Original Article

Comparative Evaluation of Quality Parameters of Platelet Stored in Additive Solution versus Plasma

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Abstract

Introduction: Extension of the shelf life of platelets remains a challenge for transfusion services, and efforts techniques are required to extend the shelf life of platelets beyond 5 days without compromising their qualities. **Materials and Methods:** The study is being done to compare *in vitro* changes of platelet indices-platelet count, mean platelet volume, platelet distribution width (PDW), pH and swirling in stored platelet concentrate (PC) with and without platelet additive solution (PAS) for 0,3,5,7 and 10 days. **Results:** Serial measurements of various parameters in PCs with and without PAS showed that PCs stored in PAS were better maintained and had optimal quality standards throughout the extended storage time as compared to the PCs without PAS. The results obtained in both categories were statistically significant ($P < 0.001$). **Conclusion:** Our study showed that the use of PAS in the PCs increased the shelf life and improved the viability of platelets as compared to the PCs without PAS.

Keywords: Additive solution, plasma, platelet concentrate

Introduction

Platelets were first identified in the year 1881,[1] and the first effort to increase the platelet counts in cases of thrombocytopenia by transfusion of whole blood was described by Duke in the year 1910.[1] General improvement of the technique to separate platelets from whole blood and availability of plastic bags in blood banking revolutionised the field of components therapy.

During the storage period, the platelet concentrate (PC) undergoes biochemical, structural and functional changes, which is collectively termed as platelet storage lesion (PSL)[2] and has a negative impact on the post-transfusion increment. Platelet indices such as the platelet count, mean platelet volume (MPV), PDW and PC are considered as representative of storage-induced shape changes in PC along with the swirling test.[3] Swirling test is used for detecting variation in shape, and its absence is highly predictive of poor post-transfusion PC increments.[4]

The occurrence of PSL is multifactorial, including the methods of collection, processing, storage and transportation after collection can result in PSL. These lesions are associated with decreased *in vivo* platelet recovery, survival and haemostatic activity after transfusion.[5]

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Aims and objectives

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 platelet additive solutions (PASs).

The aims and objective of the study were:

1. To study and compare the various morphological parameters of platelets with and without PAS on days 0, 3,5,7 and 10
2. To study and compare the pH values and sterility of platelets with and without PAS on days 0, 3,5,7 and 10.

Materials and Methods

The study sample included 130 voluntary blood donors who were selected as per the director general of health services criteria.[6] Pre-donation counselling and a medical examination

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were done, and those who did not qualify were deferred. Blood grouping of the donors was done by the ORTHO AutoVue Innova System (ortho clinical diagnostics [OCD]), a division of Johnson and Johnson Limited, USA. The study was cleared by the institutional review committee (KIM/2019/IC.234).

All the blood units were screened for human immunodeficiency virus, hepatitis B virus and hepatitis Cvirus by chemiluminescence, VITROS ECIQ OCD and malaria by enzyme-linked immunosorbent assay, (Qualpro Diagnostics Limited).

Random donor platelet preparation

Whole blood was collected in 450 ml bags containing 63 ml of Citrate-phosphate-dextrose solution with adenine (CPDA) anticoagulant, kept at room temperature (20°C–24°C), and PC was prepared by platelet-rich plasma (PRP) method within 6 h of collection. The donor's arm was selected, and blood pressure (BP) was raised by a BP cuff to locate and select a prominent vein for phlebotomy. The donor's arm was prepared by cleaning the venepuncture site at the antecubital fossa starting from centre to periphery of the selected area by the spirit and betadine. The phlebotomy was done, and pressure was set between 40 and 60 mmHg with a continuous pressing of the sponge ball by the donor's arm to maintain optimum blood flow. The entire procedure was completed within 8 min under medical supervision and 450 ml volume of blood collected (blood collection monitors, TERUMO PENPOL, Ltd., Trivandrum, India) in triple bags containing CPDA-1 anticoagulant (Hindustan Life Care Ltd., Kerala, India).

PRP was separated from whole blood by light spin centrifugation at 1750 rpm for 11 min at 21°C, with acceleration and deceleration curves of 5 and 4 min, respectively (Heraeus Kendro, Hanau, Germany 6000i).

