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Biochemical characteristics and functional performance of cold-stored platelets: an in-vitro comparative study

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ABSTRACT

Background: Platelet refrigeration could eliminate bacterial contamination and improve the hemostatic function even better than already-used room-temperature storage. This study aimed to assess the effect of cold storage, with and without agitation, on the apheresis platelets' hemostatic, metabolic, and functional activity.

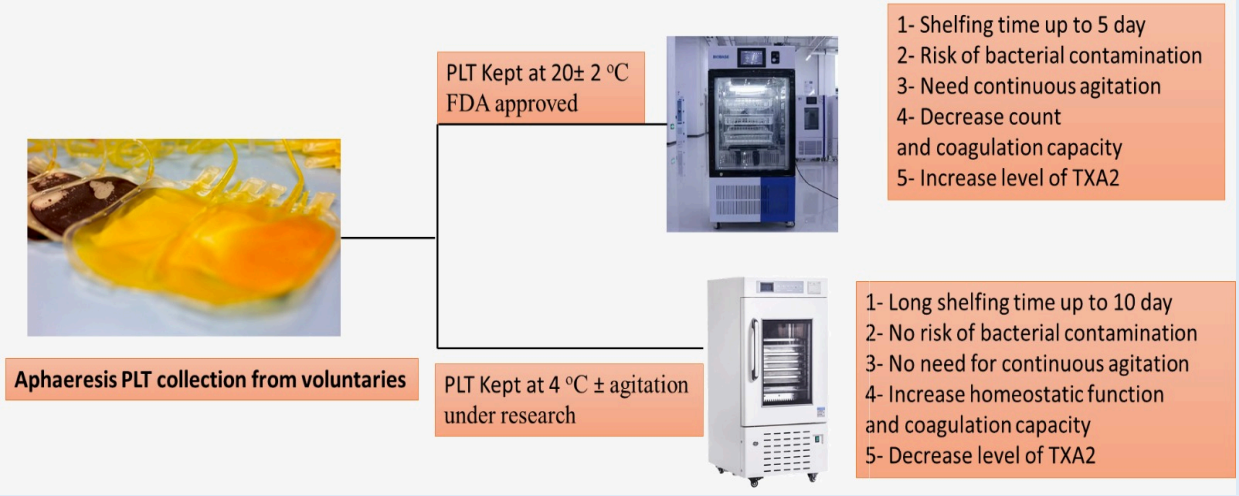
Materials and methods: The study included 10 healthy volunteer donors to collect Apheresis PLT. They were submitted to careful clinical examination and standard laboratory workup. Collected samples were processed in accordance with American Association of Blood Banks (AABB) guidelines. Every aliquot collected from each volunteer was stored for up to 5 days at one of the following storage conditions: 1. In an FDA-approved-PLT incubator with agitation at room temperature (RT + AG as a group; GI), 2. In an FDA-approved-refrigerator at 4 °C with agitation (4 °C + AG as a group; GII), 3. In an FDA-approved- refrigerator at 4 °C without agitation (4 °C – AG as a group; GIII). The following PLT workup was done; PLT count and mean platelet volume (MPV), metabolic variables, PLT aggregation studies, PLT receptors expression, and PLT pro-inflammatory mediator's release.

Results: All samples had a significant PLT count decline compared to baseline data. No changes in MPV were observed in all groups on day 3 and day 5, meaning that single PLT size remained unchanged. In addition, GI showed a mark of significant increase in metabolic activity when compared to baseline PLTs in contrast to GII, and GIII, which were more metabolically stable and less active.

Comparison between the studied groups regarding PLT aggregation revealed significantly higher PLT aggregation response to ADP and collagen in GII and GIII compared to GI on the 3rd and 5th days. Moreover, it was shown that GII and GIII samples had significantly higher CD62p expression when compared with GI on the 3rd and 5th days despite being less active and more stable. While it was found that TXB2 levels were significantly higher, nearly 3-fold, in GI as compared to GII and GIII.

