ORIGINAL RESEARCH PAPER
Volume - 9 | Issue - 9 | September - 2020 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ASSESSMENT OF PLATELET CONCENTRATE PREPARED FROM FRESH AND OVERNIGHT HELD BUFFY COATS

Medical Science

Dr. Arvind Kumar Singh* Senior Resident, Sanjay Gandhi Post Graduate Institute Of Medical Sciences, Lucknow U.P. *Corresponding Author

Dr. Prashant Agarwal Professor, Sanjay Gandhi Post Graduate Institute Of Medical Sciences, Lucknow U.P.

Dr. Anupam Verma Professor, Sanjay Gandhi Post Graduate Institute Of Medical Sciences, Lucknow U.P.

ABSTRACT

Separation of platelets within 6 hrs after collection of whole blood, kept at ambient temperature is the current practice in the preparation of platelet concentrate (PC) by different methods of platelet preparation (PRP AND BUFFY COAT methods) in India. However, the ability to hold whole blood for up to 24 hours before PC preparation would allow every unit of whole blood to be separated to its components, which is practiced in many European countries. So a study is planned to assess and to compare the platelet quality prepared from fresh whole blood derived buffy coats (within 6 hrs from collection) and overnight hold of buffy coats at room temperature i.e. 20-24°C. In this study, 30 units of PC were prepared freshly from whole blood derived buffy coats (i.e., within 6 hrs of collection) and another 30 units were prepared from buffy coats after 24 hrs of storage at 20-24°C. Each unit of PC were assessed at day 1, day 3 and day 5 of their storage for Volume, pH, total white blood cell (TWBC) count, platelet count, bacterial contamination and presence of swirling. Bacterial culture performed at 5th day of storage. **Result:** When the parameters were compared between groups, no significant difference in platelet count was found on sampling days 1, 3, and 5. WBC and pH for both groups were within the quality requirements of the Director General of Health Services requirements (DGHS) in India (>75% units tested fall within the standard). All units of PC prepared from fresh and after overnight hanged buffy coats showed the presence of swirling at 1, 3 and 5 day of storage. All PCs prepared from both the methods were sterile. Delaying whole blood processing for up to 24 hrs does not significantly affect certain in vitro quality parameters as compared with freshly prepared PC.

KEYWORDS

Platelet concentrate(PC), Platelet rich plasma(PRP), Total white blood cell count(TWBC).

INTRODUCTION

The current requirement that platelet concentrates should be separated within eight hours after the collection of the whole blood.[1] In contrast, overnight hold of the whole blood at room temperature has long been used in Europe prior to the preparation of Buffy coat platelet concentrates.[1] Platelet concentrates (PCs) can be prepared by immediate processing of fresh whole blood. For this approach the advantages are that red blood cells (RBCs) contain optimal 2,3-diphosphoglycerate acid (2,3-DPG) and adenosine tri phosphate (ATP) levels, and plasma can be frozen rapidly, leading to higher Factor (F)VIII levels, since it is known that storage of whole blood or non frozen plasma results in FVIII loss up to 1% per hour.[2-3] In a number of countries, it is mandatory to process the whole blood on the day of collection due to these concerns and bacterial contamination if blood is stored at ambient temperature for longer periods. Alternatively, PCs can be prepared from Buffy coats (BCs) separated from whole blood within a few hours of collection and then stored at ambient temperature overnight and processing of these Buffy coats following day.[1] This method of PCs preparation preserve the quality of PRBC and FFP units while preparing platelets on next day.

Advantages of the BC method are that there is less activation of platelets than in the other methods because in the BC method, platelets are cushioned against red cells during the hard spin. Thus this method both increases the yield of platelets in platelet concentrates [3,4] and it also may facilitate bacterial ingestion by white cells of any contaminating bacteria prior to leukocyte-filtration, thereby reducing the incidence of transfusion-transmitted sepsis. However, the concept that WBCs may remove an contaminating bacteria during an overnight hold of the whole blood remains controversial.[3-5] Beside this plasma units prepared from BC depleted Units have approximately 41 ml more plasma than plasma units prepared by the platelet rich plasma (PRP) method. BC depletion also results in a 13 % loss of the donated red cells[7]. In PRP method of platelet preparation PRP is subjected to hard spin leading to formation of platelet pellet. Pelleting make close contact of platelets which make them activate temporarily[6]. Platelet activation during processing and throughout storage is accompanied by surface expression of sequestered granular membrane proteins (P-selectin and CD63) and conformational changes of the fibrinogen receptor, GPIIb/IIIa[7]. P-selectin (also known as CD62P, GMP140, and PADGEM) is an integral membrane protein found in α granule that becomes expressed on the surface of activated platelets after granule release [8]. In India when RDP is prepared by the BC method, it is done so, almost entirely from the whole blood stored at room temperature, for less than 8 hours as per DGHS guidelines.

