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A Comparative Assessment of Quality of Platelet Concentrates Prepared By 3 Different Methods.

Medicine

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ABSTRACT

Aim: To compare the quality of the PC's prepared by three methods i.e Platelet Rich Plasma (PRP), Buffy Coat (BC) and Apheresis method as per the departmental SOP based on DGHS criteria.
Material & Methods: Four platelet concentrates prepared by each of the three methods were assessed every month for the following quality parameters - Swirling, Volume, Platelet count, WBC count and pH. **Results:** 48 PC of each method (Total 144) were analysed. 62.5% of PRP-PC & 58.33% of BC-PC showed Grade II swirling & 91.67% of the Apheresis-PC had grade III swirling. The mean volume of PRP-PC, BC-PC and Apheresis-PC units was 58.45 ± 13.05 ml, 60.68 ± 16.09 ml and 209.65 ±10.86 ml respectively. The mean platelet count of PRP-PC, BC-PC and Apheresis-PC units was 5.68±1.83 1010/unit, 5.13 ±1.75 x 1010 /unit and 2.72 ± 0.48 x 1011 /unit respectively. The mean WBC count of PRP- PC, BC-PC and Apheresis-PC units was 7.6 ±4.87 x 107/unit, 3.72 ± 1.63 x 107 /unit and 4.16 ± 0.71 x 106/unit & The mean pH of PRP-PC, BC-PC and Apheresis-PC units was 6.9 ± 0.32, 6.9 ± 0.33 and 7.02 ± 0.26 respectively. Conclusion: PRP-PC and BC-PC units were comparable for swirling. Apheresis-PC units showed better swirling than PRP-PC and BC-PC units. PRP-PC and BC-PC units were comparable for mean volume & Platelet count per unit; Apheresis-PC units showed better platelet counts than PRP-PC and BC-PC units. BC-PC units had less mean WBC contamination than PRP-PC units and Apheresis-PC units had least WBC contamination.

KEYWORDS:

Platelet concentrate, Platelet Rich Plasma -Platelet Concentrate, Buffy Coat-Platelet concentrate, Apheresis-Platelet Concentrate

Introduction

Platelets play a pivotal role in haemostasis through adhesion to the injured vessel wall, aggregation, propagation of coagulation & thrombus formation. Platelets were described by Addison in 1841 as extremely minute granules in clotting blood & were termed platelets by Bizzozero who also observed their tissue adhesive qualities as increased stickiness when a vascular wall is damaged.[1] Subsequently platelets are also involved in fibrinolysis & the repair of the vessel wall, restoring blood flow & vascular integrity.[2]

Platelets concentrates are prepared from three different methods i.e. Platelet Rich Plasma (PRP), Buffy Coat (BC) and Apheresis method. The quality of platelet concentrates is affected by the preparation method and the storage conditions including the duration of storage, type of storage container and storage solution (plasma or additive solution). Different in vivo and in vitro techniques can be used to analyse platelet concentrates.[3] The basic principle behind the preparation of components from whole blood is that each component has its own specific gravity and by applying differential centrifugation, each component is separated and removed, thus allowing the transfusion of the desired component according to the need of the patient.[4]

Material and Methods

The present study was conducted in the Department of Immunohematology and Blood Transfusion Medicine, Govt. Medical College, Jammu over a period of 1 year Nov. 2010 to Oct 2011.

Blood Collection (Phlebotomy)

After appropriate Donor history and physical examination, whole blood was collected in 450 ml blood bags through a single smooth venepuncture. Mechanical blood mixers were used for all donations & blood bags maintained at room temperature (20 - 24 C) until processing. All platelet concentrates were prepared within 6-8 hours of collection. The average time for collection was less than 10 minutes. Cryofuge 6000i, refrigerated centrifuge was used for preparation of random donor platelets.

Preparation of Platelet Rich Plasma - platelet concentrate (PRP-PC)

Blood bags after balancing were subjected to light spin centrifugation by Cryofuge 6000i at 1800 rpm for 10 minutes at 22C, leading to separation of two layers i.e. upper - platelet rich plasma and lower - red cells. The upper platelet rich plasma is transferred into a satellite container which is then subjected to high spin 3800 rpm for 5 minutes leading to concentration of the platelets. Subsequently, the supernatant platelet poor plasma is removed with 50-70 ml left in the bag. This platelet concentrate is left undisturbed for 1 hour and then resuspended in residual plasma.

