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Exploring Siglec-7 Expression and Apoptosis Rates in Platelet Concentrates under Various Storage Conditions

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ABSTRACT

Purpose: Platelets play a pivotal role in primary hemostasis, tissue repair, inflammation, and immunity. However, their short shelf life under storage conditions, coupled with the rising demands for platelet concentrates (PCs), has resulted in the paucity of these products in recent years. We investigated platelet apoptosis levels and the surface expressions of Siglec-7 (CD328) and P-Selectin (CD62P) on platelets at two different conditions.
Methods: In this descriptive study, PCs were stored at 4oC and 24oC conditions. The expressions of Siglec-7, Annexin V, and CD62P on stored platelets were then measured by flow cytometry in the time span of 72 hours. Additionally, platelet counts were performed at each stage of the study.
Results: The expression levels of both markers Siglec-7 and Annexin V showed a marked increase under both storage conditions over the 72-hour period. Furthermore, their expression was higher in 4oC compared to 24oC ($P<0.05$). Although CD62P expression on platelets increased throughout the experiment (4oC > 24oC), it declined by approximately 7% during the last 24 hours at 24oC.
Conclusion: The results of this study suggest a potential association between elevated Siglec-7 levels over a 72-hour period at both temperatures and platelet apoptosis. These preliminary results indicate that Siglec-7 may warrant further investigation as a molecular target for potentially extending platelet shelf life and improving the storage conditions of platelet products.

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Introduction:

Platelets are the smallest blood cells, which have an average diameter of 2.5 µm and approximate count of 140-440 x 10^3/µL, play an essential role in initial hemostasis and thrombosis. Changes in quantity or quality of the platelets lead to bleeding disorders. In addition, platelets can fight infections, encourage inflammation to induce tumor angiogenesis and metastasis pathways, produce inflammatory factors and support wound healing.[1,2] Platelet concentrates (PCs) are one of the most essential blood components, widely used to control bleeding in various cases, including thrombocytopenia, qualitative abnormalities of platelets, and patients undergoing surgery. In recent years, the demand for these products has been increasing, largely due to the rising number of patients with bone marrow failure.[2,3] Given the short shelf life of 3-7 days, platelets have the highest wastage compared to other blood products,[4] which may lead to the shortage of these products.

Platelet storage lesion (PSL) is a key factor contributing to the short shelf life of platelets. It refers to a succession of structural, functional, and biochemical variations occurring in platelets from phlebotomy to transfusion.[5,6] Apoptosis, or programmed cell death, which is the main physiological mechanism in controlling cell lifespan and elimination of damaged cells, is a principal factor influencing PSL.

Apoptosis in platelet products can occur under the influence of various external triggers such as thrombin, calcium ionophore A23187, platelets antibody, high stress induced from physical conditions, and prolonged storage time.[7-9] Unlike other blood products stored in cold conditions, platelet components are maintained at 20-24ºC with continuous gentle agitation for 3-7 days. Some studies have been shown that the amount of PSL at 4ºC is generally lower than that of 22ºC. However, the swift clearance of platelets stored at 4ºC following transfusion has led to the exclusion of this storage condition method from standard protocols.[10-13] Consequently, identifying the root cause of their rapid removal from the bloodstream is crucial.

P-selectin (CD62P) which is situated in platelets' alpha granules has an important function in thrombotic and inflammatory reactions, and is recognized as a key marker for the activation of platelets. When comparing platelets stored at 4ºC and 24ºC, it is apparent that the CD62P surface expression level at 4ºC is higher.[12,14,15] CD62P is in a close correlation with Sialic acid-binding Ig-like lectin-7 (Siglec-7).[16] Siglec-7, also known as CD328, belongs to the Siglec's family and is found on surfaces of numerous cells, including T-Lymphocytes, monocytes, NK cells and platelets. It has been demonstrated that elevated Siglec-7 expression on platelets, along with stimulation by its ligands, i.e., GD2, culminates in the apoptosis process of the platelets.[17,18] Considering the close association between P-selectin and Siglec-7, it would be possible that Siglec-7 levels may vary in platelets stored at 4°C in comparison to those stored at 24°C.

Owing to increasing necessities for platelet products in hospital wards and their short shelf-life, a critical scarcity of these products may arise; therefore, identifying an effective solution to extend the half-life of stored platelets is pivotal. Here we undertook research into the assessment of the expression of apoptosis markers including Siglec-7, Annexin V, and P-Selectin on platelets stored at two different temperatures of 4ºC and 24ºC.