From PRP, the PC was concentrated by heavy spin centrifugation at 3940 rpm for 5 min at 21°C with acceleration and deceleration curves of 9 and 5 min, respectively, along with subsequent discarding of supernatant plasma.

PC so prepared was divided into two parts by a sterile tubing welder (Terumo TSCD, SC-201 AH, Leuven, Belgium) and one portion of random donor platelets (RDPs) stored in storage solution for platelets (SSP+) (Macopharma India Transfusion Solution Private Limited, Span HealthCare Private Limited).

8 ml of PC together with 10–15 ml of residual plasma was mixed with PAS (SSP+) to give a mixture with an approximate final concentration of 80% additive solution and 20% plasma.

The volume of additive solution and stored platelets had a mean volume of around 50 ml. PAS contains Na_3-citrate $2H_2O$: 3.18 g, Na-acetate $3H_2O$: 4.42 g, $NaH2PO_4$ $2H_2O$: 1.05 g, Na_2HPO_4:3.05 g, KCl: 0.37 g, $MgCl_2$ $6H_2O$: 0.30 g and NaCl: 4.05 g at pH 7.2.

RDPs were placed in a platelet agitator (Terumopenpol Private Limited) with continuous agitation at 70 cycles/minutes during storage at 22°C. 3 ml of the sample was taken from both the groups (PC with and without additive solution) on days 0, 3, 5,7 and 10 to study the platelet indices and swirl.

The platelet count, MPV, platelet distribution width (PDW) and platelet-larger cell ratio (P-LCR) were evaluated by automated cell counter (SYSMEX KX-21 Sysmex Corporation Kobe, Japan).

Platelet swirling was done and scored as Score 0: Turbid, Score 1: Swirling only in some part of the bag, Score 2: Clear homogenous swirling in all parts of the bag and Score 3: Very clear homogenous swirling in all parts of the bag.

For microbiological cultures, additional 5 ml sample was taken to observe bacterial contamination of PC. Aerobic and anaerobic cultures were performed on all the samples on the day, on days 0, 5 and 10 using a fully automated culture system, BACTEC (BACTEC system 9240, Becton Dickinson and Company, United States).

Results

Some of the morphological parameters included in this study are (a) MPV which is an analyser–calculated measures of thrombocyte volume and expressed as femtolitres (FL). (b) PDW is an indicator of volume variability in platelets size and expressed as percentage (%). (c) P-LCR, which is an indicator of larger (>12FL) circulating platelets and expressed as a percentage (%). Table 1 shows platelet count comparison between with and without PAS. There was no significant difference in mean platelet count between the two methods at different periods.

Table 1: Platelet count comparison between with and without platelet additive solution

Storage period	Group, mean (SD)		*P*
	Platelet count without PAS	Platelet count with PAS	
Day 0	478.0 (156.7)	468.4 (192.3)	0.659
Day 3	487.4 (206.7)	457.0 (217.7)	0.250
Day 5	473.9 (192.7)	465.2 (221.6)	0.734
Day 7	463.9 (189.4)	420.6 (199.3)	0.074
Day 10	407.5 (208.8)	397.1 (224.0)	0.700

PAS: Platelet additive solution, SD: Standard deviation

Table 2: Mean platelet volume comparison with and without platelet additive solution

Storage period	Group, mean (SD)		*P*
	MPV without PAS	MPV with PAS	
Day 0	3.9 (1.1)	4.0 (3.1)	0.888
Day 3	4.5 (1.4)	4.3 (1.1)	0.238
Day 5	12.0 (1.5)	4.8 (1.5)	<0.001*
Day 7	14.5 (1.5)	5.4 (1.6)	<0.001*
Day 10	17.7 (1.3)	5.5 (1.4)	<0.001*

*P-value <0.001 considered significant. MPV: Mean platelet volume, PAS: Platelet additive solution, SD: Standard deviation

Table 2 shows that in samples without PAS up to 0–3 days, the variation in MPV is relatively minimal, whereas 3–5 days, 5–7 days and 7–10 days the variation is relatively more but in PAS containing samples, the variation in MPV from 3 to 5 days, 5 to 7 days and 7 to 10 days is minimal and gradual throughout.

