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Assessment of Qualitative and Quantitative Changes in 5 days stored Platelet Concentrates in a tertiary care Hospital of Bangladesh

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Abstract

Background: The fast growing demand for platelet concentrates (PC) necessitates the storage of these blood products prior to transfusion. Platelets are prepared as concentrates from the whole blood or by plateletpheresis. Qualitative and quantitative assessment of these PCs are an important issue in transfusion medicine. Aim of the study: To assess the qualitative, quantitative changes and bacteriological safety of 5 days stored platelet concentrates (PC). **Material & Methods:** This prospective study was conducted at the department of Clinical Pathology in collaboration with the department of Transfusion medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka during April 2008 to April 2009. A total of 65 healthy donors were included for the study as per the inclusion and exclusion criteria. Therefore, 65 platelet concentrates (bags/units) were prepared from the donors. Purposive sampling of the units was done. pH and platelet indices (PLT, MPV, PDW and P-LCR) were measured and Gram staining of PCs were performed on day 0 and 5. Statistical significant tests were done at 95% confidence interval using statistical package for social science (SPSS). **Results:** The mean (±SD) pH was 7.18±0.07 ranging from 7.0 to 7.3 during day 0. During day 5 the mean (±SD) pH was 6.77±0.11 and their range was from 6.5 to 7. The mean pH difference was statistically significant ($p<0.05$) between day 0 and day 5. The mean (±SD) PLT/unit was 70.56±15.56 x109/unit and it ranged from 38.01 to 110.6 x109/unit during day 0. During day 5 the mean (±SD) PLT/unit level was 68.46±15.52 x109/unit and it ranged from 36.82 to 107.2 x109/unit. The mean PLT/unit difference was statistically significant ($p<0.05$) between day 0 and day 5. The mean (±SD) MPV was 9.34±0.92 fl and it ranged from 7.5 to 11.5 fl during day 0. During day 5 the mean (±SD) MPV was 9.27±0.99 fl ranging from 7.0 to 11.2 fl. The mean (±SD) PDW was 10.07±1.61 fl and which ranged from 7.4 to 14.4 fl during day 0. During day 5 the mean (±SD) PDW was 10.72±1.71 fl ranging from 7.0 to 15.4 fl. The mean (±SD) PLCR was 18.28±5.67 % and it ranged from 8.0 to 32.5 % during day 0. During day 5 the mean (±SD) PLCR was 21.18±5.91 % and it ranged from 10.0 to 36.3 %. The mean PLT, PDW and PLCR difference were statistically significant ($p<0.05$) between day 0 and day 5 in unpaired t-test, however the mean MPV difference was not statistically significant ($p<0.05$) between day 0 and day 5. Gram staining of platelet concentrates on day 0 and day 5 found no bacteria. **Conclusions:** Storage-induced lesions take place in PCs, when stored for 5 days in second generation storage containers under the currently recommended conditions, but how far these change are clinically relevant need to be investigated.

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Keywords:- Platelet concentrates (PC), platelet indices (PLT, MPV, PDW and P-LCR), pH, Gram staining, storage-induced lesions.

INTRODUCTION

Platelet transfusions are being used increasingly in patients with thrombocytopenia to improve hemostatic function. Platelet transfusions were shown to reduce the risk of death from hemorrhage substantially during chemotherapy for leukemia. About 2 million PCs are transfused per year in the United States,[1] 2.9 million in Europe.[2] Many factors influence the quality of the platelets during storage. These include the preparation method of the platelets,[3] the plastic material of the storage bag and the ability of bags to exchange gas across its surface.[4,5] Other important factors that affect the quality are the storage temperature, the type of anticoagulant used and the platelet concentration in the bag. In the U.S.A, platelets derived from whole blood are produced by the platelet rich plasma (PRP) method, whereas the buffy-coat (BC) method is used in Europe.[6] Aging of platelets after in vitro storage at 220C is significantly slower than aging in vivo at 370C, a situation that makes long term platelet storage feasible.[7] Due to development of platelet storage bags and the formulation of platelet additive solution PCs can now be kept stored even beyond 5 days. Platelets have usually been stored for up to 5 days at room temperature with constant agitation, which is necessary for the maintenance of platelet viability.[4,8] Changes in pH in the PCs has been shown to effect platelet viability.[3,4,5] Recently, changes in platelet indices during storage of PC have been found to be useful parameters for monitoring the quality of PC.[] These indices are platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW) and platelet large cell ratio (PLCR). It has been shown that MPV is a reliable measure of residual platelet function in stored PC, an increased MPV representing deterioration of the product.[9] PDW is a measure of platelet volume heterogeneity and, together with MPV, provides a more complete description of the platelet volume distribution than MPV alone. Therefore, the present study was undertaken to compare and explore the changes in platelet indices, variation in pH and the bacterial safety of PC between 0 and 5 days storage period to ensure the maintenance of quality of the platelet concentrate at the end of expiry in a second generation bag with the recommended storage conditions.

