The Effect of Storage on Platelets in Platelet Rich Plasma and Platelet Concentrate

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ABSTRACT

Introduction: Platelets are routinely isolated from whole blood and stored in plasma for 5 days. The platelets undergo various storage changes starting from collection, processing to storage and the underlying conditions within the patients which may affect the therapeutic benefit to the recipient. Study aimed to assess the quality of platelets in platelet rich plasma and platelet concentrate and to evaluate the effects of storage on platelets in platelet rich plasma and platelet concentrate.

Material and methods: The study was carried out in the Department of Transfusion Medicine and Department of Medical Laboratory Technology in Government Medical College, Thiruvananthapuram after obtaining approval from the Institutional Ethical Committee. The study period was six months. The samples were collected from platelet rich plasma and platelet concentrate bags under sterile conditions. Their quality was assessed using the parameters such as swirling, volume of the platelet concentrate, platelet count, WBC count and pH on day 0, day 3 and day 5 of storage.

Results: A total of 64 samples were evaluated and of these 95% have fullfilled all 5 parameters of quality control with a score 5 and 5% had score 4. Thus in our study majority of the prepared units were of the desired quality. All the parameters were assessed and the results obtained on both the units were well above the values of recommended norms.

Conclusion: The quality of platelet concentrates were maintained well within the usual 5 days thus an extension of platelet storage time is recommended.

Keywords: Storage Effects; Whole Blood; Platelet Rich Plasma; Platelet Concentrate; Swirling; Platelet Count; WBC Count; pH.

INTRODUCTION

The platelets are small anucleated cytoplasmic fragments that play an essential role in blood clotting and wound healing. Platelets are routinely isolated from whole blood and stored in plasma for 5 days.[1] The energy supply of platelets is based on both anaerobic and aerobic metabolism by the TCA cycle and respiratory chain. The anaerobic part is associated with degradation of glucose to lactic acid, while the aerobic oxygen-dependent part results in total degradation of substrates.[2]

The platelet storage lesions still presents one of the major challenges to the transfusion centres, because of the limitations of storing platelets. The two main reasons for the 5 day shelf life for platelet is bacterial contamination at a storage temperature of 22ºC and loss of viability during storage, known as platelet storage lesions. The in vitro quality assessment of platelet products is the valuable part to evaluate the quality of platelet rich plasma and platelet concentrate.

Platelet transfusions are the primary therapy for thrombocytopenia due to various causes. There are two types of platelet concentrates are available for transfusion; one which is the product of normal blood donation i.e. random donor platelets (RDP) prepared platelet rich plasma-platelet concentrate (PRP-PC) or by Buffy coat poor-platelet concentrate (BC-PC) and the other is single donor platelets (SDPs): apheresis-PC: collected from platelet apheresis donors with the help of an automated cell separator.[3] The basic principle behind preparation of components from whole blood is that each component has its specific gravity and by applying centrifugation, each component is separated and removed, thus allowing the transfusion of desired component according to the need of the patient.

The recommended shelf life of PC in presently available platelet storage bags is 5 days at 22±2ºC with continuous agitation. The platelets undergo various storage changes starting from collection, processing to storage and the underlying conditions within the patients, which may affect the therapeutic benefit to the recipient.

The preservation of viable and functional platelets depends on the following factors-

1. Temperature: platelets should be stored at 22-24ºC with continuous gentle agitation in platelet incubator and agitator
2. pH: should be above 6.0
3. Plastic bag: Maintenance of pH and function of platelets depend on the permeability of the storage

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bag to oxygen and carbon dioxide. Platelets stored in bags made of polyvinyl chloride (PVC) with plasticizer di–(2-ethylhexyl) phthalate (DEHP) have shelf life of 3 days. New plastic bags made of polyolefin with no plasticizer (Baxter's PL732) and thin walled PVC with tri-(2- ethylhexyl) tri-mellate plasticizer (TOTM) maintain pH and functions up to about 7 days. However it is recommended to store platelets in new bags for 5 days only from the date of collection of blood.

