Original Article

Comparison of changes in platelet count, mean platelet volume and swirling in stored platelet concentrates with and without platelet additive solution

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ABSTRACT

Background: Stored platelet concentrates (PC) are in increasing demand for transfusion to patients with thrombocytopenia or disordered platelet function with active bleeding. Platelets are difficult to preserve *in vitro* for longer period of time. Different platelet additive solutions (PAS) have been formulated which can increase the survival period of PC during storage. **Study Design:** Comparative study. **Materials and Methods:** The study was carried out to compare *in vitro* changes in platelet indices [platelet count and mean platelet volume (MPV)] and extent of swirling in stored PC with and without PAS stored for 10 day. **Results:** This study included 42 samples of random donor platelet concentrates, divided into two groups. In one group, PC were stored without PAS while in other group PAS was added to PC before storage. Changes in platelets count and MPV were monitored by using automated hematology cell analyzer, in both groups on day 0, 3, 5, 7, 8, 9, and 10. Bacterial cultures were also applied to detect bacterial contamination of PC. The results showed that mean platelet count of PC stored without PAS was 5.6 + 0.1 x 1010/L on day 0 and it was 5.5 + 0.5 x 1010/L in PC stored with PAS. The difference between the two groups started becoming statistically significant by day 7. Platelet count was significantly lower in PC without PAS as compared to the PC stored with PAS (*P* value ≤ 0.001). The difference in the mean platelet volume (MPV) between two groups was highly significant during 10 days storage (*P* value ≤ 0.001). Swirling was better seen in PC stored in PAS as compared to PC stored without PAS during 10 days storage. Blood cultures were applied on 42 samples on days 0, 5 and 10. Bacterial contamination occurred in few samples in both groups, those samples were not processed further. **Conclusion:** This study showed that the use of PAS in platelet concentrate storage bags increased the shelf life and viability of platelets as compared to those without PAS.

Key words: Apheresis platelets, platelet concentrates, platelet components

INTRODUCTION

Platelets play a very vital role in hemostasis.[1] Hence the therapeutic storage of platelets is a serious problem for transfusion medicine as platelets are given prophylactically and treatment of hemorrhage in patients with thrombocytopenia or with functional disorders of platelets.[2] Platelets are usually separated from whole blood. They are stored in donor plasma at 22°C for use in transfusion therapy.[3] Several studies have shown that platelet concentrates (PC) maintain an adequate function for 5 days and thereafter a progressive loss of platelet viability and function occur during platelet storage.[4] Platelets storage remains a major challenge to transfusion services. Technologies and strategies are desired that allow extended storage without causing an unacceptable loss of product quality.[5]

During the storage period, the PC undergo biochemical, structural and functional changes, known as platelet storage lesions (PSL)[6] have a negative impact on the post-transfusion functional capacity of platelets.[7]

Platelets indices such as the platelet count, mean platelet volume (MPV) and platelets distribution width are used as a marker for maintaining quality control of PC and also considered representative of storage induced shape changes in platelets.[8] The assessment of MPV is an imminent test for studying the PSL as MPV correlates with morphological changes that occur during storage of PC.[7] Platelets that retain their shape *in vivo* at the time of transfusion are expected

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to be functionally better *in vivo*. Swirling can also be considered as a good test for determination of shape change[9] and its absence is highly predictive of poor post-transfusion increments (Bertolini *et al*., 1992).[10]

In order to increase the survival period of platelets, different platelet additive solutions (PAS) have been formulated [Table 1]. The presence of glucose is essential for platelet metabolism. Acetate reduces the production of lactate by the platelets and by the formation of bi-carbonates, it maintains stable pH during storage (Gulliksson *et al*., 2007).[11] It is seen that saline maintains osmolarity of PAS.[6] Studies have shown that potassium and magnesium have an inhibitory effect on platelet activation and metabolism.[12] The role of gluconate is not clear and some authors have shown that gluconate does not have any beneficial effect on the buffering capacity of platelets.[13] Addition of PAS to platelets is carried out after removing 60-80% of plasma. PAS can be prepared by the addition of different compositions of sodium, potassium, magnesium, acetate, citrate, gluconate and phosphate.[14] At present shelf life of the recommended PC without PAS under appropriate conditions in vitro is 3-5 days while several studies show that use of PAS can enhance this shelf life to 9-14 days.[15,16]

The present study was carried out to assess the changes in some of the in vitro parameters of PC stored for 10 days with and without PAS. Random donor platelets with prolonged and improved shelf life will help to meet logistics support of increased platelet availability for clinical transfusions.