Table 3 shows that in samples without PAS up to 0–3 days, the variation in PDW is relatively minimal, whereas 3–5 days, 5–7 days and 7–10 days the variation is relatively more but in PAS containing samples, the variation in MPV from 3 to 5 days, 5 to 7 days and 7 to 10 days is minimal and gradual throughout.

Table 4 shows that in samples without PAS up to 0–3 days, the variation in PLCR is relatively minimal, whereas 3–5 days, 5–7 days and 7-10 days the variation is relatively more but in PAS containing samples, the variation in MPV from 3 to 5, 5 to 7 and 7 to 10 is minimal and gradual throughout.

Table 5 shows that in samples without PAS up to 0–3 days, the variation in pH is relatively minimal, whereas 3–5 days, 5–7 days and 7-10 days the variation is relatively more but in PAS containing samples, the variation in MPV from 3 to 5 days, 5 to 7 days and 7 to 10 days is minimal and gradual throughout.

Table 6 shows the swirling grade comparison between with and without PAS. There was a higher grade of swirling in the PAS method than without PAS. This difference was statistically significant at all periods.

Table 3: Platelet distribution width comparison between with and without platelet additive solution

Storage period	Group, mean (SD)		*P*
	PDW without PAS	PDW with PAS	
Day 0	14.4 (1.7)	14.2 (1.6)	0.267
Day 3	15.1 (2.0)	15.2 (2.8)	0.842
Day 5	21.2 (2.9)	16.0 (2.6)	<0.001*
Day 7	21.5 (1.5)	17.3 (3.5)	<0.001*
Day 10	27.6 (3.8)	17.4 (4.1)	<0.001*

* P-value <0.001 considered significant. PDW: Platelet distribution width, PAS: Platelet additive solution, SD: Standard deviation

Table 4: PLCR comparison with and without platelet additive solution

Storage period	Group, mean (SD)		*P*
	PLCR without PAS	PLCR with PAS	
Day 0	13.7 (5.2)	12.6 (4.5)	0.089
Day 3	16.9 (5.8)	16.5 (5.6)	0.601
Day 5	25.4 (7.1)	18.0 (7.2)	<0.001*
Day 7	29.8 (6.6)	21.0 (7.7)	<0.001*
Day 10	36.5 (7.7)	21.1 (7.6)	<0.001*

* P-value <0.001 considered significant. PAS: Platelet additive solution, SD: Standard deviation, PLCR: Platelet large cell ratio

Table 5: pH comparison without and with platelet additive solution

Storage period	Group, mean (SD)		*P*
	pH without PAS	pH with PAS	
Day 0	6.4 (0.4)	6.9 (4.7)	0.291
Day 3	6.9 (5.3)	6.9 (2.7)	0.904
Day 5	8.6 (0.5)	6.5 (0.4)	<0.001*
Day 7	9.5 (0.4)	6.5 (0.4)	<0.001*
Day 10	10.5 (0.4)	6.9 (4.7)	<0.001*

* P-value <0.001 considered significant. PAS: Platelet additive solution, SD: Standard deviation

Discussion

Post-transfusion platelet increment is very important in clinical management. Reduction in platelet count during the storage can be multifactorial and may be caused by the storage medium itself or as a by-product of the platelets metabolism along with the subsequent hydrogen ions (pH) changes.[6]

Functional integrity and quality of PCs are determined by glucose and lactate dehydrogenase levels, platelet count, MPV and PDW.[7]

When exposed to stress, mechanical trauma and external surface, PCs get activated coupled with conformational changes leading to degradation of cytoskeletal proteins such as actin and myosin with the formation of platelet microvesicles.[8]

Second-generation containers prepared from polyolefin or polyvinyl chloride plasticised with compounds such as triethylexyltimelliate and butyryl trihexyl citrate may help in mitigating the deleterious effects of PSL by promoting gaseous exchange across the bags during the storage period.[9]

Continued and proper platelet agitation is essential for maintaining platelet quality because inadequate agitation may cause a significant reduction in platelet count, fragmentation of platelets and promotion of procoagulant activities.[10]

Minimisation of activation of PCs during collection, processing and storage along with reduction of the anaerobic consumption of glucose with lactate production, in addition to the presence of minimal, residual glucose, are the basic conditions for maintaining good PC qualities.[11]