Conclusions: Apheresis platelets (AP) cold storage provides a clear advantage over standard conditions regarding biochemical balance and hemostatic performance, which could markedly improve AP's clinical and economic value in different scenarios.

Keywords: Platelet aggregation, P-selectin, Thromboxane B2.



INTRODUCTION

To reduce the danger of bacterial contamination that might result in fatal transfusion-related reactions, AABB recommends that PLTs must be maintained in FDA-approved- incubators at 22 °C (room temperature) with mild agitation for not more than 5 days [1]. However, this short storage time led to many economic problems in blood banks. In addition, it resulted in the so-called platelet storage lesion which is associated with a marked decrease in platelet hemostatic function [2, 3].

Compared to room-temperature storage, platelet refrigeration may reduce the danger of bacterial contamination and better protect the hemostatic function. Other advantages include a lower risk of

transfusion-related reactions, lower costs, better hemostasis in patients with severe bleeding, and a shelf-life extension of up to 10 days [4, 5].

This research assessed the effect of cold storage on the hemostatic, metabolic, and functional activity of the Apheresis platelet (AP).

MATERIALS AND METHODS

The current research was carried out at Al-Azhar University Hospitals (AL-Husieen and Damietta), with AL-Shabrawishy Blood Bank, Cairo, Egypt. The Al-Azhar Faculty of Medicine's ethics council accepted the research procedure. Apheresis platelet (AP) units were collected in acid-citrate-dextrose (ACD)-plasma from each healthy volunteer (n=10). Before enrolling in the research, participating volunteers provided informed consent and submitted to careful clinical examination and standard laboratory workup in accordance with American Association of Blood Banks (AABB) guidelines. In addition, collected samples were subjected to the following processes.

Collection of Apheresis platelets and storage of aliquots: Trima Accel Automated Blood Collection System (Terumo BCT, Lakewood, CO, USA) was used to collect AP from healthy volunteer donors (n=10). Collected AP from each volunteer was then sub-divided into 3 equal aliquots (one for each group; GI, GII, GIII) and preserved in 120 ml platelet-transfusion bags (JMS, Lot No: 201129001) using FDA -approved blood bank welding machine (Terumo TSCD-3.USA) [6]. Every aliquot from each volunteer was stored for up to 5 days at one of the following storage conditions:

Group I: In an FDA-approved platelet incubator with agitation at room temperature (RT + AG).

Group II: In a refrigerator at 4 ^{o}C with agitation (4 ^{o}C + AG group).

Group III: In a refrigerator at 4 ^{o}C without agitation (4 ^{o}C – AG group).

Samples stored at 4 °C were allowed to come to RT for 25 min and gently massaged before testing procedures.

Assessment of platelets quality: The impact of various storage conditions on platelets aliquots was examined on the 1st day of collection (the baseline sample), on day 3, and day 5, and assessed parameters included the following:

1. Platelet count and mean platelet volume (MPV); were determined using an automated blood cell counter (Beckman Coulter Counter, Brea, CA).
2. Metabolic variables: Using an iSTAT point-of-care device (Abbot Labs, Abbott Park, IL). The following values were determined: pCO_2 (mmHg), blood pH, pO_2 (mmHg), sodium (mM), bicarbonate (mM), chloride (mM), glucose (mg/dL), and potassium (mM).
3. Platelet aggregation response; using adenosine diphosphate (ADP) and collagen as agonists (Crono-log business, USA) on platelet aggregometer (PAP-4CD Laboratory Analyzer, Biodata, Horsham, PA).
4. Cross-color flow cytometry (BD FACSCalibur, BD. Biosciences, San Diego, CA) expressed platelet surface receptors. Cell lineage-specific antibodies anti-human CD42b, also known as the glycoprotein Ib receptor, or "GPIb" were utilized to recognize

platelets (PLT gating). In addition, the CD62p receptor (P-selectin receptor) was also examined as a marker of PLT activation.

5. Platelets pro-inflammatory mediators; released in plasma using ELISA commercially available kits for thromboxane B2 (TxB2) levels (Cayman Chemicals, Ann Arbor, Michigan, USA).