OBJECTIVES

- General objective:
  - To assess the qualitative, quantitative changes and bacteriological safety of 5 days stored platelet concentrates (PC).
- Specific Objectives:
  - To estimate the platelet indices (PLT, MPV, PDW, PLCR) of PC on day 0 and day 5 of storage.
  - To measure the variation of pH of PC on day 0 and day 5 of storage.
  - To observe bacteriological contamination of the PC by Gram staining on day 0 and day 5 of storage

MATERIAL AND METHODS

This prospective study was conducted at the department of Clinical Pathology in collaboration with the department of

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Transfusion medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka during April 2008 to April 2009. A total of 65 healthy blood donors of both sexes were selected from the Department of Transfusion medicine, BSMMU, Dhaka as per the inclusion and exclusion criteria (according to National and World Health Organization (WHO) guidelines for donor selection). Platelet concentrates (bags/units) were then prepared from the donors. Purposive sampling of the units was done. After performing routine screening tests one unit of whole blood (450 ml) was collected in a triple bag system from each donor .The primary bag contains 63 ml of CPDA-1 as anticoagulant. The platelet bags used for this study consists of polyvinyl chloride (PVC) and plasticizer di(2-ethylheyl)phthalate (DEHP). The unit of whole blood was cooled to 20-240C within 2 hours and process thereafter for separation into components. PCs were prepared from whole blood by means of centrifugation. 1 ml of platelet concentrate was taken to perform Gram stain. Then the stained slide was first analyzed by bright field microscopy at 40x magnification. A minimum of 10 fields of each slide were examined microscopically for the presence of bacteria using the 100x (oil immersion) objective. The slides were scored as positive or negative for bacteria. 10 ml of PC sample was taken in a small glass beaker to measure the pH using a pH meter. Platelet indices such as PLT, MPV, PDW and P-LCR was measured using an automated cell counter. These laboratory procedures were performed on day 0 and day 5 and the results were compared. The student t –test was employed to estimate the difference between groups. Differences were considered to be highly significant when the probability, $p<0.001$. Statistical significant tests were done at 95% confidence interval using statistical package for social science (SPSS).

RESULTS

In the present study the age of the blood donors ranged from 19 to 45 years with mean (±SD) 28.2±6.3 years. Maximum 38(58.5%) donors were from 20-29 years age group [Table 1]. The male (83.1%) donors were predominant than female (16.9%) donors in this study [Figure 1]. The mean (±SD) PLT of the study donors was 317.8±50.7x 109/L and it ranged from 220 to 450 109/L, which were within the normal range [Table 2]. The mean (±SD) pH of PCs was 7.18±0.07 ranging from 7.0 to 7.3 during day 0. During day 5 the mean (±SD) pH was 6.77±0.11 and it ranged from 6.5 to 7.0. The mean pH difference of PCs was statistically significant ($p<0.05$) between day 0 and day 5 in unpaired t-test [Figure 2]. The mean (±SD) PLT/unit of PCs was 70.56±15.56 x109/unit and it ranged from 38.01 to 110.6 x109/unit during day 0. During day 5 the mean (±SD) PLT/unit level was 68.46±15.52 x109/unit and the PLT/unit ranged from 36.82 to 107.2 x109. The mean (±SD) PLT was 1027±238 (x109/L) and it ranged from 543 to 1627 (x109/L) during day 0. During day 5 the mean (±SD) PLT was 997±234 (x109/L) ranging from 526 to 1577 (x109/L). The mean (±SD) MPV was 9.34±0.92 fl and it ranged from 7.5 to 11.5 fl during day 0. During day 5 the mean (±SD) MPV was 9.27±0.99 fl ranging from 7.0 to 11.2 fl. The mean (±SD) PDW was 10.07±1.61 fl and it ranged from 7.4 to 14.4 fl during day 0. During day 5 the mean (±SD) PDW was 10.72±1.71 fl ranging from 7.0 to 15.4 fl. The mean (±SD) PLCR was 18.28±5.67 % and it

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ranged from 8.0 to 32.5 % during day 0. During day 5 the mean (±SD) PLCR was 21.18±5.91 % and it ranged from 10.0 to 36.3 %.The mean PLT, PDW and PLCR difference were statistically significant ($p<0.05$) between day 0 and day 5 in unpaired t-test, however the mean MPV difference was not statistically significant ($p<0.05$) between day 0 and day 5 [Table 4]. Gram staining of 65 platelet concentrates on day 0 and day 5 detected no bacteria.

