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IN VITRO CHARACTERISTIC OF PLATELETS STORED IN ADDITIVE SOLUTION: AN INSTITUTIONAL STUDY

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ABSTRACT
Background: 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. **Study design:** Comparative study. **Materials and Methods:** The study is being done to compare in vitro changes of platelet indices -platelet count, Mean Platelet Volume (MPV), 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 the both categories were statistically significant (p value <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: platelet concentrate, platelets additive solutions, platelet indices.

AIMS
The aims and objective of the study was
1. To study and compare the various morphological parameters of platelets with and without platelet additive solution on days 0, 3, 5,7,10.
2. To study and compare the pH values and sterility of platelets with and without Platelet additive solution on days 0, 3, 5,7,10
3. To extend the shelf life of platelets.

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 revolutionized the field of components therapy.

During the storage period, the 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, MPV, PDW and PC are considered as representative of storage induced shape changes in PC along with 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 multi factorial as several factors 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 hemostatic activity after transfusion.[5]

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 (PAS).

MATERIALS AND METHODS
The study sample included 130 voluntary blood donors who were selected as per WHO guide lines. Pre-donation counseling and medical examination was done and those who did not qualify were deferred. Blood grouping of the donors was done by ORTHO Auto Vue Innova system. (Ortho Clinical Diagnostics, a division of Johnson & Johnson Limited, USA.

All the blood units were screened for human immunodeficiency virus (HIV), Hepattis B virus (HBsAG), Hepatitis C (HCV) by chemiluminescence, VITOR ECI (ortho clinical diagnostics, OCD) and Malaria by Enzyme Linked Immuno Sorbent Assay, ELISA (Qualpro Diagnostics Limited).

Random donor platelet (RDP) preparation

Whole blood was collected in 450 ml bags containing 63 ml of CPDA anticoagulant, kept at room temperature (20-24^{o}C) and PRP-PC was prepared within 6 hours of collection. The donors arm was selected and blood pressure raised by a BP cuff to locate and select a prominent vein for phlebotomy. The donor arm was prepared by cleaning the veni-puncture site at the anti-cubital fossa starting from center to peripheral of the selected area by spirit and betadine. The phlebotomy was done and BP set between 40-60 mm Hg with continuous pressing of the sponge ball by the donors arm in-order to maintain optimum blood flow. The entire procedure was completed within 8 minutes 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. Ltd). Platelet rich plasma was separated from whole blood by light spin centrifugation at1750 rpm for 11 minutes at 21^{o}C, with acceleration and deceleration curves of 5 and 4 respectively (Heraeus Kendro, Hanau, Germany 6000i).

The platelets were concentrated by heavy spin centrifugation at3940 rpm for 5 minutes at 21^{o}C with acceleration and deceleration curves of 9 and 5 respectively along with subsequent discarding of supernatant plasma.

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

8ml of PC and 10-15 ml of residual plasma was added to give a final concentration of 80% additive solution and 20% plasma. The volume of additive solution and stored platelets had a mean volume of around 50ml. PAS contains Na_3-citrate $2H_2O$:3.18g, Na-acetate$3H_2O$:4.42g, $NaH2PO_4$ $2H_2O$:1.05g, Na_2HPO_4:3.05g, KCl: 0.37g, $MgCl_2$ $6H_2O$:0.30g, NaCl: 4.05g. 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^{o}C. 3ml of sample was taken from both the groups, (PC with and without additive solution) on day 0, 3, 5,7 and 10 to study the platelet indices and swirling.

The Hemoglobin count, platelet count, Mean platelet volume (MPV), Platelet volume distribution width (PDW) and Platelet larger cell ration (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 part of the bag and Score 3: Very clear homogenous swirling in all part of the bag.

For microbiological cultures additional 5 ml sample was taken to observe bacterial contamination of PC. Aerobic and anaerobic cultures 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).

RESULTS

Some of the morphological parameters included in this study are (a) MPV which is analyzer –calculated measures of thrombocyte volume and expressed as femtoliters (fL). (b) PDW which is indicator of volume variability in platelets size and expressed as percentage (%) (c) P-LCR which is indicator of larger (> 12Fl) circulating platelets and expressed as percentage (%).