Statistical analysis: Data were analyzed using SPSS 26.0 (SPSS Inc., Chicago, Illinois, USA), version 23.0. The quantitative data were presented as mean ±standard deviation and ranges. Qualitative variables were presented as numbers and percentages. A one-way analysis of variance (ANOVA) test was done when comparing more than two means. The confidence interval was set to 95%, and the margin of error accepted was set to 5% [7].

RESULTS

This study stored AP samples obtained from 10 healthy volunteers in three different conditions for up to 5 days.

1. Platelet count and MPV: Results showed that all samples significantly declined platelet count compared to baseline data. Moreover, it was demonstrated that GI samples had significantly higher platelet counts on the 3rd and 5th days compared to the other two groups. On the other hand, no significant differences were found between platelet count in GII and GIII on the 3rd and 5th days. Furthermore, no significant differences between all groups regarding MPV throughout the storage days were found, which means that single PLT size remained unchanged, as shown in Table 1.

Table 1. Platelets parameters in the studied groups.

	Baseline	GI (RT + Ag)		GII (4°C + Ag)		GIII (4°C – Ag)	
		3rd day	5th day	3rd day	5th day	3rd day	5th day
PLT count ×10^3 /mL	1201.2 ± 20.6	1133.2 ± 13.3*	1098.6 ± 42.9*#	1088.0 ± 29.6*§	1024.8 ± 8.6*#¥	1087.0 ± 30.2*§	1006.1 ± 12.6*#¥
MPV fl	7.0 ± 0.17	7.46 ± 0.2*	7.73 ± 0.16*#	7.37 ± 0.21*	7.66 ± 0.2*#	7.37 ± 0.24*	7.56 ± 0.15*#

Data is expressed as mean and standard deviations.

*Significant versus baseline, # Significant versus 3rd day same group, § Significant results versus GI 3rd day, ¥ Significant versus GI 5th, day, ∑ Significant versus GII 5th day.

2 Platelet aggregation: Comparison between the studied groups regarding platelet aggregation response to ADP and collagen revealed significantly higher platelet aggregation response to ADP and collagen in GII and GIII compared to GI on the 3rd and 5th days and nearly as well as baseline PLT (fresh PLT) as shown in Figure 1 A, B.

