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Evaluation of Changes in PH, Platelet Count and Swirling Score in Platelet Concentrate on Storage

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ABSTRACT

The normal concentration of platelets in the blood is between 150 and 400×10^9/L; their main function is to promote a haemostatic surface in blood vessels (primary haemostasis). Platelets are stored at room temperature, 20-24°, because below 18°C, their lipid bilayer membrane under goes a phase change which allows the aggregation of surface glycoproteins. PH should be above 6.0 and Platelet count should be less than 5.5×10^9 in 40-70 mL plasma, less than 3×10^{11} in about 300 mL plasma for plateletpheresis. This study aim to evaluate effect of storage on pH, platelet count and swirling score in platelet concentrate. Study was carried for one year from 1 jan 2023 to 1 jan 2024 in the Blood bank and Department of Pathology, Jaipur national university institute for medical science and research centre, jaipur in coordination with Department of paediatrics, JNUIMSRC, jaipur. The quality of platelet product was assessed on day 1, day 3 and day 5 using the following parameters: Swirling ,Volume of the platelet concentrate, Platelet count and pH changes. A total of 50 samples were included in this study and were subjected to assess the in vitro quality by using the parameters such as volume, using Chi Square test. All the samples showed slight changes in swirling, pH, platelet count and WBC count on day 3 and day 5 from the day 1. The changes remained within the limits of recommended quality control parameters. In general, this research found that up to 24 hours has no significant impact on in vitro quality metrics and function.

INTRODUCTION

Platelets are small discoid anucleated cells, varying size from 1 to 4 microns in diameter, which have mitochondria, produce and store adenosine triphosphate and are highly sensitive to their environment[1]. The normal concentration of platelets in the blood is between 150 and 400×10^9/L, their main function is to promote a haemostatic surface in blood vessels (primary haemostasis) following the events of vasoconstriction, adhesion, secretion and platelet aggregation. The main inducing agonists in this process are collagen, thrombin, platelet activating factor and adenosine diphosphate (ADP)[2,3].

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[4].

Platelets are given either therapeutically to stop bleeding in poly trauma patient or prophylactically to prevent bleeding in thrombocytopenia and leukaemia patient[5]. The maximum storage period before components preparation is often within 8 hours at 20 to 24°C[1].

Platelets are collected in three ways. They can be centrifuged from platelet-rich plasma, isolated from buffy coats, or collected directly from the bloodstream by apheresis. There is some evidence that the buffy coat and apheresis methods provide better platelets, with the suggestion that centrifuging platelets against the plastic bag surfaces in the platelet-rich plasma method leads to partial or complete activation of some of the platelets[6].

Platelets are stored in large flat bags with as high surface to volume ratio and on agitators to facilitate oxygen diffusion. Off agitation for more than 24 hours, the bag contents become hypoxic and metabolism shifts to anaerobic glycolysis so that the contents become acidotic and the platelets loose function[7].

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[8].

Platelets are stored at room temperature, 20-24°, because below 18°C, their lipid bilayer membrane undergoes a phase change which allows the aggregation of surface glycoproteins[9]. pH should be above 6.0 and Platelet count should be less than 5.5× 10^9 in 40-70 mL plasma, less than 3×10^{11} in about 300 ml plasma for plateletpheresis[8].

MATERIALS AND METHODS

This study is a prospective type. The study were carried for one year from 1 Jan 2023 to 1 Jan 2024 in the Blood bank and Department of Pathology, Jaipur national university institute for medical science and research Centre, Jaipur (JNUIMSRC) in coordination with Department of paediatrics, JNUIMSRC, Jaipur, India. Approvals were obtained from Institutional Ethics Committee and informed consent was taken from the patients parents participating in this study.

Sample size: Total of 50 platelet concentrate beg were taken

Calculation: Sample size formula with desired error of margin

$$\chi^2 = \sum(O-E)^2/E$$

Where, X^2 is the level of significance at 5% i.e. 95 % confidence interval

- O = observed platelet count
- E = expected platelet count

Inclusion criteria:

- All platelet concentrate bags which have minimum 50-70 ml platelet concentrate

Exclusion criteria:

- RBCs more than 0.5 mL
- Platelet less than 5.5×10^{10} in 50-70 mL plasma on day 1
- PH less than 6 on day 1

In this study, platelet concentrates prepared in Blood Bank, Department of Pathology, JNUIMSRC, Jaipur.