Table 1: Shows Platelet count comparison between with and without PAS: There was no significance difference in mean platelet count between two methods at different periods.

Table 2: In samples without PAS upto 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 3-5days, 5-7 days and 7-10 days is minimal and gradual throughout.

Table 3: 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 3-5 days, 5-7days and 7-10 days is minimal and gradual throughout.

Table 4: In samples without PAS upto 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 3-5, 5-7 and 7-10 is minimal and gradual throughout.

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

Table 6: Shows Swirling grade comparison between with and without PAS: There was higher grade of swirling in PAS method than without PAS. This difference was statistically significant at all the period.

Table 1: Platelet count comparison between with and without PAS:-There was no significant difference in Mean Platelet count between two methods at different periods of follow up.

Storage period	Group				P value
	Platelet count without PAS		Platelet count with PAS		
	Mean	SD	Mean	SD	
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

Table 2: MPV comparison between with and without PAS: There was significant difference in MPV between two methods from Day 5 till Day 7 of follow up.

Storage period	Group				P value
	MPV without PAS		MPV with PAS		
	Mean	SD	Mean	SD	
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*

Table 3: PDW comparison between with and without PAS:-There was significant difference in PDW between two methods at from Day 5 till Day 10 of follow up.

Storage period	Group				P value
	PDW without PAS		PDW with PAS		
	Mean	SD	Mean	SD	
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*

Table 4: PLCR comparison between with and without PAS:-There was no significant difference in PDW sd between two methods at different periods of follow up.

Storage period	Group				P value
	PLCR without PAS		PLCR with PAS		
	Mean	SD	Mean	SD	
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*

Table 5: pH comparison between without and with PAS:-There was significant difference in pH between two methods from day 5 till day 10 follow up.

Storage period	Group				P value
	pH without PAS		pH with PAS		
	Mean	SD	Mean	SD	
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	.5	6.5	0.4	<0.001*
Day 7	9.5	.4	6.5	0.4	<0.001*
Day 10	10.5	.4	6.9	4.7	<0.001*

Table 6: Swirling grade comparison between with and without PAS:-There was higher grade of swirling in PAS method than without PAS. This difference was statistically significant at all the period of follow up.

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

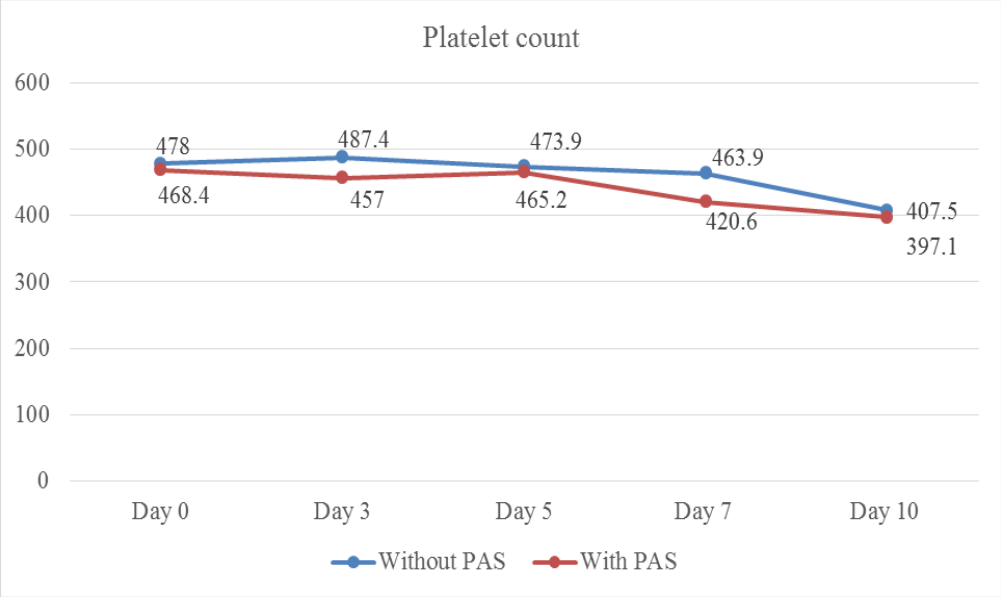

Figure 1: Line diagram showing Platelet count comparison between with and without PAS