4. The pooled platelets can be stored for 4 hours at 22-24ºC before they are transfused.
5. Platelet incubators should have a system to monitor and record the temperature continuously. Temperature recording charts should be changed regularly. They should also have alarm systems with audible signals. These facilities should have battery back up.
6. Platelet count should be less than 5.5×10^9 in 40-70 ml plasma[4]
7. In plateletpheresis count should be less than 3×10^{11} in about 300 ml plasma[4]

The functions of platelets formation of the primary haemostatic plug in maintaining normal haemostasis. Platelets forms the haemostatic plug and provide the surface on which fibrin forms in a bleeding patient resulting in: cessation of bleeding, correction of prolonged bleeding time, rise in platelet count.[3]

Study aimed to assess the quality of platelets in platelet rich plasma and platelet concentrate and to evaluate the effects of storage on platelets in platelet rich plasma and platelet concentrate.

MATERIAL AND METHODS

In this study, the following platelet products were prepared; random donor platelets (i) platelet rich plasma - platelet concentrate (PRP-PC). The study was carried out in the Department of Transfusion Medicine and Department of M.L.T in Govt. Medical College, Thiruvananthapuram after obtaining approval from the Institutional Research and Ethical Committee. The study period was six months.

Preparation of Platelet Rich Plasma and Platelet Concentrate

The blood donor was selected as per the standard operative procedure followed by the Department. After the selection of a suitable vein, the donor arm was prepared by cleaning the antecubital area starting from centre to periphery of the selected arm by betadine and spirit . The phlebotomy was done with minimal trauma after maintaining the BP at 40 mmHg.

The whole blood was collected from blood donors using 350-ml bags containing 49 ml of CPD anticoagulant (HLL Lifecare Limited, Trivandrum, India) kept at room temperature (20-24°C) and PRP-PC was prepared within 4 hours of collection . The blood collection was completed within 7 minutes from the starting time.

Platelet rich plasma was separated from whole blood by light spin centrifugation by Heraeus 6000i, Germany refrigerated centrifuge at 1600 rpm for 10 minutes at 22°C, with acceleration and deceleration curves of 9 and 4 respectively and the platelets were concentrated by heavy spin centrifugation at 3880 rpm for 10 minute at 22°C, with acceleration and deceleration curves of 9 and 4 respectively with subsequent removal of supernatant plasma. The platelet concentrate bag was left stationary with the label side down at room temperature for approximately 1 hour. The platelet poor plasma was frozen and stored as fresh frozen plasma (FFP) at or below -40°C for one year.

The quality of platelet product was assessed on day 0, day 3 and day 5 using the following parameters:
1. Swirling
2. Volume of the platelet concentrate
3. Platelet count
4. WBC count
5. pH changes

Swirling: The swirling was evaluated by examining the units against light and scored as:
Score 0: Homogen turbid and is not changed with pressure.
Score 1: Homogen swirling only in some part of the bag and is not clear.
Score 2: Clear homogenic swirling in all part of the bag.
Score 3: Very clear homogen swirling in all part of the bag.

Volume of PRP and PC: Weigh the filled bag by using calibrated blood bag weighing machine, weigh the empty bag, then calculate the volume by using a formula.

$$\frac{\text{Weight of filled bag} - \text{Weight of empty bag*}}{\text{Specific gravity of platelet*}}$$

* Weight of empty bag is approximately 27 – 30 mL; Specific gravity of platelet is 1.03

Platelet count per bag: The platelet count in the sample was analyzed using hematology analyzer (Sysmex 2000) in Clinical Pathology Lab, Medical College, Thiruvananthapuram. Alternatively count was performed manually by using Neubauer counting chamber after bulk dilution of the samples with 1% Ammonium Oxalate. The dilution is 1:20 (50 µl of sample + 950 µl of 1% Ammonium Oxalate). Platelet counting was done in 5 small squares of the central square of the counting chamber under 40X objective of microscope.