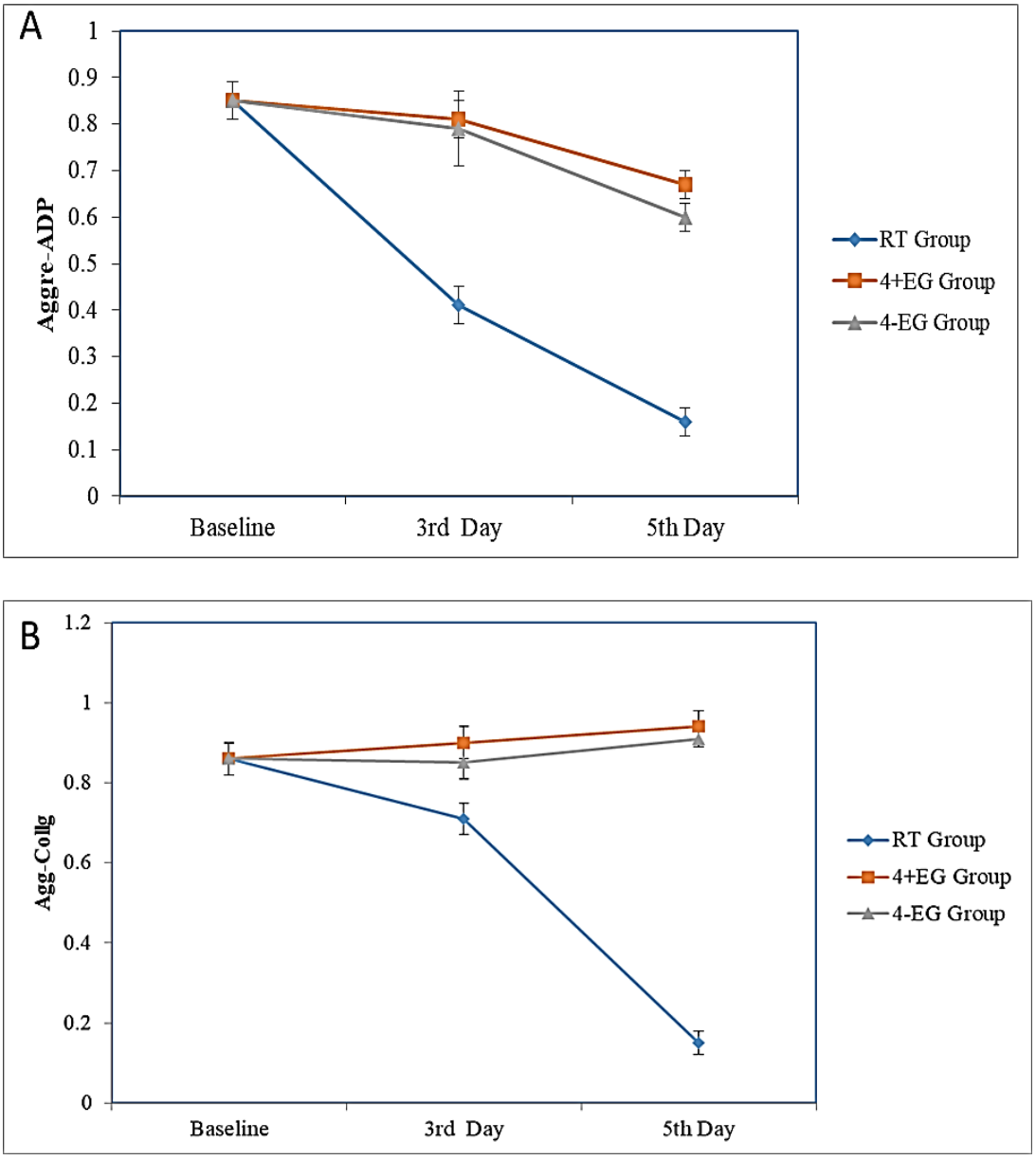


Figure 1. A) Platelets aggregation response with ADP agonist during storage, B) Platelets aggregation response with Collagen agonist during storage.

3-Platelet metabolic parameters; GI showed a marked significant increase in metabolic activity when compared to baseline PLT. However, in contrast to GII, GIII was metabolically stable and less active as follows:

There was a marked decline in the pH of G1 at day 5 (-16% decrease from baseline) when compared to GII and GIII (-3.5% drop from baseline). However, all samples were maintained within the acceptable range of pH, i.e., (6.3 and 7.3). Similar findings were noted with respect to PCO_2 and HCO_3. PO_2 showed significant elevation from baseline in all groups. Glucose levels significantly declined in all groups in comparison to baseline data. However, GII and GIII samples had significantly higher glucose values in comparison to GI since they are less active and consume less glucose, as shown in Table 2 and Figures 2 A, and

Table 2. Biochemical findings in the studied groups.

	Baseline	GI (RT + Ag)		GII (4°C + Ag)		GIII (4°C – Ag)	
		3rd day	5th day	3rd day	5th day	3rd day	5th day
pH	7.31±0.02	6.77±0.28*	6.30±0.18*#	7.11±0.19*§	7.06±0.19*¥	7.11±0.19*§	7.08±0.18*¥
PO_2 mmHg	92.49±1.83	106.42±5.85*	131.16±4.31*#	107.54±6.14*	130.40±4.07*#	106.60±6.0*	129.01±3.84*#
PCO_2 mmHg	37.82±3.23	14.12±1.24*	12.15±0.92*#	26.69±2.56*§	20.66±1.24*#¥	26.20±2.65*§	20.25±1.14*#¥
HCO_3 Mm	17.75±1.52	11.69±1.27*	6.11±0.48*#	14.41±0.93*§	12.01±0.80*#¥	14.34±0.96*§	11.85±0.52*#¥
Na mmol/l	137.66±1.08	139.89±1.17*	141.04±0.98*#	138.95±0.79*§	140.08±0.69*#¥	138.61±1.07*§	139.63±1.20*#¥
K mmol/l	3.21±0.14	3.31±0.09*	3.48±0.10*#	3.29±0.09*	3.42±0.07*#	3.29±0.07	3.48±0.07*#
Cl mmol/l	97.72±0.52	100.97±1.24*	104.72±1.11*#	97.55±0.46§	97.31±0.45¥	97.47±0.46§	97.34±0.46¥
Glucose mg/dl	335.02±9.29	269.45±12.40*	231.40±15.53*#	315.45±7.93*§	300.35±10.55*#¥	306.85±11.14*§	300.60±10.59*#¥

