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Assessment of Qualitative and Quantitative Changes in 5 days stored Platelet Concentrates in Bangabandhu Sheikh Mujib Medical University

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Abstract

Background: The fast-growing demand for platelet concentrates (PC) necessitates the storage of these blood products before transfusion. Platelets are prepared as concentrates from the whole blood or by plateletpheresis. Qualitative and quantitative assessment of these PCs is an important issue in transfusion medicine. To assess the qualitative, quantitative changes and bacteriological safety of 5 days of 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 from April 2008 to April 2009. A total of 65 healthy donors were included in 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 was performed on days 0 and 5. Statistical significant tests were done at a 95% confidence interval using the 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. On 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 $x10^9$/unit and it ranged from 38.01 to 110.6 $x10^9$/unit during day 0. On day 5 the mean (±SD) PLT/unit level was 68.46±15.52 $x10^9$/unit and it 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. 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 differences were statistically significant ($p<0.05$) between day 0 and day 5 in the 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 changes are clinically relevant needs to be investigated.

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

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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 their 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] Ageing of platelets after in vitro storage at 22°C is significantly slower than ageing in vivo at 37°C, a situation that makes long-term platelet storage feasible.[7] Due to the 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 have been shown to affect platelet viability.[3,4,5] Recently, changes in platelet indices during storage of PC are useful parameters for monitoring the quality of PC.[9] 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, with an increased MPV representing a 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 the 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 of 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 Transfusion Medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka from April 2008 to April 2009. A total of 65

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Page no- 159-165 | Section- Research Article (Pathology)

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 an 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 20-24°C within 2 hours and processed there after for separation into components. PCs were prepared from whole blood using centrifugation. 1 ml of platelet concentrate was taken to perform a 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 were 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 a 95% confidence interval using the statistical package for social science (SPSS).

RESULTS

In the present study, the age of the blood donors ranged from 19 to 45 years with a mean (±SD) of 28.2±6.3 years. A maximum of 38(58.5%) donors were from the 20-29 years age group [Table 1]. The male (83.1%) donors were more predominant than female (16.9%) donors in this study [Figure 1]. The mean (±SD) PLT of the study donors was 317.8±50.7x 10^9/L and it ranged from 220 to 450 x 10^9/L, which was 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. On 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 the unpaired t-test [Figure 2]. The mean (±SD) PLT/unit of PCs 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 PLT/unit ranged from 36.82 to 107.2 x10^9. The mean (±SD) PLT was 1027±238 (x10^9/L) and it ranged from 543 to 1627 (x10^9/L) during day 0. During day 5 the mean (±SD) PLT was 997±234 (x10^9/L) ranging from 526 to 1577 (x10^9/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 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 differences were statistically significant ($p<0.05$) between

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Page no- 159-165 | Section- Research Article (Pathology)

day 0 and day 5 in the 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)	

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

Donor PLT ($x10^9$ /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)	

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**	**%**	**0.002s**
35-54 $x10^9$	7	10.8	11	16.9	
55-74 x 10^9	36	55.4	34	52.3	
75-94 $x10^9$	15	23.1	15	23.1	
≥95 $x10^9$	7	10.8	5	7.7	
Mean±SD	70.56±15.56 $x10^9$		68.46±15.42 $x10^9$		
Range (min-max)	(38.01-110.6)		(36.82-107.2)		

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

Variables	**Day 0**	**Day 5**	**P value**
	Mean±SD	**Mean±SD**	
PLT ($x10^9$) Range (min-max)	1027±238 (543-1627)	997±234 (526-1577)	0.001s
MPV (fL) Range (min-max)	9.34±0.92 (7.5-11.5)	9.27±0.99 (7.0-11.2)	0.660ns
PDW (fL) Range (min-max)	10.07±1.61 (7.4-14.4)	10.72±1.71 (7.0-15.4)	0.003s
PLCR (%) Range (min-max)	18.28±5.67 (8-32.5)	21.18±5.91 (10-36.3)	0.001s

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

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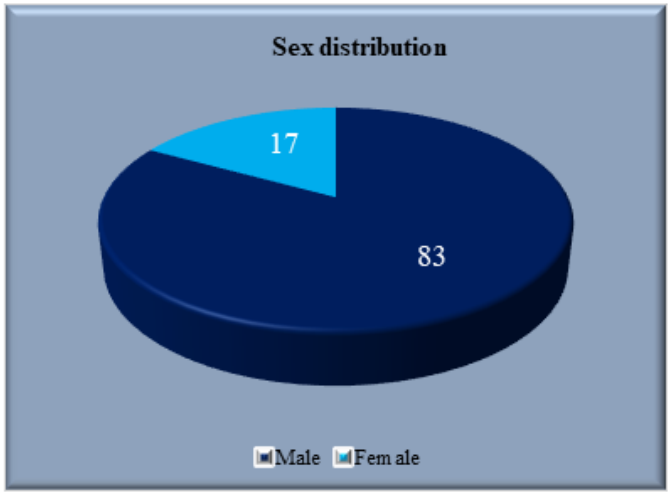

Figure 1: Pie diagram showing sex distribution of the study subjects

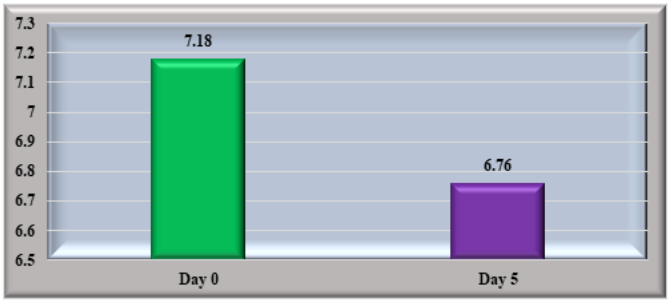

Figure 2: Bar diagram showing Comparison of pH of day 0 and day 5 of storage of PC

DISCUSSION

In this study, the maximum number (38) of platelet donors were from the age group 20-29 years, and they constituted 58.5% of the study donors. The minimum number (3) of donors were found in the age group <20 years. Rahman,[10] also reported the maximum number of 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. The predominance of male donors is also in the study of Rahman.[10] The 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 counts between 201-300 (x10^9/L) and another 31 donors had a count ranging from 301-400 (x10^9/L). Only 3 donors had a platelet count of 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. Throughout 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 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 of storage (6.70+ 0.26). Rahman10 in her study reported the fall of pH on 6.63 on day 3. As per the Drugs and Cosmetics Act of India, the minimum pH should not be <6 on 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 on day 0. On 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 the 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 counts decrease significantly on day 5 it remains in the fairly acceptable range. In the present study, MPV was found at 9.34 fL on day 0 which

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Page no- 159-165 | Section- Research Article (Pathology)

decreased to 9.27 fL on day 5. The 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 the PLCR of day 0 (18.28%) and day 5 (21.18%) in this study. Singh,[12] observed similar results. In the present study, the 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 were detected in the 65 PCs (neither on day 0 nor on day 5) in the present study. Storing the PCs beyond 5 days increases the 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 the present study, but they are up to a 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 this change is clinically relevant needs to be investigated. Although this study shows that significant qualitative and quantitative changes occur in 5 days of stored platelet concentrates it still needs further research to be performed to determine platelet viability in vivo once transfused.

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Page no- 159-165 | Section- Research Article (Pathology)

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