2. Materials and Methods:

2.1. Sample Collection

In the current study, the whole blood was taken from healthy volunteers (N=5) referred to Tabriz Blood Transfusion Organization (TBTO) and collected in citrate phosphate dextrose adenine (CPDA-1) anticoagulated bags. All healthy volunteers had a normal platelet count without taking aspirin or aspirin-like anti-inflammatory

drugs 72 hours prior to blood donation. Informed consent was obtained from all donors prior to donation. Platelet concentrates were prepared according to international standard operation procedures at TBTO.

2.2. Platelet Preparation and Handling

At time zero (T0), approximately 3 mL of content was extracted from each PC through the attached cords. Subsequently, half of each bag's contents were displaced to another attached bag by means of their cords. One of these bags was stored at 4°C (Refrigerator, RF), while the other was kept at 24°C (Room Temperature, RT) under mild agitation. In the end, using sterile syringes, sampling was done at the 24, 48, and 72-hour time points (T24, T48, and T72) for both storage conditions. All experiments were conducted under sterile conditions to ensure accuracy and reliability.

2.3. Evaluation of Platelet Markers

In order to determine platelet-related apoptosis markers, collected cells (1×10^5/ml) from each group were analyzed using flow cytometry. Initially, the cells were washed, suspended in phosphate-buffered saline (PBS), and labeled on ice (4°C) with anti-Siglec-7-PE (eBioscience Inc., San Diego, CA, USA), FITC-Conjugated Anti-Annexin V (Miltenyi Biotec), and anti-CD62P-PE (BD Bioscience, San Jose, CA) for 45 minutes. Each antibody was added at a volume of 5 µL (0.5 µg) per test, following the manufacturer's instructions.

The cells were then rewashed again and resuspended in PBS, which served as sheath fluid for flow cytometric analysis. Data were acquired by FACSCalibur flow cytometer equipped with the CellQuest software package (BD Biosciences), and subsequent analysis was conducted using Flowing software (Turk University, Finland). Additionally, FITC- and PE-conjugated mouse IgG1 (eBioscience, San Diego, CA) were used as isotype controls.

2.4. Statistical Analysis

All measurements were performed in triplicate for each sample across all temperature and time conditions. Data obtained from the study were presented as mean ± SD and analyzed by GraphPad Prism v5.00 (GraphPad Software, San Diego, CA, USA). For statistical analysis, repeated measures ANOVA was used to assess changes over time within the same storage condition. Additionally, a paired t-test was conducted to compare the two storage temperatures (4°C and 24°C) at each time point. A p-value < 0.05 was considered statistically significant for all experiments.

3. Results:

3.1. Platelet Count

In the blood samples of five volunteers, the initial number of platelets was within normal parameters (140-440 x 10^3/µL). Platelet counts for all samples were measured using a Sysmex KX-21N cell counter (Sysmex Corporation Kobe, Japan). As shown in ***Figure 1***, platelet concentrates stored at 24°C demonstrated a modest and progressive decline over the 72‑hour storage interval. In contrast, units maintained at 4°C exhibited a markedly sharper reduction, with the most pronounced drop observed at 72 hours relative to their respective T0 (fresh) values. Across all time points, platelet retention remained consistently higher under 24°C compared with 4°C storage condition; however, these differences did not reach statistical significance ($P > 0.05$).