Data is expressed as mean and standard deviations.

*Significant versus baseline, # Significant versus 3rd day same group, § Significant results versus GI 3rd day, ¥ Significant versus GI 5th, day, Ω Significant versus GII 3rd day, ∑ Significant versus GII 5th day.

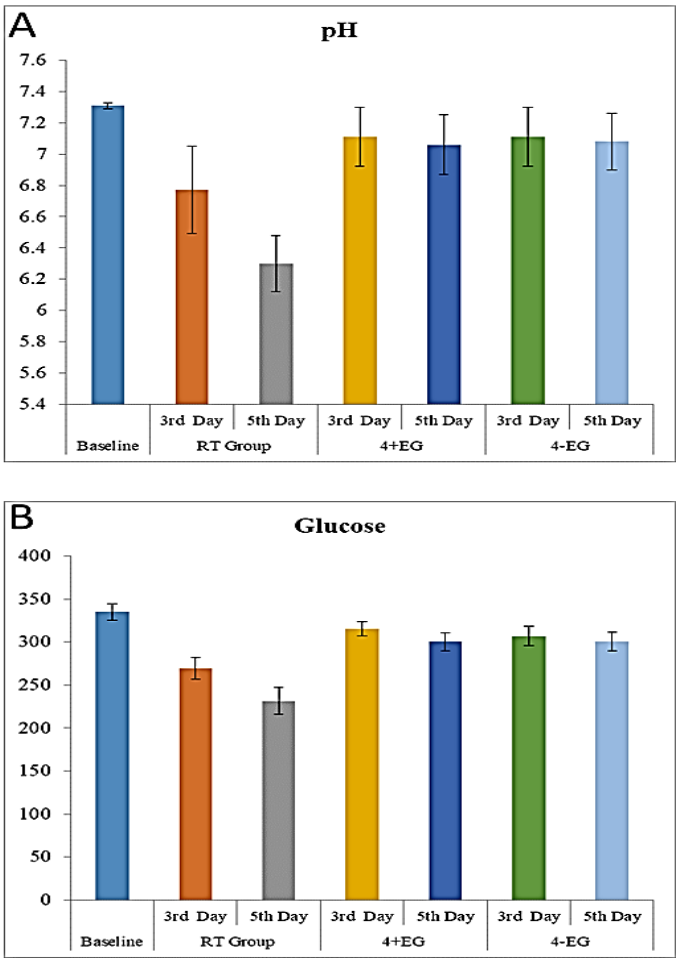


Figure 2. Comparison between baseline and other groups according to A) pH, and B) glucose.

4-Platelet activation receptor expression; It was shown that all groups expressed significantly higher CD62p expression as compared to baseline expression. Moreover, it was shown that GII and GIII samples had significantly higher CD62p expression when compared with GI on the 3rd and 5th days, as shown in Figure 3.