Calculation
Platelet count per bag = N × 106 × volume of PRP and/ or PC

WBC count per bag: The WBC count was performed manually using Neubauer counting chamber and WBC diluting Turk's fluid which contains Gentian violet to stain WBCs and Glacial acetic acid to lyse RBCs. The 50 µl of sample + 950 µl of Turk's fluid was taken (dilution is 1:20), mixing done gently and charge the chamber and wait for a minute to settle the cells. The WBC counting was done in 4 large squares of the counting chamber under 10X objective of microscope.
WBC count per bag = N × 5000 × volume of PRP and/ or PC

pH changes of PRP and PC: Analyzed using pH indicator strip. The two types of strips are used, ranging from 1 to 10

and ranging from 5.5 to 7.5. By using a sterile dropper and take one drop of each samples and pour on the pH indicator strip and the colour change occurs and results are taken after matching the colour of indicator strip.

Scoring

Scoring was done on the basis of parameters taken for quality control evaluation for PRP and PC units. Score was given according to number of parameters fulfilled the parameters of quality control by each unit.

Parameter Quality	Requirements
Volume	50 – 70mL
Platelets count	$\geq 5.5 \times 10^{10}$
pH	>6.0
RBC contamination	0.5mL
WBC contamination	$5.5 \times 10^{7} - 5 \times 10^{8}$

STATISTICAL ANALYSYS

Data analyzed using descriptive statistics. Comparison of the parameters on each day were done using McNemar chi-square test for the data analysis SPSS software was used.

RESULTS

A total of 62 samples are included in this study. 31 samples of PRP and 31 samples of PC were subjected to assess the in-vitro quality by using the parameters such as volume, using Chi Square test and all the 64 samples showed significant changes in swirling, pH, platelet count and WBC count on day 3 and day 5 from the day 0. The changes remained within the limits of recommended quality control parameters.

Platelet rich plasma parameters

A total of 31 samples of PRP 21 samples (67.7%) shows score 3 swirling and 10 samples (32.3%) shows score 2 swirling on day zero. On day 3, 13 samples of PRP (41.9%) shows score 3 swirling and 18 samples (58.1%) shows score 2 swirling. On day 5, ten samples (32.3%) shows score 3 swirling, 20 samples (64.5%) shows score 2 swirling and 1 sample (3.2%) show score 1 swirling.

Comparison of swirling scores in PRP on Day 3 with Day 0

Swirling score (PRP)		Day 0			Total
		1	2	3	
Day 3	2	0	10	8	18
	3	0	0	13	13
	Total	0	10	21	31

McNemar test p = 0.008

The observed p value is 0.008 and there is a significant difference in the scoring of swirling on day 3 compared with day 0.

Comparison of swirling scores in PRP on Day 5 with Day 0

Swirling score (PRP)		Day 0			Total
		1	2	3	
Day 5	1	0	1	0	1
	2	0	9	11	20
	3	0	0	10	10
	Total	0	10	21	31

p = 0.001

The observed p value is 0.001 and there is a significant difference in the scoring of swirling on day 5 compared to day 0.

Platelet concentrate swirling score

A total of 31 samples of PC 27 samples (87.1%) shows score 3 swirling, 3 samples (9.7%) shows score 2 swirling and 1 sample (3.2%) show score 1 swirling on day 0. On day 3, 23 samples (74.2%) shows score 3; 6 samples (19.4%) shows score 2 and 2 samples (6.5%) shows score 1. On day 5, 13 samples (41.9%) shows score 3; 15 samples (48.4%) shows score 2 and 3 samples (9.7%) shows score 1.