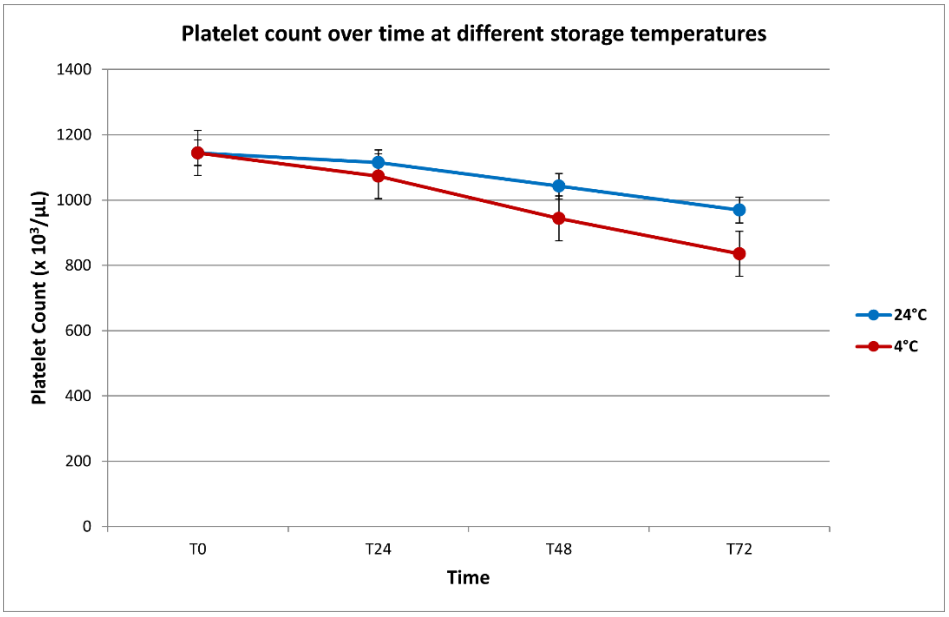

Figure 1. Platelet counts changes of stored platelets at 24°C and 4°C during a 72-hour time period. Data are presented as mean ± SD.

3.2. Siglec-7 (CD328) expression

Storage temperature significantly impacted the expression levels of Siglec-7 on platelets over a 72-hour period. While both refrigeration (RF, 4°C) and room temperature (RT, 24°C) storage resulted in a progressive increase in Siglec-7 expression, platelets stored under refrigeration consistently showed higher levels compared to those at room temperature. This difference became statistically significant by the end of the observation period (T72, P=0.03), suggesting that cold storage may promote or preserve Siglec-7 expression more effectively. (***Figure***)

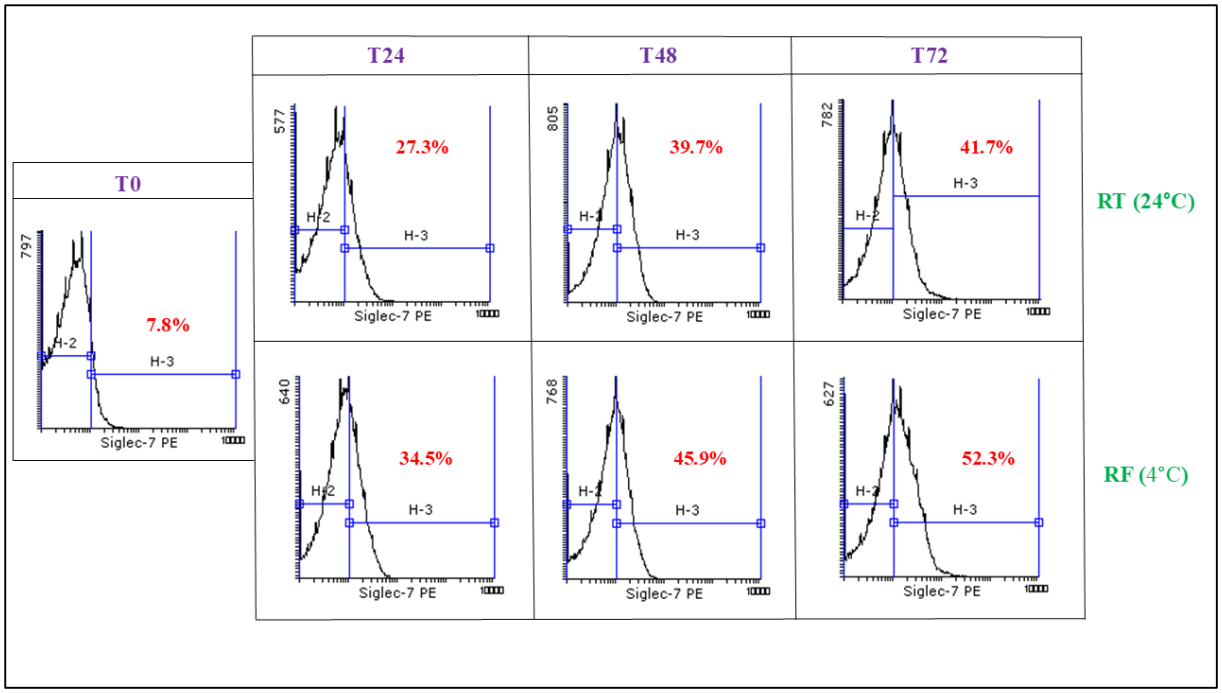

Figure 2. Flow cytometric analysis of Siglec-7 (CD328) expression in platelets stored at 4°C (RF) and 24°C (RT) over a 72-hour period. Both storage conditions showed an upward trend in Siglec-7 expression, with platelets stored at 4°C consistently exhibiting higher levels in comparison to those maintained at 24°C. Statistical analysis using a paired t-test confirmed a significant difference between the storage conditions at the T72 time point (P=0.03).