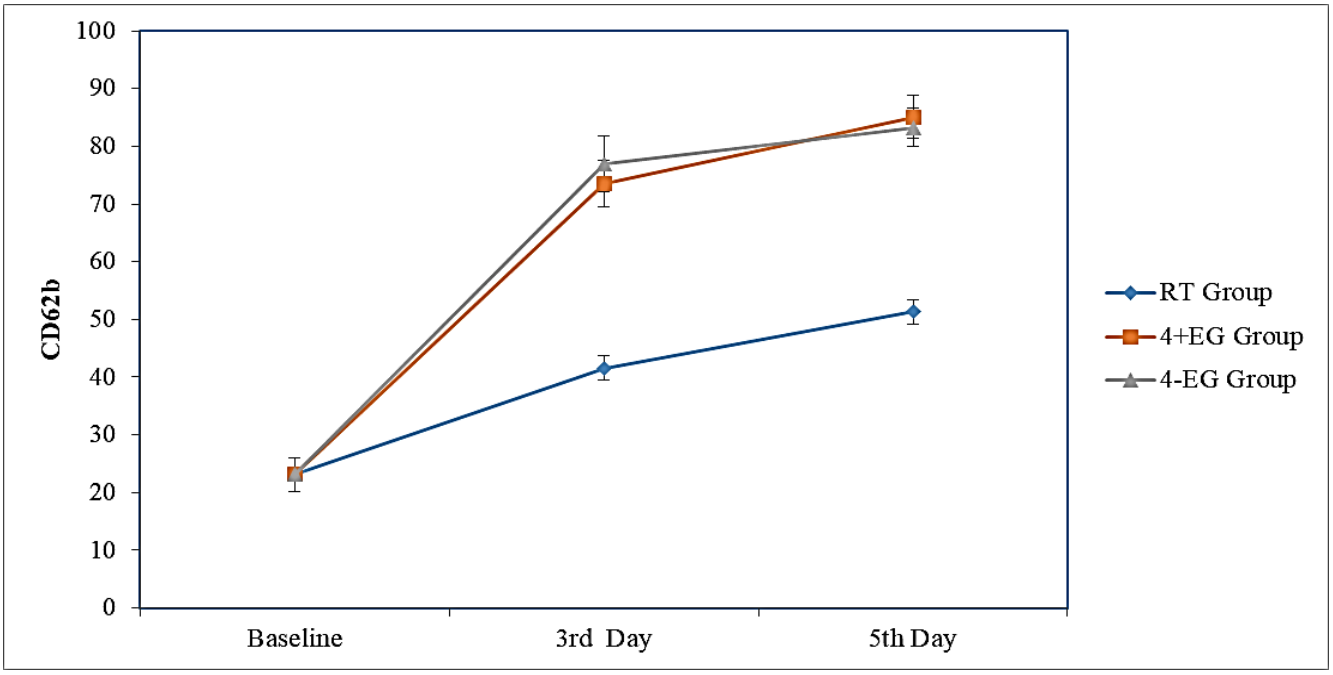


Figure 3. Platelets surface receptor CD62 (P-Selectin) expression levels during storage.

5-Platelet released pro-inflammatory mediators; It was found that all groups had significantly higher TXB2 levels compared to the baseline. TXB2 levels were significantly increased in GI compared to GII and GIII on the 3rd and 5th days, as shown in Figure 4.