Study procedure: 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 mm Hg. The whole blood was collected from blood donors using 350-mL bags containing 49 mL of CPD anticoagulant kept at room temperature (20-24°C) and PRP-PC was prepared within 4 hours of collection.

Platelet rich plasma was separated from whole blood by light spin centrifugation by REMI KBM70 PLUS, refrigerated centrifuge at 1900 rpm for 12 min at 22°C, with acceleration and deceleration curves of

9 and 4 respectively and the platelets were concentrated by heavy spin centrifugation at 3400 rpm for 10 min at 22°C, with acceleration and deceleration curves of 9 and 4, respectively with subsequent removal of supernatant plasma. The platelet concentrate bag store at room temperature. 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 1, day 3 and day 5 using the following parameters:

- Swirling
- Volume of the platelet concentrate
- Platelet count
- PH changes

Swirling: The swirling was evaluated by examining the units against light and scored as (Fig.1):

- 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 PC: Weigh the filled bag by using calibrated blood bag weighing machine, weigh the empty bag, then calculate the volume by using a formula:

Weight of filled bag-Weight of empty bag/Specific gravity of platelet

Weight of empty bag is approximately 27-30 mL; Specific gravity of platelet is 1.03.

Statistical analysis: Data was collected and entered simultaneously in statistical package for social sciences (SPSS) version 23 and coded appropriately. The data was analyzed keeping in view the aims and objectives of the study. Descriptive statistics were calculated to summarize the sample characteristics in terms of frequency and percentage. Graphs and Charts were made. Analytical and inferential analysis was applied between dependent variable and other independent variables. Significance was set at standard 0.05.

RESULTS

A total of 50 samples were included in this study and were subjected to assess the in vitro quality by using the parameters such as volume, using Chi Square test. All the 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.

In present study, majority of the participants were in the age group of 41-50 years followed by 32% each in age group of 20-30 years and 31-40 years. Present study had female preponderance (56%) (Fig. 2).

Swirling scores: Out of 50 samples 39 samples (78%) shows score 3 swirling and 11 samples (22%) shows score 2 swirling on day one. On day 3, 7 samples of PRP (14%) shows score 3 swirling and 43 samples (86%) shows score 2 swirling. On day 5 none of the samples had score 3 swirling, 38 samples (76%) shows score 2 swirling and 12 sample (24%) show score 1 swirling. The observed p value is 0.000 and there is a significant difference in the scoring of swirling on day 5 compared to day 1 (Table 2).

Table 1: Distribution of participants according to gender

Gender	Count	Column N%
Male	22	44
Female	28	56

Table 2: Percentage of Swirling Score on Day 1, Day 3 and Day 5

Day	Swirling Score			
	Score 0: Homogeneous turbid and is not changed with pressure`	Score 1: Homogeneous swirling only in some part of the bag and is not clear	Score 2: Clear homogeneo in all part of the bag	Score 3: Very clear homogeneous in all part of the bag
Day 1	0 (0%)	0 (0%)	1 (22%)	9 (78%)
Day 3	0 (0%)	0 (0%)	3 (86%)	(14%)
Day 5	0 (0%)	12 (24%)	8 (76%)	(0%)

Comparison of swirling scores in PRP on Day 3 with Day 1

	Day 1			
Swirling score	Score 2	Score 3	Total	p-Value
Day 3				
Score 2	11	32	43	0.153
Score 3	0	7	7	
Total	11	39	50	

Comparison of swirling scores in PRP on Day 5 with Day 1

	Day 1			
Swirling Score	Score 2	Score 3	Swirling Score	p-value
Day 5				
Score 1:	11	1	12	0.000*
Score 2:	0	38	38	
Total	11	39	50	