In an additive solution unit, the final medium contains 20%–30% donor plasma, which provides glucose for platelet metabolism.[12] Apart from this, PAS contains acetate, which serves as a second metabolic fuel and also acts as a buffer.[13]

Magnesium and potassium are also present in PAS, which inhibits platelet activation and aggregation, although their exact mechanism of action is unknown.[14]

Although the radiolabelled studies of PCs are the best way to determine the various *in vivo* functions of transfused PCs, they are expensive, time-consuming and complex to perform and are mainly restricted for research purposes. Hence, morphological parameters of PCs such as platelet count, MPV, PDW and P-LCR are essential for routine quality control purposes.[1]

In this study, important morphological parameters, platelet count [Table 1 and Figure 1], MPV [Table 2 and Figure 2], PDW [Table 3 and Figure 3] and PLCR [Table 4 and Figure 4], are well maintained in the PAS even during the extended storage period. Similarly, pH levels [Table 5 and Figure 5] and the swirling movement [Table 6] are within the acceptable limits in the PAS-treated PCs.

The results of our study are similar to that of Singh *et al*.[2] and Nasiri and Khosroshahi[15] which highlight that MPV of stored PC is inversely related to pH.

Our study also highlights the fact that PDW, together with the MPV, provides a complete description of the platelet volume distribution than MPV alone because PDW is a measure of platelet volume heterogeneity. The same finding has also been reported by Chandra *et al*.[16]

Similar to the studies conducted by Bashir *et al*.,[17] our study shows that the viability of the PC shelf life stored in PAS can be increased to at least 10 days in contrast to 5 days when stored in plasma alone.

Our results of swirling were scored, and it was observed that the swirling score was much better for the PCs stored in PAS as compared to that of plasma alone. This is similar to the results obtained by Bashir *et al*.[17]

In a study conducted by Mathur *et al*.,[18] it was observed that PAS-treated PC were found to be statistically superior with respect to transfusion-related adverse reaction rate as they have not received a single report of adverse reaction from 1674 units PAS-treated PC, respectively. Similar findings were also reported by de Wildt-Eggen *et al*.[19]

In our study, the results of aerobic and anaerobic culture of PC with and without PAS show sterile results, which have to be further validated by clinical trials.

Our study highlights the fact that the morphological platelet indices can be considered as routine quality control markers of PCs as they are less time-consuming and can be readily performed by using an automated haematological analyser. A major limitation of our study is that no *in vivo* measurements were done to investigate whether platelet properties were negatively affected during storage. Hence, further investigations are required to monitor PSL during PC storage by other platelet quality markers.

The study proves that PAS can partially replace plasma in preparation of PCs and decrease adverse reactions associated with plasma transfusion along with the feasibility of extending the shelf life of PC by (a) improving functionality of platelets, [1] (b) maintaining membrane stability, [1] (c) help prevent platelet aggregation[2] and (d) reduce platelet activation and metabolism[1]

Conclusion

In our study, the viability of PCs stored PAS is better maintained for additional 5 days (i.e., up to 10 days) without compromising their functional abilities. Hence, all efforts must be made to extend the shelf life of platelets using PAS for

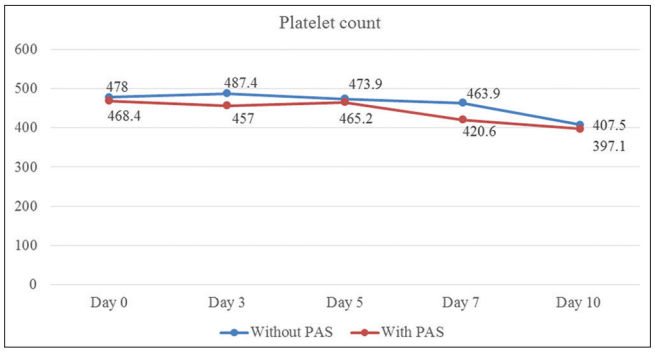

Figure 1: Platelet count comparison with and without platelet additive solution

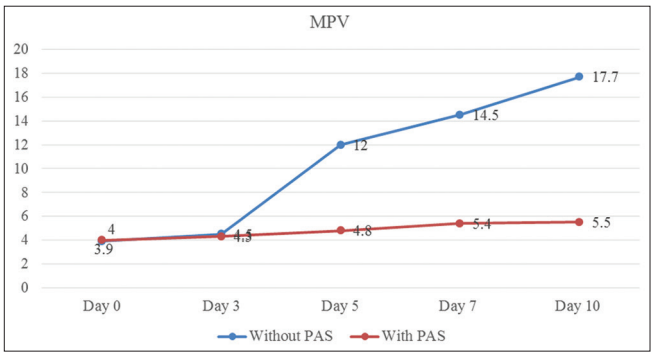

Figure 2: Mean platelet volume comparison with and without platelet additive solution

