

Study of storage induced Morphological and functional changes in Platelet concentrate at 22°C-24°C for 5 days

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Received: 30-11-2021 / Revised: 08-12-2021 / Accepted: 01-01-2022

Abstract

Background and objective: Platelets are routinely isolated from whole blood and stored in plasma for 5 days. The platelets undergo various storage changes which include biochemical, structural and functional changes that occur during platelet storage under blood bank conditions. Study aimed to assess the quality of platelets in platelet concentrate and to evaluate the effects of storage on platelets in platelet concentrate. **Material and methods:** The study was carried out in the Department of Pathology, GMERS Medical College, Gandhinagar. The samples were collected from platelet concentrate bags under sterile conditions. Total 54 platelet samples were assessed using the quality parameters such as swirling, platelet count, WBC count, MPV, PDW and pH on day 0 and day 4 of storage. **Result:** A total of 54 samples were evaluated and of these 95% have fulfilled all 6 parameters of quality control. 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 well maintained within the usual 5 days storage under 22°C to 25°C thus an extension of platelet storage time is recommended.

Keywords: Platelet storage, Mean Platelet Volume, Platelet Distribution Width, Swirling

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Introduction

The platelets are derived from cytoplasmic fragments of bone marrow cells called megakaryocytes that play an essential role in blood clotting and wound healing. They measure 2-3 µ in diameter. The volume of platelet normally ranges from 7-11 fl (called mean platelet volume)[1]. Platelets play a very vital role in hemostasis[2]. Platelets are routinely isolated from whole blood and stored in plasma for 5 days[3]. The energy supplies 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].

The two main reasons for the 5 day shelf life for platelet is bacterial contamination at a storage temperature of 22°C 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 concentrate[5], increasing the storage time of platelet concentrate have always been a challenge in transfusion medicine and many studies have been carried out with the aim of improving it[6,7,8].

Stored platelets experience a progressive decline in function accompanied by characteristic morphologic changes. Studies document up to 20% loss of platelet recovery through five days of storage[9].

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Platelet indices such as platelet count(plt), mean platelet volume(MPV), platelet distribution width (PDW) and platelet-large cell ratio (P-LCR) have been used as a marker for the quality control of platelet concentrate, as these reflect storage induced shape changes in platelet[10]. The assessment of MPV is an imminent test for studying the Platelet Storage Lesion as MPV correlates with morphological changes that occur during storage of PC[11]. The recommended shelf life of PC in presently available platelet storage bags is 5 days at 22±2°C with continuous agitation. The platelets undergo various storage changes include biochemical, structural and functional changes, which may affect the therapeutic benefit to the recipient[12]. The preservation of viable and functional platelets depends on the following factors-

1. Temperature: platelets should be stored at 22-24°C 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 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) maintains 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[12].

4. Platelet count should be more than 4.5×10^{10} in 40-70 ml platelet concentrate[13].

5. WBC count should be less than $5.5 \times 10^7 - 5.0 \times 10^8$ in 40-70 ml platelet concentrate[13].

6. Swirling: Visual inspection of swirling correlates with platelet morphology; the presence of swirling indicates discoid morphology and absence is indicative of spherical morphology[12].

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.

7. MPV: Mean Platelet Volume is a measure of the size of your platelet. MPV is increased when platelet destruction occurs during storage of platelet concentrate[1].

8. PDW: Platelet Distribution Width is a measure of degree of variation of platelet size storage of platelet concentrate[1].

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, and rise in platelet count[14].

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

Material and method

The study was carried out in the Department of Pathology, GMERS Medical College, Gandhinagar. The samples were collected from platelet concentrate bags under sterile conditions. Total 54 platelet samples were assessed using the quality parameters such as swirling, platelet count, WBC count, MPV, PDW and pH on day 0 and day 4 of storage.