MATERIALS AND METHODS

Sample Collection

The present study was conducted on samples of forty two blood donors, both male and female donors. These donors were selected after a complete medical history and examination. Platelet donors having negative screening test for transfusion transmitted infections such as malaria, hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV) and syphilis were included in the study.

Table 1: Composition of different PAS (mmol/l)

Chemicals	Plasmalyte A	PAS II	PAS III	PAS IIIM	Composol
NaCl	90	115.5	77.3	69.3	90
KCl	5	-	-	5	5
MgCl	3	-	-	1.5	1.5
Na citrate	-	10	10.8	10.8	27
Na acetate	27	30	32.5	32.5	27
Na phosphate	-	-	28.2	28.2	-
Na gluconate	23	-	-	-	23

This table is adopted from Tyngard., 2008. PAS=Platelet additive solutions

Method of Preparation of PC

All the PC were obtained from the whole blood by routine phlebotomy into 450 mL bags containing 63 mL of citrate phosphate dextrose A1 anticoagulant in triplet bags (JMS PTE Ltd, Singapore).

PC were prepared from platelet-rich plasma method within 4-6 h of collection. Platelet rich plasma was separated from whole blood by light spin centrifugation at 2500 rpm for 3 min at 20°C. Two groups of PC (21 PC in each group) were formed: In group I approximately 35-50 mL of plasma was removed and an equal volume of PAS was added. Random donor PC were stored in PAS (Plasmalyte A) which was prepared and standardized using the following constituents: NaCl (Honeywell Riedel-de-Haen. Germany), MgCl (Honeywell Riedel-de-Haen Germany), KCl (Merck Millipore Chemicals Pakistan), Na gluconate (Merck Millipore Chemicals, Pakistan) and Na acetate (Honeywell Riedel-de-Haen, Germany). The pH of PAS was kept at 6.8-7.2. While in Group II., samples were left without removing plasma and without the addition of PAS.[17]

All of these PC were kept in a platelet incubator (HELMER Company, China) at 22°C and were constantly agitated for 10 days. A volume of 3 ml of sample was taken from both groups on day 0, 3, 5, 7, 8, 9 and 10 to record study parameters of total platelet count, MPV and Swirling. The platelet count and MPV were evaluated by automated cell counter (SYSMEX KX-21, Sysmex Corporation Kobe, Japan). Platelet swirling was observed by using 50-100 watt white light source. The results of platelet swirling test were scored as score 0-3.

Score 0: Turbid, Score 1: Swirling only in some part of the bag, Score 2: Clear homogenous swirling in all part of the bag and Score 3: Very clear homogenous swirling in all part of the bag.[18]

For microbiological cultures additional 5 mL sample was taken to observe bacterial contamination of PC. Aerobic culture was performed on all the samples on the day on day 0, 5 and 10 using fully automated culture system, BACTEC (BACTEC System 9240, Beton Dicknson and Company, United States).

Statistical Analysis

The data was entered and analyzed using PASW 18.0 (formerly known as SPSS) (Predictive Analytics Software). Continuous data was expressed as mean ± standard deviation and categorical data was expressed in frequencies and percentages. The independent-*t*-test

was applied to compare the PC in both groups on day 0, 3, 5, 7, 8, 9 and 10. Analysis of variance (ANOVA) for repeated measures was also used to analyze the data. $P \leq 0.05$ was considered to be statistically significant.

RESULTS

The results showed that the mean platelet count in random donor PC stored without PAS was 5.6 ± 0.1 × 10^{10}/L on day 0 which decreased noticeably to 3.3 ± 0.7 × 10^{10}/L by day 10. Whereas the mean platelet count in PC stored with PAS was 5.5 ± 0.5 × 10^{10}/L on day 0 and was only reduced to 4.8 ± 0.5 10^{10}/L by day 10 [Table 2]. The difference between two groups was highly significant (0.001). This difference was also observed with repeated measures using ANOVA as shown in the Figure 1.

MPV in random donor PC stored without PAS was 8.0 ± 0.1 fL on day 0 and was found to increase considerably to 13.0 ± 0.4 fL on day 10. On the other hand, the MPV in PC stored with PAS was maintained during 10 days storage with statistically significant difference of 0.001 between two groups [Table 3]. This difference was also appreciated by using ANOVA as shown in the Figure 2.

The swirling results showed that it was better observed during 10 days storage in PC with PAS when compared to PC without PAS [Table 4].