Table 1: Age distribution of the donor (n=65)

Age in years	No of donor	Percentage (%)
<20	3	4.6
20-29	38	58.5
30-39	19	29.2
>39	5	7.7
Mean ±SD	28.2	±6.3
Range (Min-Max)	(19	-45)

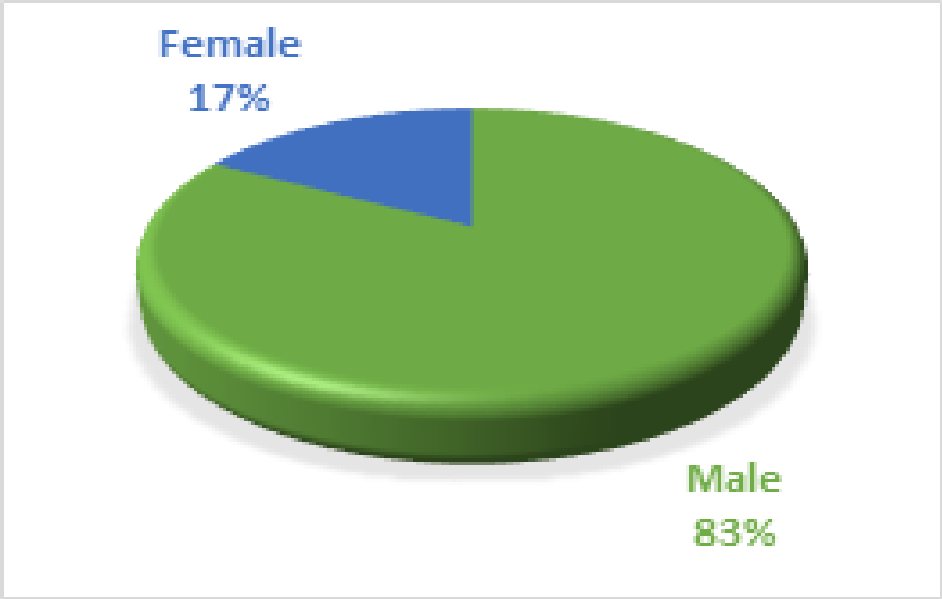

Figure 1: Pie diagram showing sex distribution of the study subjects

Table 2: Platelet count (PLT) of the study donor (n=65)

Donor PLT (x109/L)	No of donor	Percentage (%)
201-300	31	47.7
301-400	31	47.7
>400	03	4.6
Mean±SD	317.8	±50.7
Range (Min –max)	(220	-450)

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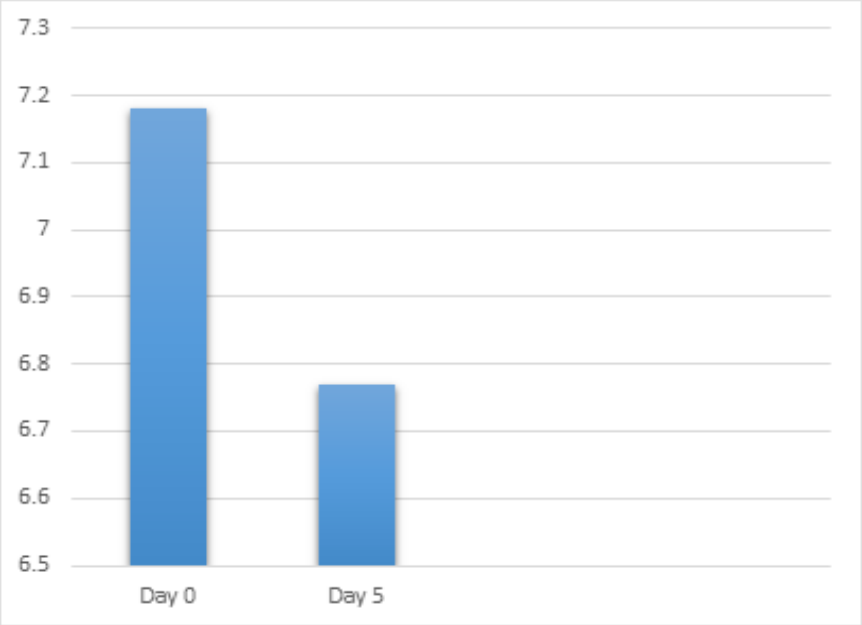

Figure 2: Bar diagram showing Comparison of pH of day 0 and day 5 of storage of PC

Table 3: Comparison of PLT /unit of day 0 and day 5 of storage of PC (n=65).

PLT/unit	Day 0		Day 5		P value
	n	%	n	%	
35-54 x109	7	10.8	11	16.9	
55-74 x109	36	55.4	34	52.3	
75-94 x109	15	23.1	15	23.1	
≥95 x109	7	10.8	5	7.7	
Mean±SD	70.56	±15.56 x109	68.46	±15.42 x109	0.002s
Range (min-max)	(38.01	-110.6)	(36.82	-107.2)	

Table 4: Platelet indices during storage of PC (n=65).