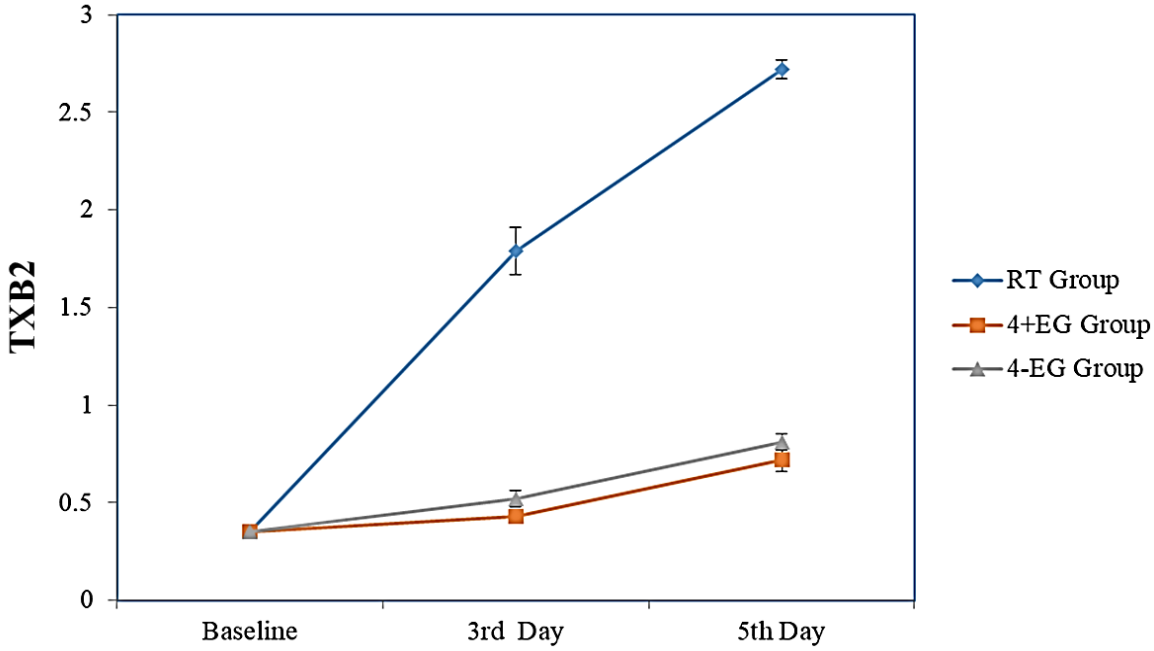


Figure 4. ELISA quantitative assessment of soluble factors TXB2 released by platelets during storage.

DISCUSSION

Despite shifting from CP to RP in the 1970s, at this time, CPs were better for severely bleeding patients to stop bleeding, as they shortened the bleeding time by making stronger PLT clots [8].

Our study demonstrated that AP stored at cold temperatures (CP) is superior in metabolic and hemostatic functions, and aggregates are more effective than platelets stored under standard RT.

However, it was found that continuous PLT agitation does not generally produce any effect on the function and quality of CP unlike RP. RP is of great economic benefit due to the reduction in the cost of transportation and storage of platelets together with the prolongation of shelving time of CP and eliminating the risk of bacterial contamination. This makes CP an excellent choice to stop acute hemorrhage when immediate hemostasis is required.

During PLT storage, a lot of changes occur. Therefore, they are collectively called PLT storage lesions, which include PLT activation, degranulation, and release of pro-inflammatory mediators, along with declined PLT aggregation capacity and clot strength. Also, storage lesions reduce the in-vivo transfused PLT activity, survival, and recovery [9].

Factors responsible for PLT changes during storage include donor selection, collection methods, storage conditions and duration, leukoreduction, and pathogen reduction [10].

In the current investigation, RP showed a significant decline in pH and bicarbonate levels at day 5 compared to a hardly detectable decline in HCO_3 in CP, as shown in Table 2; Figure 2A. This could be the result of RP vigorously metabolizing glucose via the glycolytic pathway, producing lactate and free hydrogen ions in these processes, and trying to buffer this acidity by increasing HCO_3 consumption with the production of CO_2, reported similar findings [11, 12].

Despite that, both RT and CP storage conditions promote PLT activation. However, they show different degrees of sensitivity to chemical agonists. For example, cold-stored PLT aggregate is markedly better when activated with ADP and collagen than RT-stored PLT, as shown in Figure 1. This is consistent with prior research on collecting PLT using buffy coat and apheresis procedures and holding them at room temperature, which was explained by PLT aging and progressive decline in the energy-generating mechanism in PLTs [13-15].

Conversely, CP aggregates to ADP and collagen agonists are almost as effective as fresh PLT, which may be because cold-induced PLT activation by various methods that "primes" the PLT to a state of heightened responsiveness without impairing the initial reaction to stimuli. This was attempted to be explained by [16], who investigated how cold storage affected PLT response to ADP. They discovered that owing to persistent P2Y12 activity and concurrent breakdown of inhibitory pathways, which allows for a better reactivity of stored platelets to ADP under cold circumstances. PLT is metabolically active during storage. Increased metabolic activity together with glucose consumption during storage leads to apoptosis and loss in mitochondrial membrane potential with consequent release of a-granule contents (CD62P released from Plts a-granules), which is externalized from the inner leaflet of the platelet membrane to the surface. Upon activation, the CD62p on the PLT surface plays a major role in hemostasis, coagulation, and wound healing [17].