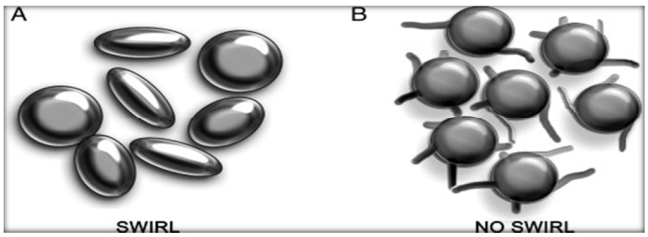

Fig 1: Swirling was evaluated by examining the units against light and scored

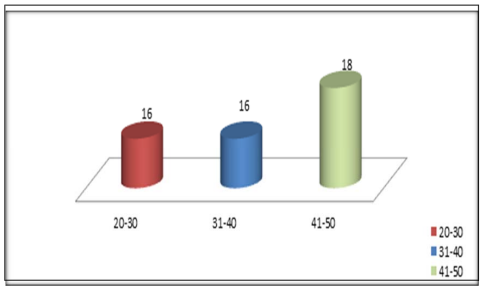

Fig. 2: Distribution of participants according to age group

Table 3: Mean Comparison of Platelet Volume on Day 1, Day 3 and Day 5

	Platelet volume		
Day	Mean		Standard deviation
Day 1	8.06		0.20
Day 3	8.34		0.21
Day 5	8.68		0.19
	Paired difference		
Paired t Comparison	Mean	SD	p-value
Day 1 - Day 3	0.28600	0.06704	0.000*
Day 3 - Day 5	0.33800	0.05675	0.000*
Day 1 - Day 5	0.62400	0.08466	0.000*

Table 4: Mean Comparison of Platelet count/Unit on Day 1, Day 3 and Day 5

	Platelet count/Unit		
Day	Mean		Standard Deviation
Day 1	5.46		0.16
Day 3	5.79		0.26
Day 5	5.57		0.32
	Paired difference		
Paired t comparison	Mean	SD	p-value
Day 1 - Day 3	0.33400	0.23177	0.000*
Day 3 - Day 5	0.22600	0.23803	0.000*
Day 1 - Day 5	0.10800	0.27168	0.007

Table 5: Mean Comparison of pH on Day 1, Day 3 and Day 5

	pH		
Day	Mean		Standard deviation
Day 1	7.02		0.31
Day 3	7.71		0.54
Day 5	7.86		0.59
	Paired difference		
Paired t comparison	Mean	SD	p-value
Day 1 - Day 3	0.68600	0.32514	0.000*
Day 3 - Day 5	0.15400	0.27863	0.000*
Day 1 - Day 5	0.84000	0.32888	0.000*

PH variations: The pH of all samples shows variation on day 1, day 3 and day 5. The standard deviation falls within the limit i.e. 0.31, 0.54 and 0.59. A progressive increase in pH was seen between Day 1, day 3 and day 5. When comparison done by data on day 1 with day 3; day 1 with day 5 and day 3 with day 5 shows a significant difference with paired difference SD is 0.32514, 0.27863 and 0.32888. The observed p value on paired comparison o f these days is <0.001 (Table 5).

Platelet count variations: The variation of platelet count on day 1, day 3 and day 5 shows no significant change in SD. In paired comparison on day 1 to day 3 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. Although comparison of day 1 and day 5 was not statistically significant (Table 4).

Platelet volume variations: The platelet volume of all samples showed slight mean variation on day 1(8.06), day 3(8.34) and day 5(8.68). The standard deviation falls within the limit i.e. 0.20, 0.21 and 0.19. When comparison done by data on day 1 with day 3; day 1 with day 5 and day 3 with day 5 shows a significant difference with paired difference SD is 0.06704, 0.05675 and 0.08466. The observed p value on paired comparison of these days is < 0.001 (Table 3).

DISCUSSION

Platelets are typically held for up to 5 days prior to transfusion, though this can be extended to 7 days in some blood services. PLT and storage medium undergo modifications during storage, which might lead to PLT activation and malfunction. The clinical significance of these modifications is unknown[14]. Platelet activation, metabolic modifications, and platelet senescence are the three broad categories in which platelet changes are characterized. Normal platelet senescence is most likely only a small part of the storage lesion. Platelet concentrates that have been prepared well and then platelet activation. Following Platelet concentrates preparation, visual and electronic quality check is performed on all units prepared[15,16].