The observed p value is < 0.001, significant change occur in day 5 compared to day 0.

pH variation in Platelet rich Plasma

The pH of all 31 samples of PRP did not show significant variation on day 0, day 3 and day 5. The standard deviation falls within the limit i.e.0.23, 0.26 and 0.30. When comparison done by data on day 0 with day 3; day 0 with day 5 and day 3 with day 5 shows a significant difference with paired difference SD is 0.098, 0.103 and 0.157. The observed p value on paired comparison of these days is < 0.001

Platelet count variation in PRP

The variation of platelet count in PRP on day 0, day 3 and day 5 shows no significant change in SD. In paired comparison on day 0 to day 3; day 0 to day 5 and day 3 to 5 shows a change in SD and observed p value is < 0.001. The values are fall within the limits of recommended quality control values.

WBC count variation in PC

The 31 samples of PC, WBC count variation seen on day 0 and day 3 shows no significant change in SD, but on day 5 there is a change in the SD. The paired difference SD of day 0 – day 3, and day 5 SD shows no significant change and observed p value on day 0 - day 3 is 0.051; day 3 and day 5 is < 0.001; day 0 – day 5 is 0.005 shows a significant change. All results were within the limit.

Mean of observed values on day 0, day 3 and day 5:

Adequacy of platelets in PC with reference to PRP

None of the samples showed the values exceeding the limit of quality control parameters. On comparing the mean values of all the parameters selected for assessment in both PRP and PC, no significant difference was observed from day 0 to day 5 of storage period. All the samples of PRP and PC were assessed for evaluating the volume, pH, swirling, platelet count and WBC count, the observed mean values and standard deviation of all the samples are acceptable and all are within the limits of standards of transfusion medicine.

DISCUSSION

The ability of transfused platelets to circulate and function is dependent on both the effect of the ex-vivo storage lesions that undermines platelet functionality.[5,6] In thrombocytopenic patients factors affecting platelet consumption may be so strong in their influence on platelet recovery and survival that they may outweigh the effects of in vitro platelet storage.[7,8] Nevertheless it has long been recognized that changes in platelets that occur during storage can contribute to poor platelet function and decreased post transfusion survival. The platelet changes are classified into three broadly defined

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	N	pH		Paired comparison	Paired difference		P
		mean	SD		Mean	SD	
Day 0	31	7.18	0.23	Day 0- Day 3	.081	.098	<0.001
Day 3	31	7.10	0.26	Day 3- Day 5	.094	.103	<0.001
Day 5	31	7.00	0.30	Day 0- Day 5	.174	.157	<0.001

	N	Platelet count in 10^{13}		Paired comparison	Paired difference in 10^{13}		P
		mean	Sd		Mean	sd	
Day 0	31	6.50	2.17	Day 0- Day 3	.210	.183	<0.001
Day 3	31	6.29	2.15	Day 3- Day 5	.255	.206	<0.001
Day 5	31	6.03	2.17	Day 0- Day 5	.465	.250	<0.001

	N	WBC count 10^8		Paired comparison	Paired difference n 10^{13}		P
		Mean	SD		Mean	SD	
Day 0	31	2.87	2.24	Day 0- Day 3	-.652	1.782	0.051
Day 3	31	3.53	2.37	Day 3- Day 5	2.045	2.852	<0.001
Day 5	31	1.48	0.95	Day 0- Day 5	1.394	2.545	0.005

Parameter Quality	Requirements	Observed values	
		PRP	PC
Volume	50 – 70mL	99 - 195 mL	49 – 75 mL
Platelets count	$\geq 5.5\times10^{10}$	$\geq 6.27 \times 10^{13}$	$\geq 5.97 \times 10^{13}$
pH	>6.0	7.1	7.1
WBC contamination	$5.5\times10^7 - 5\times10^8$	$3.24 \times10^7 – 1.83 \times10^8$	$3.53 \times10^7 - 1.48 \times10^8$
swirling	3 or 2	2	2

categories: platelet activation, metabolic alterations and platelet senescence. Normal platelet senescence is most likely a relatively minor component of the storage lesion. PCs that have been gently prepared and then immediately transfused without a significant without a significant storage interval (within 24 – 48 hours of donation) have uniformly high recovery, good survival and preserved function.[9] The storage of platelets was found to be associated with higher of platelet activation (about 10% of the release was associated with the preparation and about 30% with the subsequent storage period).[10]

Although 5 – day storage of platelet concentrate is generally practiced in the developed countries in our department platelet concentrates are usually issued within 24 - 72 hrs of preparation to minimize chances of bacterial contamination and preserve function. Following PC preparation, QC is performed on all units prepared using both visual and electronic means.