Preparation of Platelet Concentrate

The blood donor was selected as per the standard operative procedure followed by the Department. After the selection of a suitable vein blood was collected in blood bag and kept at room temperature and PC was prepared within 4 hours of collection.

The quality of platelet product was assessed on day 0, day 4 using the following parameters:

1. Swirling
2. pH changes
3. Platelet count
4. WBC count
5. Mean Platelet Volume
6. Platelet Distribution Width

Calculation

Platelet count per bag: The platelet count in the sample was analyzed using hematology analyzer (hemax 330) in Clinical Pathology Lab, GMERS Medical College, Gandhinagar.

Platelet count per bag = (volume of PC x concentration of platelet in bag x 10⁶)

WBC count per bag: The WBC count in the sample was analyzed using hematology analyzer (hemax 330) in Clinical Pathology Lab, GMERS Medical College, Gandhinagar.

WBC count per bag = (volume of PC x concentration of WBC count in bag x 10⁹)

pH changes of PC: Analyzed using pH indicator strip.

Swirling: Evaluation of swirling is a simple non can be performed invasive method that by visual inspection.

Table 1: Quality Control of platelet concentrates were analysed using following criteria

Parameter	Quality Requirements
Volume	50-70 ml
Platelet Count	≥ 4.5x 10 ¹⁰
pH	>6
RBC Contamination	< 0.5 ml
WBC Contamination	< 5.5x 10 ⁷ – 5 x 10 ⁸

Result

Swirling in PC: Score 3 swirling was observed in 3.7% and 1.9% on respective day 0 and day 4. Score 2 swirling was noticed in 90.7% and 85.2 % on respective day 0 and day 4. Only 7 (12.9%) unit showed score 1 swirling on day 4. This drop of swirling could be due to lesions that are known to occur during platelet preservation. But 95% of the units show the swirling score within the recommended limits of quality requirements.

Table 2: Comparison of swirling grading on Day 0 and Day 4

Grading of Swirling	Day 0	Day 4
Score 0	00 (00)	00 (00)
Score 1	03 (5.6 %)	07 (12.9 %)
Score 2	49 (90.7 %)	46 (85.2 %)
Score 3	02 (3.7%)	01 (1.9 %)
Total	54 (100 %)	54 (100 %)

pH, Mean platelet volume and platelet distribution width

In the present study of 54 units of PC, mean of pH on day 0 was 6.98 and on day 4 were 6.61. The standard deviation falls within the limit of 0.26. The pH ranged from 6.5 to 7.6 and no significant difference

was being observed in these 4 days. The observed values of pH fulfilled the quality control criteria for platelet concentrate.

Observed value of MPV was higher and had shown significant change at day 4. SD was 2.14PDW has also shown significant increase at day 4 with SD of 1.51.

Table 3: Comparison of parameters (pH, Mean platelet volume, platelet distribution width) on Day 0 and Day 4

Parameters	Day 0 (Mean)	Day 4 (Mean)	SD
pH	6.98	6.61	0.26
Mean platelet volume	10.57	13.61	2.14
platelet distribution width	15.40	17.54	1.51

Platelet count and WBC contamination

The variation of platelet count in PC on day 0 and day 4 showed no significant change in SD. The values are fall within the limits of recommended quality control values of platelet concentrate. In 54 samples of PC, WBC count variation between day 0 and day 4 has not shown any significant change in SD, with SD of 0.49.

Table 4: Comparison of Platelet count and WBC Count on day 0 and day 4

Parameters	Day 0 (Mean)	Day 4 (Mean)	SD
Platelet count	7.30	6.3	0.71
WBC contamination	3.95	4.64	0.49

Conclusion

Platelets storage remains a major challenge to transfusion services. Platelets indices such as pH, platelet count, WBC 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 changes in platelets.

In our study all units showed adequate swirling and platelet count in PCs. All the platelet concentrates units had well maintained pH at day 4. 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. Platelets showed significant changes in shape and size even after adequate storage condition.

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