3.3. The levels of Annexin V expression

Annexin V expression, a marker of platelet activation and apoptosis, exhibited a progressive increase under both storage temperatures (4°C and 24°C) throughout the 72-hour study period. Although both conditions showed a rise in expression, platelets stored at 4°C consistently displayed higher levels of Annexin V compared to those stored at 24°C, with the difference reaching statistical significance at the 72-hour mark (P=0.04). These observations indicate that temperature-dependent variations in storage conditions significantly influence the rate of platelet apoptotic changes. (***Figure***)

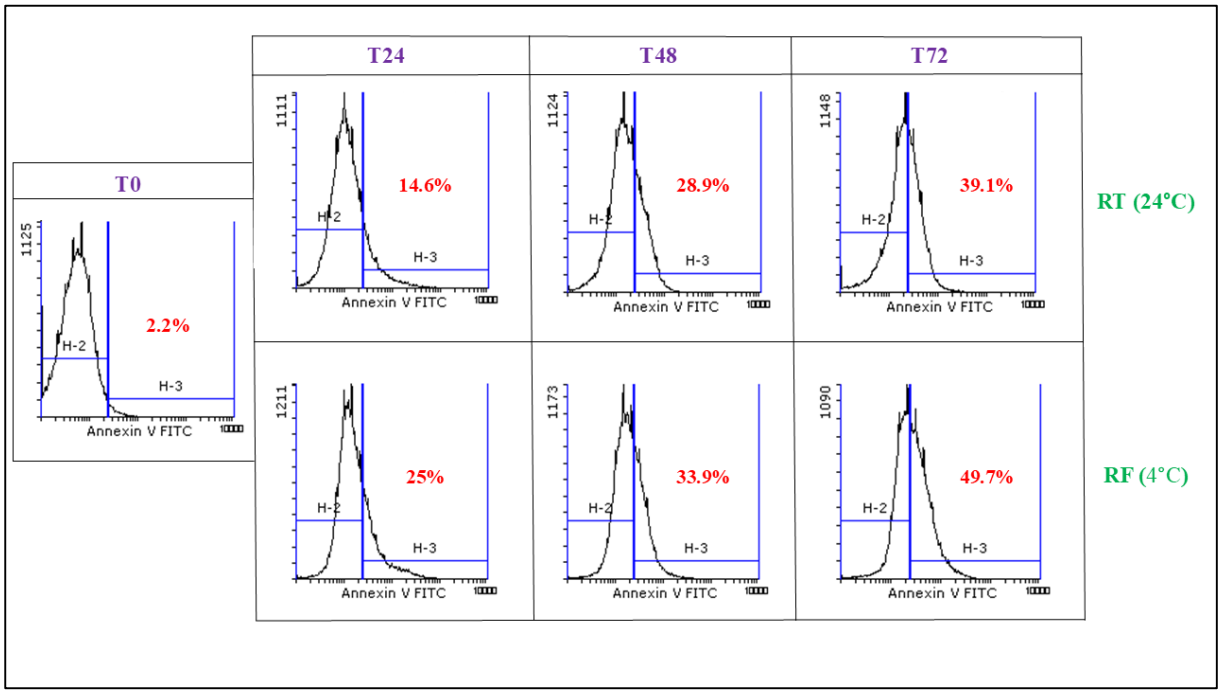

Figure 3. Flow cytometric evaluation of Annexin V expression in platelets stored at 4°C (RF) and 24°C (RT). Over the 72-hour storage period, Annexin V expression levels increased under both temperature conditions. However, platelets stored at 4°C steadily exhibited a higher expression level compared to that maintained at 24°C.

3.4. Evaluation of P-Selectin Expression

P-selectin (CD62P) expression, an indicator of platelet activation, increased during storage in both conditions. Refrigerated units (4°C) showed a more sustained rise throughout the 72-hour period, whereas room temperature storage (24°C) exhibited an increase followed by a modest decline after the peak. Throughout the experiment, CD62P expression in the RF condition consistently exceeded that in the RT condition; however, the difference was not statistically significant (P=0.13) (***Figure***).