The selection of preparation methods of PCs production should be based on availability of resources and its efficacy. As a result of different preparation methods there is substantial variability of the quality control of the products with regards to platelet content, residual leukocyte count, pH etc. Sampling for quality control provides early assurance that production is within specifications which were decided. Adequate quality control parameters gives assurance that the processing system in use maintains the integrity of a closed system .Non adherence to quality control parameters should be viewed as a potential processing problem and needs through investigation. In Europe there is approximately 50:50 split between the use of BC-PC and Apheresis PCs. In Canada 70% of PCs are derived from whole blood donation. Denmark, Finland and Netherlands prepare 85-95% of their platelet concentrates by the BC-PC method.[9]

This study was designed to investigate the effect of overnight storage of BCs on the preparation of PCs by the Buffy coat (BC) method, by evaluation of in vitro quality parameters of PCs like swirling, volume, platelet count and WBC count per bag and pH changes as per the recommended quality control parameters by DGHS, India.

MATERIAL AND METHODS:

The present study was carried out in the department of Transfusion Medicine, Sanjay Gandhi Postgraduate Institute of medical sciences, Lucknow over a period of two years from November 2013 to October 2015.The study protocol was approved by ethical committee of institute. A total of 60 platelet concentrates PC were included in the study.30 PCs prepared from fresh buffy coat (within 6 hours of whole blood collection) bags and 30 PCs prepared from overnight hanged buffy coat bags at room temperature.

Whole blood was collected from healthy donors in accordance with current Sanjay Gandhi Postgraduate Institute of medical sciences standard operating procedures. All donors gave written informed consent for participation in the study. Units were collected into top-and-top blood collection systems manufactured by Terumo Penpol Ltd, Puliyarakonam , Trivendrum, India (quadruple top and top blood pack unit, citrate-phosphate-dextrose [CPD] 63 ml, saline-adenine-glucose-mannitol [SAGM] 100ml, in 450 mL of whole blood .Collection was completed within 7 to 10 min to minimize platelet

activation and bacterial contamination .Then platelet concentrates were prepared from whole blood kept at room temperature (22-24 0C) by using buffy coat method.

Thirty whole blood was collected in a 450-ml quadruple bag containing 63 ml of CPD anticoagulant (TERUMO PENPOL, Ltd., Puliyarakonam, Trivandrum, India). The whole blood was first subjected to "hard spin" centrifugation (Cryofuge3000i, Thermo Scientific Germany) at 3100 RPM for 9 minutes at 22 °C with acceleration and deceleration curves of 9 and 4 respectively within 6 hour of collection. Whole blood was separated into following components according to their specific gravity: (1)The top layer-platelet poor plasma (120-160 ml),(2)Middle layer- buffy coat, containing approximately 90% of platelets, 70% of WBCs and 10% of red cells.(3)Bottom layer-packed red cells.

After centrifugation the whole blood was processed on Component Extractor (TACE-II TERUMO PENPOL-JAPAN). Platelet poor supernatant was expressed into one satellite bag and Buffy coat into another satellite bag. About 20-30 ml of plasma was returned to buffy coat with the aim of cleaning the tubing from residual cells and obtaining an appropriate amount of plasma in the BC. The SAGM solution was added to the red cells. The bags containing red cells and plasma were then removed .Red cells were placed at 4^0C and platelet poor plasma in to the blast freezer for processing as fresh frozen plasma (FFP). The Buffy coat was gently mixed with the plasma and hang for two hours and then again subjected to 'light spin" centrifugation at 900 rpm for 6 minutes at 22 °C, with acceleration and deceleration curves of 5 and 4 respectively, along with one empty satellite bag. The supernatant, platelet rich plasma was expressed into empty platelet storage bag and then tubing was sealed. This PC were stored at room temperature for one hour and then shifted to platelet agitator. Thirty PCs were prepared by the hanging the Buffy coat bags for overnight at room temperature (20-24^{0}C), in spite of 2 hour hanging period for fresh PCs. The quality assessment of platelet concentrates prepared from Buffy coat method fresh hang only for 2 hours and overnight hanged at room temperature, were performed by the following parameters:

- platelet concentrate volume
- Swirling
- Platelet count per bag
- White blood cells WBC count per bag
- pH changes
- Sterility
- Mean platelet volume (MPV)
- Platelet distribution width (PDW)

Exclusion criteria:

- Lipemic plasma in platelet concentrates
- Visible RBC contamination in platelet concentrates
- Transfusion transmitted infection reactive units
- Bacterial contaminated units

Sampling:

Sampling was done on day 1, 3 and day 5 from each type of PC after properly mixing the bag and proper stripping of segment. 2 ml of sample from platelet concentrates was collected in a K2 EDTA vial for platelet -count, WBC count, Ph, MPV, PDW and 10 ml for bacterial culture.

Statistical analysis

Statistical analysis was done by using SPSS 20 software .independent sample t test was used for comparing the mean of different parameters of different method like volume, platelet count, WBC count, pH, MPV, PDW. For comparison of storage effect on PC at the end of allowable storage time (5 days) of an individual method paired t test was used, p value < 0.05 was taken significant.

RESULTS:

Table 1 : Comparison of mean volume, platelet count per unit, mean WBC count/unit, mean MPV, mean PDW and mean pH of fresh BC-PC and overnight BC-PC

Parameters	Days	Fresh BC-PC n=30 (mean ± SD)	Overnight BC–PC n=30 (mean ± SD)	P-value
Volume(ml)		72.73±1.07ml	73.02±.95ml	0.730
Platelet count/unit (10e8)	1	664.82±46.71	770.33 ±39.78	0.089
	3	593.60±41.67	662.63±30.66	0.186
	5	489.79±31.41	614.83±31.31	0.006
WBC count/unit(10e6)	1	80.38±14.33	36.60±10.41	0.016
	3	20.86±3.21	16.72±2.85	0.339
	5	9.95±.1.6	8.40±.61	0.277
MPV(fl)	1	9.75±.28	10.14±.30	0.352
	3	9.80±.25	10.17±.26	0.309
	5	9.31±.33	9.86±.22	0.183
PDW(fl)	1	15.20±45	15.53±.44	0.609
	3	14.96±.39	15.70±.39	0.189
	5	14.87±.38	15.28±.36	0.442
pH	1	7.15±.01	7.35±.04	0.001
	3	7.11±.04	7.24±.04	0.034
	5	7.16±.04	7.07±.04	0.173

individual unit and scored as score 1 to 3 according to subjective observation. In fresh BC-PC grade 3 swirling was present in all PC units at day 1 and day 3, grade 3 and grade 2 swirling was present in 92% and 8% of PC units at day 5. Grade 3 swirling was present in 100% of overnight BC-PCs, both on day1 and 5. Statistically no significant difference between fresh BC-PC and overnight BC-PC units with regard to swirling was observed. As per the quality requirement according to DCGI, all the units of both the methods fulfill the criteria of presence of swirling at day 5 of storage, in all the platelet units.

DISCUSSION:

Various parameters have been studied by many researchers in the past to detect the effect of ambient temperature storage on quality of platelet concentrate. In the present study along with volume and sterility, parameters of platelet storage lesions like change in platelet count during storage, WBC contamination of PC, pH change, swirling and platelet indices like MPV and PDW were assessed.

REFERENCES:

