt to raise the platelet count in thrombocytopenic patients by transfusion of whole blood was described by Duke in 1910.

*Corresponding author: Chitra, M.,

Assistant Professor, Department of Transfusion Medicine, Government Kilpauk Medical College and Hospital, Chennai, India.

Availability of plastic bags in blood banking and the general improvement in the technique to separate components revolutionized the field of component therapy. Platelet transfusions are the primary therapy for various causes of thrombocytopenia (Singh, 2009). Two types of platelet concentrates (PC) are available, Random donor platelets (RDP) which is collected as a co-product of whole blood donation while Single donor platelets (SDP or Apheresis-PC) is collected from voluntary donors with the help of automated cell separator. RDPs can be separated by two methods, Platelet rich plasma method (PRP-PC) and Buffy coat poor method

(BC-PC) (Perrota, 2003). Buffy coat method of platelet production was developed by investigators in the Netherlands and Sweden in the mid-1970s, which reverses the sequence of centrifugation steps compared to PRP method. A hard spin is used initially to separate whole blood into three components: plasma, red blood cells (RBCs), and a Buffy coat layer. Using automated extraction, the most common configuration uses a so-called top-and-bottom collection set in which plasma and RBCs are transferred to storage containers and the buffy coat is left in the donation bag. This BC contains PLTs, white blood cells (WBCs), plasma, and some RBCs. The BC is then given a soft spin, and the PC is extracted with or without leukofiltration (Levin, 2008). In contrast to PRP-PCs which are centrifuged against the plastic bag, BC-PCs are centrifuged against cellular elements of the whole blood unit. This may lead to less platelet activation during BC-PC production relative to PRP-PCs. Although both methods produce similar platelet products, they may have different in-vitro characteristics. There is a noticeable quality improvement in laboratory markers of BC-PCs relative to PRP-PCs. This is characterized by a higher proportion of discoid PLTs, lower CD62 expression and better hypotonic shock response (HSR) at the end of storage. Metcalfe and coworkers indicated that PRP-PCs have a higher level of the activation markers CD62 and annexin V, 1 hour after the end of the separation process. BC-PCs are leukoreduced well below acceptance level, reducing risk for non-hemolytic transfusion reactions and alloimmunization.

There is a remarkable improvement in the quality of platelets prepared by laboratories using Buffy coat (BC-PC) method relative to platelets produced by PRP (PRP-PC). The close contact between platelets in the platelet pellet and also with non-biological surface of blood bag, during the second phase of centrifugation for separation of platelets by PRP-PC method, leads to reversible platelet aggregation. In the absence of “biologic cushion” (WBC and RBC as a barrier), separation of platelets by PRP-PC method may stimulate more degranulation or activation of platelets during hard spin centrifugation. But BC-PC method does not involve significant close cell contact between platelets in a pellet and also with blood bag (Simon, 2009). In our hospital, Platelet concentrates were usually prepared by PRP method and recently Buffy coat method has been introduced. This study focuses on in-vitro quality assessment between PRP-PCs and BC-PCs so that if BC-PCs are superior, the Buffy coat method can be fully implemented for the benefit of patients.

MATERIAL AND METHODS

This was a prospective study done from March 2016 to August 2016 at Government Kilpauk Medical College Hospital, Chennai, Tamilnadu, India. A total of 48 platelet concentrates (24 units of PRP-PC and 24 units of BC-PC) were selected randomly and tested for quality at the end of expiry. 2-3 mL of samples were collected from the selected platelet bags in plain test tubes using aseptic precautions and tested for the following parameters: 1. Volume of PC. 2. Swirling. 3. Platelet count per bag. 4. Leukocyte count per bag. 5. pH changes.