Preparation of Buffy Coat- Platelet concentrates (BC-PC)

The blood bags after balancing were subjected to hard spin at 3800 rpm for 5 minute at 22C. Whole blood separated into three layers: The upper layer - platelet poor plasma, middle layer - buffy coat, containing app. 90% of platelets,70% of WBCs and 10% of red cells & the lower layer - red cells. The platelet poor plasma was transferred into a transfer bag by breaking the upper clamp & stored in the deep freezer as fresh frozen plasma. The packed cells were transferred by breaking the lower clamp into the satellite bag containing adsol leaving behind the buffy coat in the primary bag. The primary bag containing buffy coat and plasma is left hanging for about 1 hour at room temperature. The buffy coat was then again subjected to light spin at 1,100 rpm for 6 minutes. The upper platelet rich plasma was then expressed into the platelet transfer bag and the primary bag containing residual WBC's and red cells was discarded.

Single Donor Platelets (SDP) or Apheresis -PC

All plateletpheresis were done using the automated cell separator - CS3000 plus with TNX-6 separation chamber (Baxter, Fenwal division); a continuous flow centrifugation machine. All procedures were done using double venous access.

In the present study, the following criteria of Quality Control of Platelets as recommended by DGHS (Directorate General of Health Services), India was followed[5] **Table 1**.

Table 1- DGHS Criteria

Parameters	Quality Requirement for PRP-PC	Quality Requirement for BC-PC	Quality Requirement for Apheresis-PC	Frequency of Control
Swirling	Present	Present	Present	4Units/month
Volume	40 - 70 ml	70 - 90 ml	200 - 300ml	4Units/month
Platelet count/unit	≥5.5 × 1010	≥5.5 × 1010	≥3 × 1011	4Units/month

WBC count/unit	5.5 × 107 - 5 × 108	5.5 × 107 - 5 × 108	<5×106	4Units/month
pH	> 6.0	> 6.0	> 6.0	4Units/month

The platelet units were randomly selected and tested for following parameters:

1. **Platelet concentrate volume**

Vol. = Wt. of the full bag(g)-wt. of the empty bag(g)
Specific gravity

Weight is expressed in grams.
Specific gravity of PC is 1.03

2. Swirling

Swirling was evaluated by examining the units against light and graded as positive or negative.

Positive swirling was graded as:

3 - Very clear homogen swirling in all parts of the bag.

2 - Clear homogenic swirling in all part of the bag.
1 - Homogen swirling only in some part of the bag and is not clear.
0 - Homogen turbid and is not changed with pressure.[4]

3. Platelet count/unit

The platelet count/unit was calculated by multiplying platelet concentration (count) with platelet concentrate volume. The platelet count was determined with automated cell counter, Celtech.

4. WBC count/unit

The WBC count/unit was calculated by multiplying WBC concentration with platelet concentrate volume. WBC count was determined with automated cell counter, Celtech.

5. pH

pH was determined with the pH meter (Labtronics) at the end of maximum storage.

Results

Quality Parameter No.1: Swirling

Table2: Intergroup comparison of Swirling

Swirling Grade	PRP-PC	BC-PC	Apheresis-PC
Grade 1	2(4.17%)	5(10.42%)	_
Grade 2	30(62.5%)	28(58.33%)	4(8.33%)
Grade 3	16(33.33%)	15(31.25%)	44(91.67%)

Quality Parameter No.2: Volume

Table3: Intergroup comparison of Volume

Volume per unit(ml)	PRP-PC	BC-PC	Apheresis –PC
Mean ± SD	58.45 ± 13.05	60.68 ± 16.09	209.65± 10.86
Range	33.98 - 97.08	24.13 - 87.38	189.32-233.01

Quality Parameter No.3: Platelet count per unit

Table 4: Intergroup comparison of Platelet count per unit

Platelet count per unit	PRP-PC (1010)	BC-PC (1010)	Apheresis-PC (1011)
Mean± SD	5.68 ± 1.83	5.13 ± 1.75	2.72 ± 0.48
Range	1.5 - 9.38	0.61 - 9.23	1.86 - 3.66

Quality Parameter No.4: WBC count per unit

Table 5: Intergroup comparison of WBC count per unit

WBC count per unit	PRP-PC (107)	BC-PC (107)	Apheresis-PC (106)
Mean ± SD	7.6 ± 4.87	3.72 ± 1.63	4.16 ± 0.71
Range	3.7 - 34.95	1.26 - 7.48	2.04 - 5.68