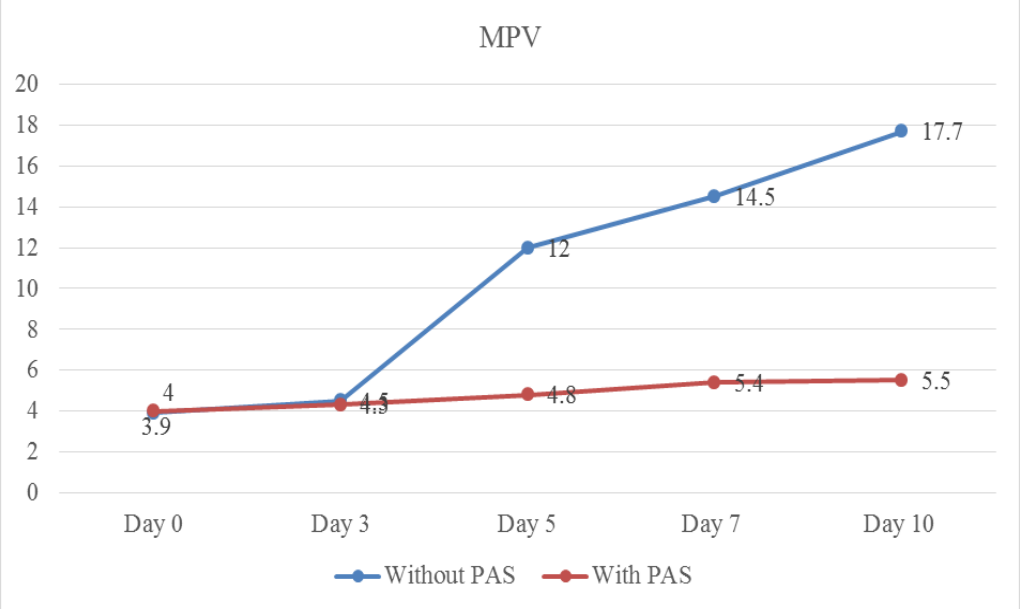

Figure 2: Line diagram showing MPV comparison between with and without PAS

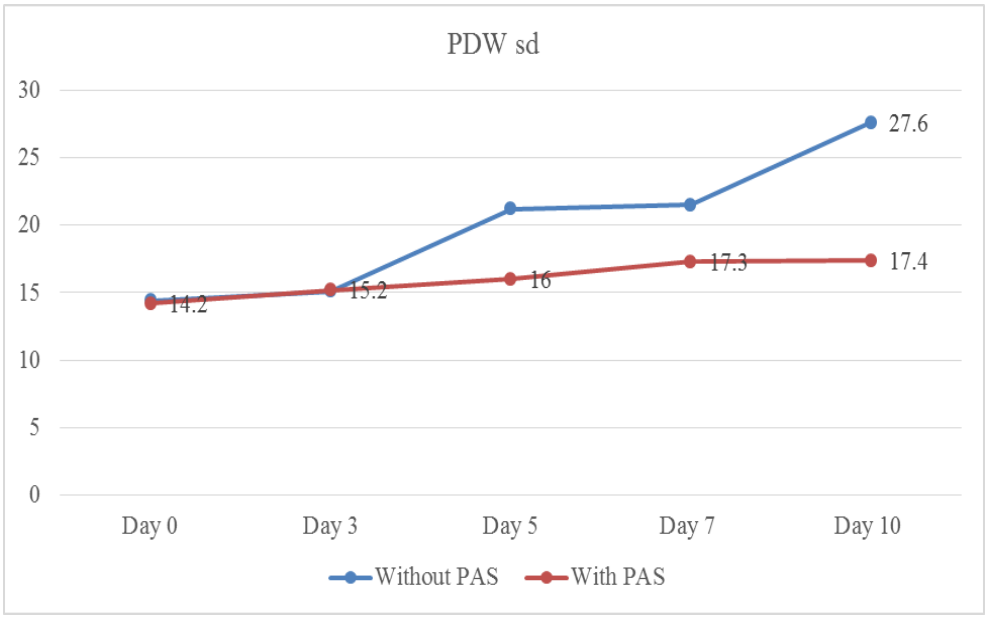

Figure 3: Line diagram showing PDW sd comparison between with and without PAS

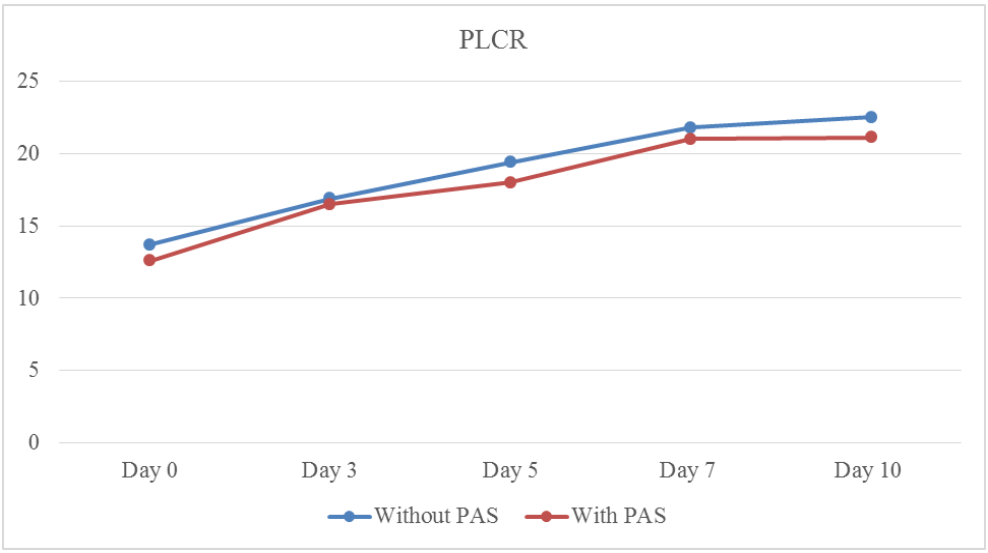

Figure 4: Line diagram showing PLCR comparison between with and without PAS