In our study 50 samples were subjected to assess the storage effects by observing swirling, volume, pH and platelet count. Platelet whirling is a non-invasive technique for evaluating platelet-rich plasma quality (PRP). Light diffraction generated by the alignment of regular discoid shaped platelets causes platelet swirling. When these discoid align, diffracted light takes on a hazy or whirling look. The presence of the

swirling phenomenon is useful to define platelet concentrates that are suitable for transfusion[17]. In present study, Score 3 swirling was observed in 78% and 14% on respective day 1 and day 3. Score 2 swirling was noticed in 22, 86 and 76 on day 1, day 3 and day 5 respectively. Only 12 unit shows score 1 swirling on day 5. After 5 days of storage the score 3 swirling decreased to none. This drop of swirling could be due to lesions that are known to occur physiologically during platelet preservation. Raveendran *et al.* reported, 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%[18]. Bashir et al in their study reported, on day 0 81% had score 3 and 19% had score 2. On day 3, all the units showed score 2 and on day 5 95% had score 2 and 1% had score of 1[19]. Authors also observed significant difference in the scoring of swirling on day 5 compared to day 1 which is in accordance with the results reported by Raveendran *et al.*[18].

Platelet function deteriorates during production, characterized by aberrant shape alterations, aggregation, and secretory response. Lesions linked with preparative handling and storage are the most common cause of platelet function degradation during preparation[19]. In present study, mean platelet count on day 1, day 3 and day 5 was 5.46, 5.79 and 5.57 respectively. n paired comparison on day 1 to day 3 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 not statistically significant. In a study done by not statistically significant. In a study done by Raveendran *et al*., authors reported On day 1, day 3, and day 5, the mean platelet count of PRP was 6.50 1013/unit, 6.29 1013/unit, and 6.03 1013/unit, respectively. There was a statistical difference between day 1, day 3, and day 5 (p 0.001)[18]. Bashir *et al* in their study reported, mean platelet count in random donor PC stored without PAS was 5.6±0.1×1010/L on day 1 which decreased noticeably to 3.3±0.7×1010/L by day 10(19). Similar results were reported by Horsney *et al.*[20] and Meer *et al*.[21] Another study validated our findings, indicating that platelet survival decreased with increasing storage time, whether platelets were preserved in PAS or plasma, but platelets were better maintaiFebruary 10, 2024ned in PAS. However, Coêlho MJ et al reported a slight increase of 5% from the first to the third day of storage, from 5.45 x 1010 platelets/U on day 1 to 5.80×1010 platelets/U on day 3[11].

During storage, the pH drops. Increased platelet glycolysis causes a drop in pH, which is linked to a significant loss of viability. The pH is a significant indicator of platelet quality in vitro because platelets become spherical at pH values below 6.8 and this shape change is permanent when the pH falls below 6.2. When pH levels fall below 6.0, platelet metabolism stops completely[18]. In the present study Mean±SD value of pH on day 1 was 7.02±0.31, on day 3 was 7.71±0.54 and on day 5 was 7.86±0.59. There was an increase in the pH from 7.02 on day 1 to 7.86 on day 5. Also, When comparison done by data on day 1 with day 3; day 1 with day 5 and day 3 with day 5 shows a significant difference. In accordance to results of our study, Coêlho reported increase in the pH from 7.4 on day 1 to 7.7 from day 3 and also there was a statistically different (p<0.001) from the third and fifth days[11]. Raveendran *et al.* in their study reported, 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[18].

CONCLUSION

On day 5 of preservation, platelet functions appear to be with in normal limits. pH increased somewhat between day 1 and day 5. Although there was no significant variation in platelet count on days 1, 3, and 5, there was a modest mean variation in platelet volume. Variable factors such as the donor's factor, the mixing of blood during collection, and the time delay between phlebotomies and processing may all impact platelet count concentration. In general, this research found that up to 24 hrs has no significant impact on in vitro quality metrics and function. We also urge that quality product implementation begin from the outset of the procedure, i.e. donor selection, and continue throughout the entire process in the department of laboratory medicine/transfusion medicine, including phlebotomy, component separation (centrifugation), and storage (temperature and agitation).

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