Quality Parameter No.5: pH

Table6: Intergroup comparison of pH

pH	PRP-PC	BC-PC	Apheresis -PC
Mean ± SD	6.9 ± 0.32	6.9 ± 0.33	7.02± 0.26
Range	6.4 - 7.5	6.4 - 7.4	6.5 - 7.6

Discussion

Quality Parameter No.1: Swirling

Swirling is a simple & non-invasive procedure performed visually. Its presence indicates pH within the required range adequate for in vivo survival.

In the present study, **Score 3** swirling was seen in **33.33%, 31.25%** and **91.67%**; **score 2** swirling was seen in **62.5%, 58.33%** and **8.33%** of **PRP-PC, BC-PC** and **Apheresis-PC units** respectively while **Score 1** swirling respectively was seen in **4.17% & 10.42% of PRP-PC** and **BC units**. Statistically no significant difference was observed between PRP-PC and BC-PC units for swirling. Apheresis-PC units showed better swirling than PRP-PC and BC-PC units; this difference was statistically significant **(p 0.0001).**

In a similar study by **Singh RP et al.**[4] score 3 swirling was observed in 79.7%, 83.9% and 90% while score 2 swirling was noticed in 20.3%, 16.1% and 10% of PRP-PC, BC-PC and Apheresis-PC units respectively. No unit had score 1 swirling.

Quality Parameter No.2: Volume

The mean volume of **PRP-PC, BC-PC** and **Apheresis-PC units** was **58.45 ± 13.05 ml**, **60.68 ± 16.09 ml** and **209.65 ± 10.86 ml** and ranged from **33.98 - 97.08 ml, 24.13 - 87.38 ml** and **189.32 - 233.01 ml** respectively. PRP-PC and BC-PC units were comparable in terms of volume (p1.00); whereas a statistically significant difference was found between Apheresis-PC units and PRP-PC & BC-PC units **(p 0.0001). 81.25% (39/48), 27.08% (13/48) and 85.42%(41/48)** of PRP-PC, BC-PC and Apheresis-PC units respectively fulfilled the quality control criteria of volume. On comparing the maximum number of units meeting the desired quality control criteria of volume for PRP-PC units, BC-PC units & Apheresis- PC units [39(81.25%) vs 13(27.08%) vs 41(85.42%), a statistically significant difference was found between PRP-PC units & BC-PC units (p 0.0001) and also between BC-PC units & Apheresis-PC units (p 0.0001).

In a study by **Chopra G et al.**[6] volume of BC-PCs was higher (71.6 ± 5ml) than PRP-PCs (57 ± 5ml). **Choudhary R et al.** [7] evaluated 94 plateletpheresis procedures on CS 3000 (Baxter) & found a mean volume of 200.6 ± 14.6 ml. **Das SS et al**.[8] on analysing the quality of 267 platelet concentrate units found a mean volume per unit of 54 ml. In the study by **Ali SF**[9], the mean volume of BC-PCs and Apheresis-PCs was 70.6 ± 20.3 ml and 225 ± 15.6 ml respectively.

Quality Parameter No.3: Platelet count per unit

The mean platelet count of PRP-PC, BC-PC and Apheresis-PC was **5.6 ± 1.83 × 10^{10}/unit**, **5.13 ± 1.75 × 10^{10}/unit** and **2.72± 0.48 × 10^{11}/unit** and ranged from **1.5 - 9.38 × 10^{10}/unit, 0.61 - 9.23 × 10^{10}/unit** and **1.86 - 3.66 × 10^{11}/unit** respectively. The mean platelet count of PRP-PC and BC-PC was comparable and statistically no significant difference was observed; whereas a statistically highly significant difference was found between Apheresis-PC units and PRP- PC & BC-PC units (p 0.0001)**. 66.7% (32/48), 52.08% (25/48) and 35.42% (17/48)** of of PRP- PC, BC-PC and Apheresis-PC units respectively fulfilled the quality control criteria of platelet count/unit. **Choudhary R et al.**[7] in a study on 94 plateletpheresis procedures on CS 3000, Baxter found a mean platelet yield of 2.8 ± 0.73 ×10^{11} /unit. In the study by **Das SS et al**.[8], 267 units of platelet concentrates were subjected for quality analysis and a net platelet count of 5.4 × 10^{10} platelets per unit was found. **Ali SF**[9]found that the mean platelet count of the BC-PC and APC units was 6.5 ± 1.8 x 10^{10} /unit and 2.8 ± 1.8 x10^{11} /unit respectively.