The visual investigations includes:
1. The recording of the donation number
2. Date of pooling and expiry date
3. Lot and reference numbers of the storage bag
4. The number of buffy coats used to prepare the unit
5. Observation for red cell contamination
6. Checking for any incidence of the swirling phenomenon.

The electronic investigations include:
1. Volume
2. Platelet count and concentration
3. Residual leukocyte content

4. Residual erythrocyte count
5. pH measurement.

The products which were found to fail these parameters were discarded. pH readings are recorded for all products on the first day and on the last day of storage.

In our study 31 bags of PRP and 31 samples of PC prepared from PRP were subjected to assess the storage effects. In vitro quality of PRP and PC was assessed by observing swirling, volume, pH, platelet count and WBC count per unit. We also assessed the yield of platelets in PC which were prepared from PRP of 31 samples. The platelet concentrates are stored at 22 to 24 with continuous agitation are recommended, until the time of issue.

Swirling in PRP

Evaluation of swirling is a simple non invasive method that can be performed by visual inspection and is useful for routine quality control. Visual inspection of swirling correlates with platelet morphology; the presence of swirling indicates discoid morphology and absence is indicative of spherical morphology. Score 3 swirling was observed in 67.7%, 41.9% and 32.3% on respective day 0, day 3 and day 5. Score 2 swirling was noticed in 32.3%, 58.1% and 64.5%. Only one unit shows score 1 swirling on day 5. After 5 days of storage the score 3 swirling decreased to 35.4%.

In case of PC, score 3 swirling was noticed in 87.1%, 74.2%, 41.9%. Score 2 swirling was noticed in 9.7%, 19.4% and 48.4%. Score 1 swirling was noticed in 3.2%, 6.5% and 9.7%. After 5 days of storage the score 3 swirling was decreased to 77.4%. This drop of swirling could be due to lesions that are known to occur during platelet preservation. But 95% of the

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units show the swirling score within the recommended limits of quality requirements.

Volume

Platelets prepared from whole blood collections are typically suspended in 40 to 70 mL plasma, which serves as a buffering agent to maintain pH. The major reason for using this volume range was based on early studies with PCs stored in first generation PVC containers. Because of the insufficient permeability of these containers to oxygen, there was a risk of fall in pH in the PCs from anaerobic conditions and elevated lactic acid production. The platelet- suspending volume was therefore maximized to increase buffering capacity while maintaining as little volume as possible, to minimize the risk of volume overload of the recipient's circulatory system.

In the present study the mean volume of PRP observed was 155 mL, the minimum volume attained was 115 mL and maximum volume attained was 195 mL. The mean volume of PC observed was 74 mL, the minimum volume attained was 49 mL and maximum volume attained is 96 mL. All the results are within the recommended quality requirements.

Platelet count

During the preparation of PCs there is deterioration of platelet function manifested by abnormal shape changes, aggregation and secretory response. The main cause of deterioration of platelet function during preparation is lesions associated with the preparative manipulation and storage.

In the present study the platelet count in PRP and PC did not show significant difference from the fresh units to the units of day 5. The mean platelet count of PRP on day 0, day 3 and day 5 observed was 6.50×10^{13}/unit, 6.29×10^{13}/unit and 6.03×10^{13}/unit respectively. Statistical difference was observed between day 0, day 3 and day 5 ($p < 0.001$). All units of PRP met the desired quality control criteria for platelet count/units. The mean platelet count of PC on day 0, day 3 and day 5 observed was 6.23×10^{13}/unit, 6.0×10^{13}/unit and 5.68×10^{13}/unit respectively. Statistical difference was observed between the day 0 vs day 3 vs day 5 ($p < 0.001$). All units of PC met the desired quality control criteria for platelet count per units. No such difference was observed in the present study and the mean platelet count/unit in PRP and PC and statistically no significant difference was observed.