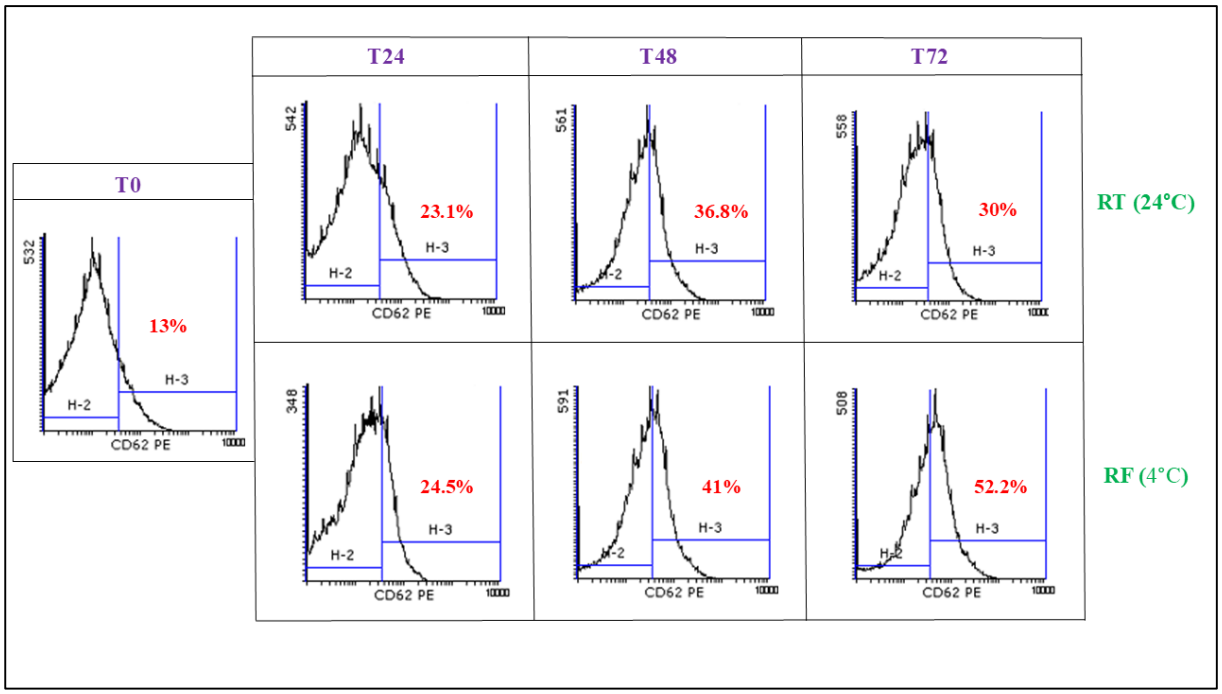

Figure 4. Histograms showing flow cytometry results of CD62P expression in platelets stored at 4°C (RF) and 24°C (RT). Over 72 hours, CD62P expression increased in platelets stored at 4°C. While platelets stored at 24°C showed an increase in CD62P expression from 13% to 36.8% by day 2 (T48), the level later dropped to 30% after 72 hours. Throughout the experiment, CD62P expression was constantly higher in platelets held at 4°C than in those preserved at 24°C.

4. Discussion:

Apoptosis is a crucial factor in platelet storage lesion (PSL), significantly impacting both quantity and quality of PLTs. The Siglec family, a group of transmembrane proteins, plays a central role in immune modulation, cell signaling and apoptosis through binding to their sialoglycan ligands.[16] Siglec-7 (CD328), a member of the Siglec family, was first reported to be intrinsically expressed on the surface of platelets by Nguyen et al. in 2014. Their study revealed that Siglec-7 binds to its ligands, i.e., GD2, brings about the apoptosis of platelets via four key procedures: i) the increased expression of pro-apoptotic proteins (Bak and Bax) and decrease in the level of anti-apoptotic protein (BCL-2), ii) depolarization of the inner mitochondrial membrane, iii) exposure of phosphatidylserine, and iv) formation of microparticles.[19] As it has been proven that Siglec-7 is found on the surface membrane of platelets, its overexpression during platelet storage is speculated to accelerate apoptosis; therefore, the primary objective of our current research was to assess the impact of Siglec-7 on platelet apoptosis rate under various storage conditions, providing insights into its role in platelet preservation.