The current investigation discovered that CP expressed more CD62p than RP despite RP being more metabolically active, as shown in Figure 3. In addition, RP released nearly 3 times more TxB2 than CP after 5 days of storage, which correlates with PLT activation, as shown in Figure 4. No significant difference between agitated and non-agitated CP was observed. Similar findings were documented in previous studies [18, 19].

This could be explained by distinct PLT activation mechanisms that are activated at various storage temperatures. At room temperature, PLT are metabolically active, which results in the generation of oxidative stress products that stimulate apoptosis and the dying of PLT. PLT kept at cold temperatures appear to be less activated than those kept at room temperature and subjected to cytoskeletal changes that include rapid, irreversible disc-to-sphere shape changes, GPIb receptor clustering, CD62p (P-selectin) exposure, and a set of biochemical pathways that are distinct from aging and apoptosis in RP [20-23].

Additionally, PLT that have been cold-stored and cryopreserved exhibit better clot strength and durability because cold-temperature storage 'primes' platelets to a state of activation and enhanced responsiveness [24, 25].

During PLT activation, arachidonic acid undergoes oxidation, resulting in TxB2. It functions as a strong platelet activator and plays an important role in coagulation and wound healing, raising the possibility of coagulation defects during transfusion and transfusion-related acute lung injury (TRALI) [26, 27]. In the current investigation, Low TxB2 release from CP suggests that arachidonic acid is relatively preserved, which may account for keeping the aggregation response to ADP and collagen almost as nearly as fresh platelets. This allows us to suggest that CP may be a safer transfusion option than RP because it may cause fewer adverse events, such as TRALI.

Our findings lend credence to the idea that cold-stored platelets are a superior treatment option in cases of severe bleeding. There are three basic causes for this. Firstly, cold storage considerably slows the development of bacteria and other microbes in platelet units, decreasing loss from out-of-date products. Secondly, CP has been shown to have stronger aggregation and clot strength than RT-PLTs in terms of hemostasis. Finally, CP does not seem to need mechanical agitation during storage, but R.T.-PLT does. By doing this, mechanical shaker costs are eliminated, and logistical handling for delivering platelets is improved.

Additionally, recent research on using PLT for transfusion has shown that the requirement for therapeutic PLT transfusions is much higher than the need for preventive transfusions. This is despite the post-transfusion short half-life of cold PLTs when compared with room temp- stored PLT [28-30]. However, superior hemostatic function and longer potential shelf-life make CP promising for actively bleeding patients to save lives and control bleeding [31, 32].

CONCLUSION

Apheresis platelets cold storage provides a clear advantage over standard conditions, being less metabolically active and having better hemostatic performance. In addition, aggregates are more effective than PLTs stored under standard RT, even near fresh PLT. However, continuous PLT agitation is not required in CP, unlike RP. RP provides great economic benefit due to the reduction in the cost of transportation and storage of PLT together while prolonging the shelving time of CP. This reduction could markedly improve the clinical and economic value of AP used in different scenarios. However, further investigation might solve the problem of the post-transfusion in-vivo short half-life of cold PLT

List of Abbreviations: 4C: Storage at 4 °Celsius; 4C+AG: Storage at 4 °Celsius with gentle agitation; 4C-AG: Storage at 4 °Celsius with gentle agitation; AABB: American Association of Blood Banks; ACD: Acid-citrate-dextrose: ADP: Adenosine diphosphate; AP: Apheresis platelets; CD62P: Cluster of differentiation 62 p; CP: Cold PLT; ELISA: Enzyme-linked immunosorbent assay; FDA: Food and Drug Administration; GPIb: Glycoprotein Ib

receptor; MPC: Mean platelet component; MPV: Mean platelet volume; PLT: Platelets; RBC: Red blood cell; RT: Room temperature; TRAP: Thrombin receptor activating peptide; TxB2: Thromboxane B2

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