DISCUSSION

The limited life span of PC under present storage condition is due to possible risk of bacterial contamination and development of PSL.[19] During the last 2 decades, a lot of work has been done on PAS to increase the shelf life of stored platelets.[20]

The present study was undertaken to compare the PC stored in PAS with those stored without PAS and

Table 2: Platelet count with and without PAS (10^{10}/L)

Platelet storage days	Platelet count		*P* value
	Without PAS	With PAS	
Day 0 (baseline)	5.6±0.1	5.5±0.5	0.42
Day 3	5.4±0.2	5.3±0.6	0.79
Day 5	5.0±0.3	5.2±0.7	0.43
Day 7	4.5±0.4	5.2±0.4	0.001
Day 8	4.1±0.6	5.1±0.5	0.001
Day 9	3.5±0.8	4.8±1.4	0.01
Day 10	3.3±0.7	4.8±0.5	0.001

Results shown as mean±SD of samples with or without PAS by using independent *t*-test. SD=Standard deviation; PAS=Platelet additive solutions

Table 3: MPV with and without PAS (fl)

Platelet storage days	MPV		*P* value
	Without PAS	With PAS	
Day 0 (baseline)	8.0±0.1	7.8±0.1	0.08
Day 3	8.4±0.2	8.5±1.0	0.6
Day 5	9.4±0.64	8.7±1.1	0.06
Day 7	11.5±0.8	8.8±1.1	0.001
Day 8	12.0±1.0	8.9±1.0	0.001
Day 9	12.2±1.0	9.1±1.0	0.001
Day 10	13.0±0.4	9.6±1.4	0.001

Results shown as mean±SD of samples with or without PAS by using independent *t*-test. SD=Standard deviation; PAS=Platelet additive solutions; MPV=Mean platelet volume

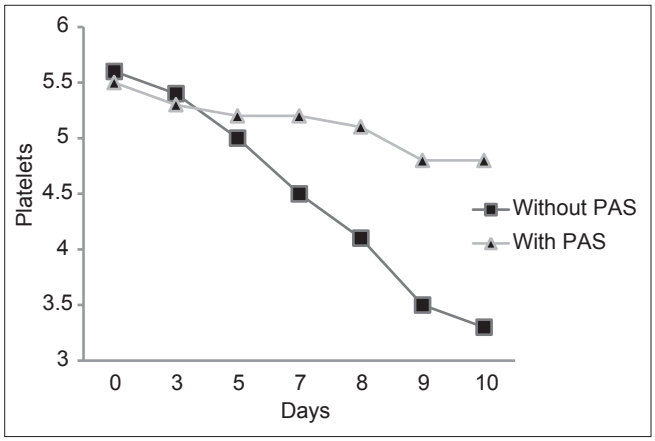

Figure 1: Comparison of platelet count in random donor platelet concentrates in two groups (with and without platelet additive solutions) stored for 10 days. This graph compares the mean values of platelet count between two groups on day 0, 3, 5, 7, 8, 9 and 10 respectively. The difference between two groups during 10 days of repeated measures using ANOVA was not significant till day 5 but it started becoming significant by day 7

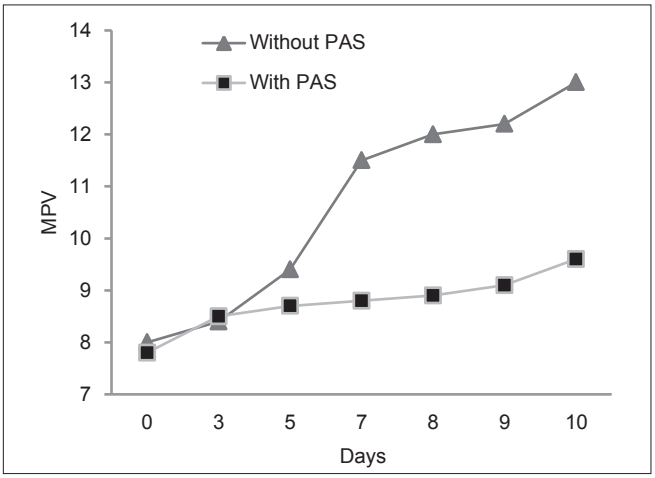

Figure 2: Comparison of mean platelet volume in random donor platelet concentrates in two groups (with and without platelet additive solutions) stored for 10 days. This graph compares the mean platelet volume between two groups on day 0, 3, 5, 7, 8, 9 and 10 respectively. The difference between two groups during 10 days of repeated measures using ANOVA was not significant till day 5 but the difference started becoming significant by day 7