Platelet separation methods: Platelet concentrates were prepared by PRP and BC method. In the PRP method, whole blood units were centrifuged using an initial ‘soft spin’ to concentrate the platelets in the supernatant plasma (platelet rich plasma) which is transferred to a satellite bag. After

pelleting the platelets by "hard-spin" centrifugation of the PRP, platelet poor plasma was removed for freezing (fresh frozen plasma). Platelets were re-suspended and stored as platelet concentrate (PC) in the reduced volume of remaining plasma. In buffy coat method, whole blood was first subjected to “hard spin” centrifugation whereby whole blood is separated into different components according to their specific gravity. The top layer contained platelet poor supernatant plasma, middle layer - buffy coat, was composed of approximately 90% platelets, 70% WBCs and 10% red cells while the bottom layer contained packed red blood cells. Platelet poor supernatant plasma and packed red cells were separated into different satellite bags, while buffy coat was retained in the same bag. The buffy coat was gently mixed with the plasma and again subjected to ‘light spin’. The supernatant platelets were expressed into platelet storage bag while leukocytes and red cells were discarded.

Platelet Concentrate volume: The volume was determined by subtracting the weight of the empty bag from that of full bag. To convert weight to volume, the resultant weight was divided by 1.03 (specific gravity of PRP-PC)

or 1.06 (specific gravity of BC-PC).

$$\text{Volume of the Concentrate (ml)} = \frac{\text{Wt. of the full bag} - \text{Wt. of empty bag}}{\text{Specific gravity}}$$

Swirling: Swirling was evaluated by examining the platelet units against light.

Platelet count: The platelet count in the bag was calculated using automated cell counter.

RBC count: RBC count in the bag was calculated using automated cell counter.

WBC count: WBC count in the bag was performed manually using a Neubauer counting chamber and WBC diluting Turk’s Fluid (RBC lysing fluid with Gentian violet and 2% glacial acetic acid). Method: 500 µL of whole blood was mixed with equal quantity of Turk’s fluid, giving a dilution of 1:2, mixed for 2 minutes following which the Neubauer counting chamber was charged. Charged chamber was left for further 2 minutes for the leukocytes to settle and then the counting was done in four large squares of the chamber under microscope (x 40 objective).

Calculation:

$$\begin{aligned} \text{WBC count (per ml)} &= \frac{\text{No of cells counted}}{\text{Volume of the chamber (}\mu\text{L)}} \times \text{dilution} \times 1,000 \\ &= \frac{N}{0.4} \times 2 \times 10^3 \\ &= N \times 5,000 \end{aligned}$$

The WBC count/bag was calculated by: WBC count/bag = N x 5,000 x platelet concentrate volume

pH determination: pH for PCs was measured using pH meter.

Sterility test: Sterility was performed on Day 5 of the PCs shelf-life.

Statistical analysis: Descriptive details were given in summary statistics. We performed statistical comparison by using student t-test for multiple groups. A probability of $p < 0.05$ (two-sided) was used to reject null hypothesis.

RESULTS

In this prospective study, quality of the platelet concentrates was assessed by observing swirling, volume, platelet count, WBC count, pH and sterility in every unit.

Swirling: Swirling was observed in all platelet units ($n=48$) at the end of day 5 of the PCs shelf-life.

WBC contamination per unit: Leukocyte count of individual units ($n=48$) was calculated and analyzed. The mean leukocyte count in PRP-PC units ($n=24$) was $14.7 \pm 12.6 \times 10^7$ cells per unit with a range from 5.8×10^7 to 47×10^7 cells while the mean leukocyte count in BC-PC units ($n=24$) was $0.7 \pm 0.1 \times 10^7$ cells per unit with a range from 0.6×10^7 to 0.95×10^7 cells [Table 3]. Even though all the units ($n=48$) fulfilled the recommended quality criteria for leukocyte count in PCs, there was a statistically significant reduction in leukocyte count in BC-PC units when compared to PRP-PC units.

pH changes: All platelet units ($n=48$) were analyzed for changes in pH at the end of day 5.

Table 1. Criteria for quality assessment of PRP-PC, BC-PC (DGHS, India recommendation)

Parameters	Frequency of control	Quality requirement for PRP-PC and BC-PC
Swirling	All units	Present
Volume	All units	40-70 ml (PRP-PC), 70-90 ml (BC-PC)
Platelet count/ unit	Monthly	$>5.5 \times 10^{10}$ in 75% of unit tested
WBC count/unit	Monthly	$5.5 \times 10^7 - 5 \times 10^8$
RBC contamination	Monthly	PRP-PC-0.5ml BC-PC-Traces to 0.5ml
pH	Monthly	>6.0 at the end of maximum days of storage in 100% of units tested.