1. N, de Korte D, Reesink HW, Dekker WJ, van den Ende A, Loose JA. Storage of whole blood for up to 24 hours at ambient temperature prior to component preparation. Vox Sang.1989 ; 56:45–50.
2. Thibault L, Beausejour A, de Grandmont MJ, Lemieux R, Leblanc JF. Characterization of blood components prepared from whole-blood donations after a 24-hour hold with the platelet-rich plasma method. Transfusion 2006 ; 46:1292-9
3. van der Meer PF, Pietersz RN. Overnight storage of whole blood: a comparison of two designs of butane-1,4-diol cooling plates. Transfusion 2007 ; 47:2038-43.
4. Hogman CF, Gong J, Eriksson L, Hambraeus A, Johansson CS. White cells protect donor blood against bacterial contamination. Transfusion 1991;31:620-6.
5. Sanz C, Pereira A, Vila J, Faundez AI, Gomez J, Ordinas A.Growth of bacteria in platelet concentrates obtained from whole blood stored for 16 hours at 22 degrees C before component preparation. Transfusion 1997;37:251-4.
6. Heaton A, Keegan T, Holme S. In vivo regeneration of red cell 2,3diphosphoglycerate following transfusion of DPG depleted AS-1, AS-3 and CPDA-1 red cells. Br J Haematol 1989 ; 71 : 131-6.
7. Levin E, Culibrk B, Gyongyossy-Issa MI, Weiss S, Scammell K, LeFresne W, Jenkins, Devine DV. Implementation of buffy coat platelet component production: comparison to platelet-rich plasma platelet production. Transfusion 2008; 48:2331-7.
8. Dijkstra-Tiekstra MJ, Kuipers W, Setroikromo AC, deWildt- Eggen J. Overnight or fresh buffy coat-derived platelet concentrates prepared with various platelet pooling systems. Transfusion 2008;48:723-30
9. Murphy S, Heaton WA, Rebulla P (1996) Platelet production in the Old World—and the New. Transfusion 36:751–754.
10. Perez-Pujol S, Lozano M, Perea D, Mazzara R, Ordinas A, Escolar G. Effect of holding buffy coats 4 or 18 hours before preparing pooled filtered PLT concentrates in plasma. Transfusion 2004 ; 44: 202-9.
11. GA Adams, SD Swenson and G Rock .Survival and recovery of human platelets stored for five days in a non- plasma medium.Blood 1986 67:672-675
12. Holme S1, Heaton A In vitro platelet ageing at 22 degrees C is reduced compared to in vivo ageing at 37 degrees C. ..Br J Haematol. 1995 Sep;91(1):212-8.
13. Scott.Murphy The efficacy of synthetic media in the storage of human platelets for transfusionTransfusion Medicine Reviews ,Volume 13, Issue 3, July 1999, Pages 153-163
14. S. Murphy MD L. Kagen S. Holme Platelet storage in synthetic media lacking glucose and bicarbonateVolume31, Issue1 Transfusion January 1991 Pages 16-20
15. Singh RP1, Marwaha N, Malhotra P, Dash S 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 Transfus Sci. 2009 Jul;3(2):86-94. doi: 10.4103/0973-6247.53882.
16. Rob Fijnheer et al In vitro Evaluation of Buffy-Coat-Derived Platelet Concentrates Stored in a Synthetic Medium Vox Sanguinis 60(1):16-22 • February 1991 16Transfusion. 1988 May-Jun;28(3):261-4.
17. Hirosue A1, Yamamoto K, Preparation of white-cell-poor blood components using a quadruple bag system.
18. R.N.L. Pieetersz. Pooled platelet concentrates. An alternative to single donor platelets. Transfusion and Apheresis science. 2009, 41,115- 119
19. Pietersz RN. Storage of whole blood for up to 24 hours at ambient temperature before component preparation: Implementation in the Netherlands. Transfusion 2011;51 Suppl 1:3S-6.
20. Dijkstra-Tiekstra MJ, van der Meer PF, Cardigan R, Devine D, Prowse C, Sandgren P, et al. Platelet concentrates from fresh or overnight – stored blood, an international study. Transfusion 2011;51 Suppl 1:38S-44.
21. Jerard Seghatchian et al Platelet storage lesion and apoptosis: are they related? February 2001Volume 24, Issue 1, Pages 103–105
22. Bertolini F1, Murphy S. A multicenter evaluation of reproducibility of swirling in platelet concentrates. Biomedical Excellence for Safer Transfusion (BEST) Working Party of the International Society of Blood Transfusion. Transfusion. 1994 Sep;34(9):796-801.
23. Abdulmajeed Albanyan Storage of platelets at 370 C accelerates platelet apoptosis: A

possible model for the platelet storage lesion Clinical Laboratory Sciences Department, College of Applied Medical Sciences, King Saud University, Saudi Arabia2013 | Volume : 4 | Issue : 2 | Page : 53-57
24. Dijkstra-Tiekstra MJ, van der Meer PF, Cardigan R, Devine D, Prowse C, Sandgren P, et al. Platelet concentrates from fresh or overnight – stored blood, an international study. Transfusion 2011;51 Suppl 1:38S-44.
25. Rácz Z, Baróti C. Storage of platelet concentrates from overnightstored blood and overnight-stored buffy coat: In vitro studies. Vox Sang 1995;68(3):160-3.
26. Tynngård N. Preparation, storage and quality control of platelet concentrates. Transfus Apher Sci 2009;41(2):97-104.