WBC count

WBCs in PC have a detrimental effect on the storage medium, resulting in a significant drop in pH, increase in glucose consumption, lactic acid production and LDH release during storage. As a result, in the PCs with high concentration of leukocytes, the platelet condition up to 5 days of storage was also significantly affected.

In this study the mean WBC count in PRP on day 0, day 3 and day 5 observed was 1.83×10^8/unit, 3.54×10^8/unit and 2.62×10^8/unit respectively. Statistical difference observed between day 0 vs day 3 and day 5 ($p < 0.001$, 0.038, 0.038). The mean WBC count in PC observed was 2.87×10^8/unit, 3.53×10^8/unit and 1.48×10^8/unit respectively. Statistical difference between day 0 vs day 3 (p 0.051), between day 3 vs day 5 ($p < 0.001$) and between day 0 vs day 5 (p 0.005). All

the units including PRP and PC fulfilled the recommended quality control criteria for WBC count. PC derived from PRP, the leukocyte contamination can be diminished if the handling of the bags and the transfer of the PRP are performed carefully.

pH changes in PRP and PC

The pH decreases during storage depending on the stabilizer in plastic platelet storage bags and storage conditions used. Increased platelet glycolysis resulting in a fall in pH to levels approaching 6.0 in PC stored in plasma is associated with substantial loss of viability. The majority of fresh, un-stimulated platelets are discoid with few projections. In the early observations of PCs stored at 20 to 24, a gradual disc to sphere transformation was seen during storage.

In the present study of 31 units of PRP mean + SD value of pH on day 0 was 7.2± 0.17, on day 3 was 7.0 ± 0.32 and on day 5 was 6.9 ± 0.34. The pH ranged from 7.2 to 6.8 and no significant was observed on these 3 days. The observed values of pH on PRP and PC fulfilled the quality control criteria for pH per units.

In this study the scoring was done on the basis of parameters used for quality control evaluation of platelet concentrates. A total of 62 samples were evaluated and of these 95% are fulfilled all 5 parameters of quality control had score 5 and 5% had score 4 with minute variations. Thus in our study majority of the prepared units were of the desired quality. All the parameters were assessed and the results obtained on both the units are well above the values of recommended norms. The quality of platelet concentrates were maintained well within the usual 5 days thus an extension of platelet storage time is recommended.

CONCLUSION

The study infers that platelet functions are maintained within normal levels on day 5 of storage at 22. In our department, the maximum shelf life of platelets is 96 hrs. The parameters assessed for quality in this study from prepared platelet concentrates, met the criteria for platelet count, residual white cell count, platelet concentrate volume set by National Blood Transfusion Society. All units showed better swirling and platelet count in PRP-PCs. All the platelet concentrates units had pH well maintained There are variable factors such donor's factor, adequacy of mixing of blood during collection, time difference between phlebotomy and processing may exist, and these could affect the concentration of platelet count. In general, this study showed that the delay in whole blood processing up to 24 hrs has no significant effect on certain in vitro quality parameters and function compared with freshly prepared PCs. The products need regular reassessment since new developments may have impact on the quality of various product types.

The implementation of quality product should start from the commencement of the procedure i.e. from the donor selection and each and every step in the whole process in the department of transfusion medicine including phlebotomy, component separation (centrifugation), storage (temperature and agitation).

The objective of the study was to identify the quality of PRP, because in our department PRP-PC method was implemented to prepare the platelet concentrate as well as the quality of the PC after the assessment of 5 parameters. The % yield of PC with reference to PRP was also in acceptable range and above the recommended norm. Thus we conclude that platelet concentrates storage can be extended to 5 days at a temperature of 22. However further large scale study with more standardization is required to better delineate the findings.

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