Table 2. Comparison of QC variables for PLTs produced by PRP and BC methods (DGHS Standards)

Parameters	PRP-PCs (mean \pm SD)($n=24$)	BC-PCs (mean \pm SD) ($n=24$)	P value
Volume (ml)	65.1 ± 3.0	79.5 ± 5.3	<0.05
PLT yield ($X10^{10}$ /unit)	$5.5 \times 10^{10} \pm 0.2$	$8.2 \times 10^{10} \pm 0.7$	<0.05
Residual WBCs ($X10^7$ /unit)	$14.7 \times 10^7 \pm 12.6$	$0.7 \times 10^7 \pm 0.1$	<0.05
pH	6.9 ± 0.1	6.5 ± 0.1	<0.05

Table 3. Comparison of volume, platelet counts and WBC counts of PCs with other studies

Study groups	Volume (ml) (mean \pm SD)		PLT yield ($X 10^{10}$ /unit) (mean \pm SD)		Residual WBCs ($X 10^7$ /unit) (mean \pm SD)		pH (mean \pm SD)	
	PRP-PC	BC-PC	PRP-PC	BC-PC	PRP-PC	BC-PC	PRP-PC	BC-PC
Singh <i>et al</i> ¹ .	62.3 ± 22.68	68.81 ± 22.95	7.6 ± 2.97	7.3 ± 2.98	4.05 ± 0.48	2.08 ± 0.39	-	-
Levin E <i>et al</i> ³ .	60.9 ± 3.7	344.5 ± 19.5	83.5 ± 23.6	302.8 ± 54.3	0.3 ± 1.1	0.3 ± 1.0	7.14 ± 0.18	7.30 ± 0.13
Fijnheer <i>et al</i> ⁵ .	67.8 ± 4.0	56.2 ± 6.7	70 ± 10.1	59 ± 12.2	19.8 ± 15.8	0.7 ± 0.8	-	-
Ali SF ⁶	50.6 ± 15.3	70 ± 20.6	5.8 ± 1.1	5.9 ± 1.0	44 ± 0.65	28 ± 0.5	6.9 ± 0.12	6.6 ± 0.12
Present study	65.1 ± 3.0	79.5 ± 5.3	5.5 ± 0.2	8.2 ± 0.7	14.7 ± 12.6	0.7 ± 0.1	6.9 ± 0.1	6.5 ± 0.12

Volume (ml): The volume of individual units was calculated and analyzed. The mean volume of the PRP-PC units ($n=24$) was 65.1 ± 3.0 ml with a range from 56 to 69 ml. The mean volume of BC-PC ($n=24$) was 79.5 ± 5.3 ml with a range from 70 to 89 ml. Even though all the units ($n=48$) fulfilled the quality criteria for volume of PCs, there was a statistically significant increase in volume of BC-PC units when compared to volume of PRP-PC units.

Platelet count per unit: Platelet count of individual units ($n=48$) was calculated and analyzed. The mean platelet count of PRP-PC units ($n=24$) was $5.5 \pm 0.2 \times 10^{10}$ cells per unit with a range from 5.2×10^{10} to 5.7×10^{10} cells while the mean platelet count of BC-PC units ($n=24$) was $8.2 \pm 0.7 \times 10^{10}$ cells per unit with a range from 6.8×10^{10} to 9.2×10^{10} cells [Table 2]. Even though all the units ($n=48$) fulfilled the quality criteria for platelet count in PCs, there was a statistically significant increase in platelet count of BC-PC units when compared to PRP-PC units.

RBC contamination per unit: All platelet units ($n=48$) were tested for red cell contamination. Neither PRP-PC units ($n=24$) nor BC-PC units ($n=24$) were contaminated with red cells. Both types of platelet concentrates fulfilled the recommended quality control criteria for Red cell contamination in PCs and there was no statistically significant difference.