Table 4: Percentage of swirling without and with PAS

Platelet storage days	Without PAS (%)				With PAS (%)			
	Score 0	Score 1	Score 2	Score 3	Score 0	Score 1	Score 2	Score 3
Day 0	0 (0)	0 (0)	3 (19)	13 (81)	0 (0)	0 (0)	2 (13)	14 (87)
Day 3	0 (0)	0 (0)	16 (100)	0 (0)	0 (0)	0 (0)	16 (100)	0 (0)
Day 5	0 (0)	1 (6)	15 (94)	0 (0)	0 (0)	0 (0)	16 (100)	0 (0)
Day 7	0 (0)	3 (19)	13 (81)	0 (0)	0 (0)	0 (0)	16 (100)	0 (0)
Day 8	0 (0)	16 (100)	0 (0)	0 (0)	0 (0)	0 (0)	16 (100)	0 (0)
Day 9	16 (100)	0 (0)	0 (0)	0 (0)	0 (0)	2 (13)	14 (87)	0 (0)
Day 10	16 (100)	0 (0)	0 (0)	0 (0)	0 (0)	4 (25)	12 (75)	0 (0)

PAS=Platelet additive solutions

to investigate whether the use of PAS increases the viability and storage time of PC. Optimized synthetic storage media might help to attenuate the PSL and to facilitate its extended storage.[21]

Our data shows decreasing platelet count in PC of both groups during 10 days storage but with significantly increased reduction of platelets in concentrates stored without PAS. Hornsey also observed decreased platelet counts when platelets were stored in plasma as compared to PAS during 9 days storage.[15] Similar results have been reported by van der Meer *et al*., 2004.[6] Findings, contrary to our were also observed by Gupta in his study which showed no significant difference in platelet counts when PC were stored in plasma or PAS.[21] Another study confirmed our results showing progressive decrease in platelet survival in both group with increasing storage time whether the platelets were stored in PAS or plasma, but platelets were found to be better maintained in PAS.[22]

Our study presented here revealed that MPV is increased in PC of both groups, but MPV in platelets stored in PAS was comparatively better sustained than those PC stored in plasma. This is inconsistent to findings of studies conducted earlier observing in vitro quality of platelets stored with and without PAS. Production occurred. Hornsey *et al*.[15] observed that the in vitro quality of the platelets stored in SSP^+ was maintained until day 9. Glucose was depleted by day 12, a rapid fall in pCO_2, a rise in pO_2 and a cessation of lactate production adenosine triphosphate and bicarbonate concentrations fell and the platelets began to swell and MPV increased and the ability to swirl decreased (van der Meer *et al*., 2004, Hornsey *et al*., 2006).[6,15] Where as in a study carried out by Sandgren *et al*.[23] they failed to observe any significant difference in MPV during 9 days storage of PC in both plasma and PAS of InterSol.[23]

Platelet discoid shape correlates with in vivo viability after transfusion.[24] Stored PC can be monitored for the loss of discoid shape before they are transfused and for this swirling is a reliable method of quality control as it correlates with platelet function.[9] Consumption of glucose causes reduction of pH leading to swelling of platelets. The PC slowly become turbid showing decreased swirling.[6] Our results of swirling were scored and it was observed that the swirling score was much better in all the PC stored in PAS as compared to plasma. Similar results were also reported by two other groups.[12] Blackwell Publishing Ltd.

We applied cultures on PC stored in both plasma and PAS on day 0, 5 and 10 to see the contamination in samples during storage. As contamination could alter the results such as the platelet count and other parameters which were observed in this study, so these contaminated samples (found in equal number in both groups) were excluded from further evaluation. Bacterial contamination was also observed by other study groups during their storage at room temperature.[25,26] It has been concluded in a study that if platelet storage has to be extended beyond 5 days it would require an appropriately validated bacterial detection system. Such system would not only involve in vitro testing with pooled products but should also include trials in clinical surroundings.[27]

Based on the results of our study during 10 days of platelet storage, the shelf life of the PC can be increased to at least 10 days in PAS as compared to plasma where life span of platelets is limited to only 5 days. PAS have been developed as a substitute to plasma which can increase the survival of platelets.[11] The most probable explanation for this extended life period of platelet in PAS could be that the carried over plasma after addition of PAS provides glucose for platelet metabolism in addition to acetate present in PAS, which serves as a second metabolic fuel. Magnesium and potassium present in PAS inhibit platelet activation and aggregation and also plays a vital role in maintaining the platelet membrane potential.[21]

CONCLUSION

We conclude from our study results that platelet morphology and other parameters were better maintained in PC stored in PAS. Significantly less

decrease in platelet counts and comparatively slight increase in MPV were observed in PC stored with PAS when compared to PC without PAS.

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