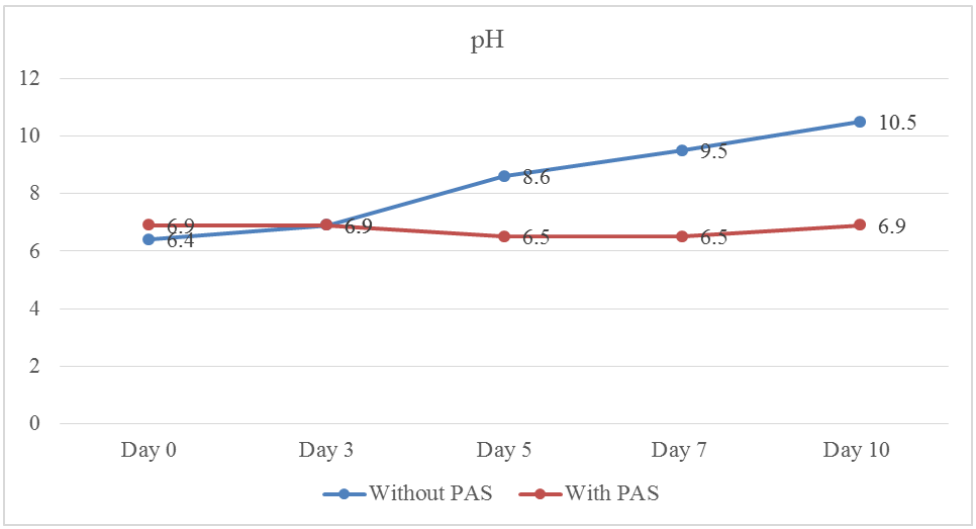

Figure 5: Line diagram showing pH comparison between with and without PAS

DISCUSSION

Post transfusion platelet increment is very important in clinical management. Reduction in platelet count during storage can be multi-factorial 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 (LDH) levels, platelet count, MPV and PDW.[7]