The results of our investigation indicate that platelet quantity decreased in both storage conditions (24°C and 4°C) over the storage period. While platelet count was higher at 24°C compared to 4°C, the difference was not significant. Similarly, Wood et al. (2016) reported a decrease in platelet number in both 22°C and 2-6°C storage conditions. Contrary to our findings, platelets stored at 2-6°C exhibited a slightly higher count, though the difference was not statistically significant.[20]

Additionally, our results demonstrated that Siglec-7 expression on platelets increased throughout the storage period at both 24°C and 4°C, with a greater increase observed at 4°C (4°C > 24°C), suggesting that both storage duration and temperature directly influence its expression. The upregulation of Siglec-7 may explain the reduction in platelet count, as previous studies have demonstrated its role in initiating platelet apoptosis.[19,21] The increased apoptosis rate in our study was evaluated based on Annexin-V binding to procoagulant phosphatidylserine (PS)

on the surface of activated platelets, which exhibited an upward trend in both groups throughout the storage period. Consistent with our findings, Marini et al. demonstrated that keeping platelets at cold temperatures enhances the expression of platelet apoptotic markers more significantly than room temperature.[22]

Due to the fact that P-selectin (CD62P) and Siglec-7 are co-located in the alpha granules of platelets,[20] we also assessed the CD62P expression on platelets during the storage period. As a marker of platelet activation, CD62P expression increased at both 24°C and 4°C; however, the difference between them was not statistically significant. In a similar work, CD62P expression was higher in platelets stored at 4°C in comparison to 24°C.[14] Interestingly, in the third 24 hours of storage, the percentage of platelets expressing CD62P at 24°C was declined. This phenomenon could be attributed to ectodomain shedding, a process known to reduce the quantity and quality of stored platelets and contribute to PSL.

PSL is an ordinary phenomenon that occurs during platelet storage, particularly at higher temperatures. In addition, RT-kept platelets may increase the hazard of bacterial contamination,[23] whereas those kept at 4°C exhibit a lower metabolic rate and reduced infection risk.[3] While our study indicated a higher apoptosis rate in 4°C-kept platelets, Reddoch et al. found that cold-stored platelets could retain viable metabolic features, release fewer inflammatory components, and demonstrate superior hemostatic efficiency compared to RT-stored ones.[14,24] However, another study disclosed that platelets stored at RT had a longer shelf life *in vivo* and greater hemostatic efficiency than those maintained at the colder storage conditions. Also, platelet viability temperature was dropped at 4°C,[25] suggesting that colder conditions impacts the average life-span of platelets, biochemical characteristics, and overall function.

Our results indicate that although colder temperatures increase the expression of the apoptosis marker Siglec‑7 on platelets, they do not significantly influence platelet activation compared to ambient conditions. These findings suggest that Siglec‑7 may play a role in platelet responses during cold storage; however, further studies using direct interventions are required to determine whether modulating Siglec‑7 could be a viable strategy for improving platelet storage at 4°C.

5. Conclusions:

Previous studies have demonstrated the indispensable role of Siglec-7 in platelet apoptosis. The present study investigates the underlying cause of PLT count reduction in platelet concentrates. Our study highlights the crucial role of Siglec-7 in platelet apoptosis, particularly under colder storage conditions. The decline in platelet count at lower temperatures appears to be driven by Siglec-7 overexpression, which activates apoptotic pathways. Additionally, the accelerated protein shedding at room temperature suggests a complex relationship between storage temperature and platelet integrity.

Our findings indicate that increased Siglec‑7 expression is associated with markers of platelet apoptosis during cold storage. This correlation highlights the need for targeted mechanistic studies to clarify the role of Siglec‑7 in platelet preservation. Future research using Siglec‑7 inhibitors or blocking antibodies may help determine whether modulating this pathway could improve storage conditions and enhance platelet viability.

Ethical Approval:

The experimental methods were performed by the relevant guidelines and approved by the Ethics Committee of Tabriz University of Medical Sciences (Ethical Approval Code: **TBZMED.REC.1394.873)**

Authors' Contributions:

This research work has been done by Asra Amelirad; Davoud Pashoutan Sarvar analyzed the data and wrote the article with the assistance of Asra Amelirad; Parvin Akbarzadehlaleh edited the final version; Karim Shamsasenjan designed and supervised the study, whole correspondence during the paper submission. All authors read and approved the final manuscript.

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Conflict of interest:

The authors express no conflict of interest.

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