Table 6: Swirling grade comparison between with and without platelet additive solution

Storage period	Group (%)						*P*
	Swirling without PAS			Swirling with PAS			
	2	3	4	2	3	4	
Day 0	66 (50.8)	64 (49.2)	0	0	64 (49.2)	66 (50.8)	<0.001*
Day 3	65 (50)	65 (50)	0	17 (13.1)	65 (50)	48 (36.9)	<0.001*
Day 5	67 (51.5)	63 (48.5)	0	0	66 (50.8)	64 (49.2)	<0.001*
Day 7	65 (50)	65 (50)	0	0	64 (49.2)	66 (50.8)	<0.001*
Day 10	66 (50.8)	64 (49.2)	0	1 (0.8)	66 (50.8)	63 (48.5)	<0.001*

* P-value <0.001 considered significant. PAS: Platelet additive solution

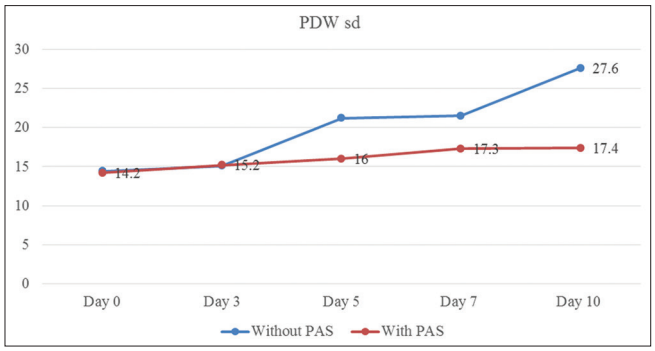

Figure 3: Platelet distribution width standard deviation comparison between with and without platelet additive solution

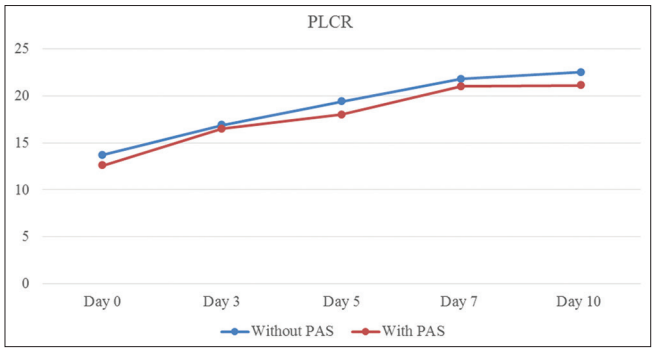

Figure 4: Platelet large cell ratio comparison between with and without platelet additive solution

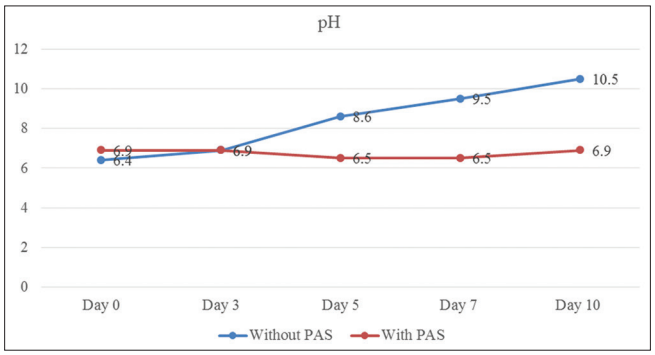

Figure 5: pH comparison between with and without platelet additive solution

more than 5 days because of its numerous clinical advantages and better inventory management, particularly in a rural and resource-constrained setup similar to ours.

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Conflicts of interest

There are no conflicts of interest.

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