Quality Parameter No.4: WBC count per unit

The mean WBC count of PRP-PC, BC-PC and Apheresis-PC was **7.6 ± 4.87 × 10^{7}/unit, 3.72 ± 1.63 × 10^{7}/unit** and **4.16 ± 0.71 × 10^{6}/unit** and ranged from **3.7 - 34.95 × 10^{7}/unit**, **1.26 - 7.48 × 10^{7}/unit** and **2.04 - 5.68 × 10^{6}/unit** respectively. On comparison, BC-PC units had less mean WBC contamination than PRP-PC units and the difference was statistically highly significant **(p<0.0001)**. Apheresis-PC units were found to have least WBC contamination as compared to PRP-PC and BC-PC units and the difference was statistically highly significant **(p<0.0001)**.

All PRP-PC and BC-PC units met the desired quality control criteria of WBC count per unit whereas the recommended quality control criteria of WBC count per unit was met in **91.67% (44/48)** of Apheresis-PC units.

Choudhary R et al.[7] in his study on 94 plateletpheresis procedures on CS 3000, Baxter found a mean WBC count of $5.3 \pm 0.6 \times 10^8$ /unit. In the study by **Ali SF** [9], **t**he mean WBC contamination in BC-PC and APC units was $41 \pm 0.4 \times 10^6$ /unit and $3.1 \pm 0.7 \times 10^6$/unit respectively.

Quality Parameter No.5: pH

The mean pH of PRP-PC, BC-PC and Apheresis-PC was **6.9 ± 0.32, 6.9 ± 0.33** and **7.02 ± 0.26** and ranged from **6.4 - 7.5, 6.4 - 7.4** and **6.5 - 7.6**. All PRP-PC, BC-PC and Apheresis-PC units met the recommended quality control criteria of pH. **Chopra G et al.**[6] found an adequate pH within range of 7.4 - 6.6 in their study. **Ali SF**[9] observed that the mean pH was 6.8 ± 0.1 and ranged from 6.6 - 7.01.

The quality of platelet concentrates is affected by various factors such as donor variables, technique used and personal skills. Blood collection, processing and storage conditions significantly influence the quality of platelet concentrates**.** Over past few decades, the developed countries are making a gradual transition from WBD-PC (PRP-PC & BC-PC) units to Apheresis-PC units. This transition is largely due to the better quality and reduced donor exposure of Apheresis-PC units.

Our study has also shown that Apheresis-PC units had a better quality with respect to swirling, volume, platelet count per unit & had least WBC contamination as compared to PRP-PC & BC-PC units. The number of Apheresis-PC units meeting the quality control criteria for platelet count per unit was 35.42%. This may be explained on the basis that Apheresis is a technical procedure requiring expertise affected by many variables like baseline platelet count of the donors, the collection time & the volume of blood processed during the procedure. A significant proportion of Indian donors have borderline haemoglobin and platelet counts.[10] Therefore, to meet the desired platelet yield, we need to select the donors with higher base line platelet counts, increase the processing time & also increase the blood volume processed.

On comparing the cost effectiveness, quality & reduced rate of complications of BC-PC units with PRP-PC units, it is recommended that BC-PC units should be used rather than PRP-PC units for the wide range of patients being treated at our centre since it is a Regional Tertiary Care Centre catering to a large subset of population belonging to variable socio-economic strata.

Conclusion

Our study suggests that Apheresis-PC units have a better quality with respect to swirling, volume, platelet count per unit & least WBC contamination as compared to PRP-PC & BC-PC units. Therefore, we should make a gradual transition from WBD-PC units to Apheresis-PC units at our centre.

Lack of awareness regarding the benefits of Apheresis-PC units poses a major hindrance in the usage of Apheresis-PC units. Moreover, donor needs to spend 60 - 90 minutes of his or her time for the procedure. And last but not the least the cost of the closed system Apheresis kits is high. Therefore, to increase the recruitment of plateletpheresis donors, we should counsel the donor regarding the better quality & cost effectiveness of Apheresis-PC units over WBD-PC units.

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