	Day 0	Day 5	P value
	Mean±SD	Mean±SD	
PLT	1027±238	997±234	0.001s
Range (min-max)	(543-1627)	(526-1577)	
MPV	9.34±0.92	9.27±0.99	0.660ns
Range (min-max)	(7.5-11.5)	(7.0-11.2)	
PDW	10.07±1.61	10.72±1.71	0.003s
Range (min-max)	(7.4-14.4)	(7.0-15.4)	
PLCR	18.28±5.67	21.18±5.91	0.001s
Range (min-max)	(8-32.5)	(10-36.3)	

ns= Not significant (p>0.05), s= significant (p<0.05).

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DISCUSSION

In this study, maximum number (38) of platelet donors were from age group 20-29 years, and they constituted 58.5% of the study donors. Minimum number (3) of donors were found in age group <20 years. Rahman,[10] also reported the maximum donors from 20-29 age groups (42.9%) among platelet donors in her study. Out of 65 donors, 54 (83.1%) were male and 11(16.9%) were female. Predominance of male donors are also in accordance with the study of Rahman.[10] Average platelet count of the donors in the present study was 317.8±50.7x10^9/L which ranged between 220 and 450x10^9/L. 31 donors had platelet count between 201-300 (x10^9/L) and another 31 donors had the count ranging 301-400 (x10^9/L).Only 3 donors had platelet count more than 400 x10^9/L. Rahman,[10] found this average donor platelet count as 317± 31.19x10^9/L) in her study. The pH of all units of PCs produced in the present study ranged from a mean value of 7.18 on day 0 to 6.77 on day 5. Over the course of the study the lowest pH value of PCs obtained was 6.5. Though, a significant drop in pH was observed after 5 days, these values were adhered to the AABB (American Association of Blood Bank) and FDA (Food and Drug Administration) standard of minimum pH (≥ 6.0). Sing et al,[11] found similar pH of PRP – PC after 5 days storage (6.70± 0.26). Rahman[10] in her study reported the fall of pH to 6.63 in day 3. As per the Drugs and Cosmetics Act of India, minimum pH should not be <6 at any given day of storage (Singh, Chaudhary & Ray).[12] In this present study, the mean (±SD) PLT/unit was 70.56±15.56 x10^9/unit and it ranged from 38.01 to 110.6 x10^9/unit during day 0. During day 5 the mean (±SD) PLT/unit level was 68.46±15.52 x10^9/unit and the level ranged from 36.82 to 107.2 x10^9/unit. The mean PLT/unit difference was statistically significant ($p<0.05$) between day 0 and day 5 in unpaired t-test. This result coincides with the result of Singh.[12] Out of 65 units of day 0 PCs 58 units (89.23%) contain ≥55x10^9 platelets per bag and approximately 86% (56 out of 65 units) of the PCs of day 5 contained ≥55x10^9 (AABB recommended count) platelets per bag. Though the platelet count decreases significantly on day 5 it still remains in fairly acceptable range. In the present study, MPV was found 9.34fL on day 0 which decreased to 9.27 fL on day 5. Gradual decrease in MPV is caused by discoid/ spherical conversion and microvesiculation (Seghatchian).[13] Changes in PDW in 5 days stored PCs are found significant in this study. PDW increased to 10.72 fL on day 5 from the day 0 value of 10.07 fL indicating the increase in platelet anisocytosis. PLCR is a measure of large cell percentage among the platelets. Significant changes were found in PLCR of day 0 (18.28%) and day 5 (21.18%) in this study. Singh,[12] observed the similar results. In the present study, Gram stain method was employed to screen the PCs on day 0 and day 5. Although the bacterial load necessary for consistent detection by the Gram stain is very high, this method is capable of detecting clinically significant levels of contamination in platelet concentrates. Lesser amounts of bacterial contamination are thought to be tolerated and/or cleared by the patient's immune system (Steen et al).[14] However, no bacteria was detected in the 65 PCs (neither in day 0 nor in day 5) in the present study. In fact, storing the PCs beyond 5 days increases the

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risk of bacterial contamination and therefore extension of platelet storage is not recommended by FDA (Slichter).[15]

Limitations of the Study

The major difficulty encountered during this study was the unavailability of sufficient amounts of platelets which could be made available for this study and which limited the supply of samples. Measurement of pH with pH meter instead of arterial blood gas analyzer is another limitation of this study.

CONCLUSIONS

It is almost certain that platelet storage for 5 days shows detriment in present study, but they are up to certain extent maintaining some internationally accepted values. In conclusion, storage-induced lesions take place in PCs, when stored in second generation storage containers under the currently recommended conditions, but how far these change are clinically relevant need to be investigated. Although this study shows that significant qualitative and quantitative changes occur in 5 days stored platelet concentrates it still needs further research to be performed to determine platelet viability in vivo once transfused.

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