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

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

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

Minimization 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 of 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 for 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 inhibit 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 platelet-large cell ratio (P-LCR) are essential for routine quality control purposes.[1]

The results of our study are similar to that of Seghatchian et al[6] and Nasiri et al.[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 more complete description of the platelet volume distribution than MPV alone because PDW is measure of platelet volume heterogeneity. The same finding has been also 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 our study the results of aerobic and anaerobic culture of PC with and without PAS show sterile results, which has to be further validated by clinical trials.

Our study highlight the fact that the morphological platelet indices can be considered as routine quality control marker of PCs as they are less time consuming and can be readily performed by using an automated hematological analyzer 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.

CONCLUSION

In our study the viability of PCs stored PAS are 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 more than 5 days because of its numerous clinical advantages and better inventory management particularly in a rural and resources constrain set up similar to as.

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REFERENCES

1. Shrivastava M. The platelet storage lesion. Transfus Apher Sci 2009; 41: 105-13.
2. Singh H, Chaudhary R, Ray V. Evaluation of platelet storage lesions in platelet concentrates stored for seven days. Indian J Med Res 2003; 118: 243-6.
3. Murphy S. Utility of *in vitro* tests in predicting the *in vivo* viability of stored PLTs. Transfusion 2004; 44: 618-9.
4. Mathai J, ResmiKR, SulochanaPV, SathyabhamaS, Baby Saritha G, Krishnan LK. Suitability of measurement of swirling as a marker of platelet shape change in concentrates stored for transfusion. Platelets 2006; 17: 393-6.

5. Murphy s, Heaton WA, Rebulla P. Platelet Production in the World and the New Transfusion 1996; 36: 751-4.
6. Seghatchian J, Krailadsiri P. The platelet storage lesion. Transfus Med Rev 1997; 11: 130-44.
7. Singh H, Chaudhary R Ray V. Platelet indices as quality markers of platelet concentrates during storage. Clin lab Hemat 2003; 25: 307-10.
8. Devine DV, Serrano K. The platelet storage lesion. Clin Lab Med 2010; 30: 475-87.
9. Yuasa T, Ohto H, Yasunaga R, et al. improved extension of platelet storage in a polyolefin container with higher oxygen permeability. Br J haemetol 2004; 126: 153-9.
10. Bannai M, Mazda T, Sasakawa S. The Effect of pH and Agitation on Platelet Preservation. Transfusion. 1985; 25: 57-9.
11. Gulliksson H. Defining the optimal storage conditions for the long-term storage of platelets. Tranfus Med Rev. 2003; 17: 209-15.
12. Sweeney J, Kouttab N, Holme S, Kurtis J, Cheves T, Nelson E. Storage of platelet-rich plasma-derived platelet concentrates pools in plasma and additive solution. Transfusion 2006; 46: 835-40.
13. Bertolini F, Murphy S, Rebulla P, Sirchia G. Role of acetate during platelet storage in a synthetic medium. Transfusion 1992; 32: 152-6.
14. Diedrich B, Sandgren P, Jansson B, Et Al. In Vitro and In Vivo Effects of Potassium and Magnesium on Storage Up To 7 Days of Apheresis Platelet Concentrates In Platelet Additive Solution. Vox Sang 2007; 94: 96-102.
15. Nasiri S, Khosroshahi BN, Lyophilization of human platelet and study of its aggregability. International Journal of Drug Delivery. 2012; 3: 241-4.
16. Chandra T, Gupta A, Kumar AS. Morphological and function changes in random donor platelets stored for seven days in platelet additive solution. Int J Blood Transfus Immunohematol. 2011; 1: 20-5.
17. Bashir, Mohsin S, Amin H, Rehman M, Hussian S, Saeed T. Comparison Of changes in platelet count, mean platelet volume and swirling in stored in stored platelet concentrates with and without platelet additive solution. J Appl Hematol 2014; 5(1): 10-4.