The mean pH of the PRP-PC units ($n=24$) was 6.9 ± 0.1 . The mean pH of BC-PC ($n=24$) was 6.5 ± 0.1 . Even though all the units ($n=48$) fulfilled the quality criteria for pH in PCs, there was a statistically significant improvement in maintenance of pH in BC-PC units when compared to PRP-PC units.

Sterility: No growth was observed in all the PCs units ($n=48$).

DISCUSSION

Assessing the quality of platelet concentrates is an important step to evaluate ex-vivo functional viability of platelet concentrates, post transfusion recovery and survival in recipients. Platelet concentrates were stored at $20-24^\circ\text{C}$ with continuous agitation as recommended, until the time of issue. Various parameters are used for routine ex-vivo quality assessment of platelet concentrates such as swirling, volume, platelet count, WBC count and pH. This study was performed to determine and compare the in-vitro quality of platelet concentrates, prepared by two different methods, at the end of 5 days shelf life. These comparisons were made using the standard DGHS guidelines of the blood center. As the comparison in Table 3 shows, the results of the present study were comparable to the results of other researchers.

The Buffy coat method of platelet separation offers significant advantages when compared to PRP platelets like better process control due to automation, increased recovery of plasma for fractionation, easy bacterial testing as it is a pre-storage pooled product and also more efficient use of laboratory personnel. Although there are relatively few in vivo data to support the suggestion that the BC-PC is a superior product to the PRP-PC, the data reported here are consistent with a number of in vitro studies in the literature, suggesting that this type of platelet product is superior. In terms of benefits to both patients and the manufacturers of whole blood derived platelets, Buffy Coat method has proven its value worldwide. In 2004, the Canadian Blood Services initiated a switch from PRP to BC production because of perceived advantages, the most important of these being logistical. Newly available automated pooling technology contributes to process control, making BC production even more economical (Simon, 2009). In Europe, there is an approximately 50:50 split between the use of BC- and apheresis-derived PCs. In Canada (Quebec excepted), 70% of platelets are derived from whole blood donation. Denmark, Finland and the Netherlands prepare 85% to 95% of their concentrates by the BC method. These countries demonstrate that a national platelet supply can be derived almost entirely from collected whole blood rather than relying upon apheresis PC production (Simon, 2009).

Conclusion

Our study, in concordance with similar studies, proved that even though BC-PC and PRP-PC fulfilled the desired quality control parameters, Buffy coat platelets were superior to PRP platelets in terms of increased platelet count, PC volume and decreased leukocyte count. There is reduced production related damage and improved platelet quality during storage, with BC-PC.

It would be prudent to shift from PRP-PC to BC-PC method in whole blood component separation as the benefits like increased platelet yield, better quality platelets, reduced bacterial contamination and cost-effectiveness outweigh the reduction in packed red cell volume.

Conflict of Interests: The authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES

- Ali SF. 2012. Platelet Activation in Stored Platelet Concentrates: Comparison of Two Methods Preparation. *J Hematol.*, 1(1):15-19.
- Fijnheerr R, Pieterszd NI, Kort D *et al.*, 1990. Platelet activation during preparation of platelet concentrates: a comparison of the platelet-rich plasma and the buffy coat methods. *Transfusion.*, 30(7) :634-638.
- Levin E., Culibrk B., Gyöngyösy-Issa MIC. *et al.* 2008. Implementation of buffy coat platelet component production: comparison to platelet-rich plasma platelet production. *Transfusion* 48: 2331-2337.
- Perrota PL, Anderson KC. 2003. Blood banking and transfusion medicine. Basic Principles and Practice. Philadelphia: Churchill Livingstone. p. 181-205.
- Simon TL, Snyder EL, Solheim BG *et al.* 2009. Rossi's Principles of Transfusion Medicine. 4thed. A John Wiley & Sons, Ltd, Publication.
- Singh RP., Marwaha N, Malhotra P *et al.* 2009. Quality assessment of platelet concentrates prepared by platelet rich plasma-platelet concentrate, buffy coat poor-platelet concentrate (BC-PC) and apheresis-PC methods. *Asian J Transf Sci.*, July 3(2):86-94.
