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Effect of Platelet Functions Studies During 3 Days of Storage of Single Donor Platelets Obtained from Platelet-Apheresis

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Abstract

Objective: To investigate the effect of platelet function during a 3-day storage period of single donor platelets obtained through plateletpheresis. and to assess how the storage conditions may impact the functionality of these platelets after 72 hours.

Methodology: This study was conducted at the Department of Hematology, Children's Hospital Lahore, after obtaining informed consent from the donor by a trained staff member. Samples were selected regardless of gender and were matched with the exact age of the cases. All samples with a platelet count of less than 250,000/µL were excluded. A total of 92 samples were collected based on the sample size estimated by the WHO. Platelet concentration and aggregation were evaluated using various concentrations of inducing agonists, including ADP (7.0 µg/mL). For each test, 400 µL of platelet-rich plasma was used, each in a separate cuvette after allowing for spontaneous aggregation. The aggregation curve was observed after five minutes of stimulation by the inducing agonists. Chrono-log aggregometer was utilized for aggregation studies on day 0 and day 3. SPSS was employed for data analysis.

Result: The average age of donors was 29.38(yr). More then half were female donor. Mean platelet count was 270300 µL. When aggregation of the platelet count was evaluated, it was found that 24(26.1%) have aggravated platelet at day 0. While on day 3, platelet aggregation was fond in 11(12%) of cases The percentage decrease in aggregation of platelet was 49(53.3%).

Conclusion: There is reduction in platelet aggregation in the patients when they were evaluated at day0 and day 3.

Key words: Platelet count, aggregation, apheresis

Introduction

Megakaryocytes are particles responsible for generation of platelets. The size of the platelet varies form 1-4 microns in diameters which are discoid enucleated cells by structure.[1]

The adult range of platelets is 150 to 450 x 109 /L with a basic role of keeping homeostatic surface in vessels.[2] Now there is opening of new horizon by use of hemodynamic components for patient management in case of hemorrhage and other bleeding indications.

Hematologists commonly practice the segregation of the platelet form the whole blood while extracting it form the donor. There are two common procedure employed, one is differential centrifugation and other is platelet apheresis.[3]

Apheresis is most effective method that is used for collection of required amount of platelet from a single donor hindering the exposure of the patients with multiple donors and their platelets. A single donor can generate minimum count of 3 x 1011 /L suspended in 200 to 300 ml of plasma. But mostly they are not instantly transfused so they are stored in a temperature-controlled incubator (20-24 degree) with constant agitation for up to 5 days. [4, 5]

This storage puts the platelet into a process named as platelet storage lesion which changes the structure, function and biochemical profile of the platelets. So they become less functional as the storage times increases.[6]

It becomes viable to check the function and biochemical profile of the stored platelet before transfusion in the patients. there are various test that can be utilize for this purpose. The most common test

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is light transmission aggregometery which gives a value form 0% minimum absorption to 100% maximum absorption.[7]

In order to understand it further, A Brazilian study has evaluate that here was significant decrease in the platelet aggregation at day 0 and at day 3 and day 5. They further noted that the aggregation rate goes on a continuous decrease as the storage duration increases and the maximum decrease which was observed at day 5 was 9%.[8]

In another study performed in Eskisehir Osmangazi University, Turkey the similar results were noted which states that they platelet should not be stored for more than 24 hours. If possible than every cases who is in need of transfusion of the palates should be given fresh platelets.[9]

With this background, this study intended to check the results in Pakistani population at day 0 and day 3. In this study, platelet aggregation testing using collagen was performed on day 0 and 3rd of their storage. If a higher prevalence of platelet function defects is found in our set up at day 3 of the storage, then this may lead to the revision of protocols regarding the storage of the platelets.

Methodology

Ninety-two samples that met the inclusion and exclusion criteria were enrolled in this study, conducted at the Department of Hematology, Children's Hospital Lahore. Informed consent was obtained from each donor by a trained staff member, and a Performa was completed for each donor before their inclusion in the study. The following tests were conducted on the samples obtained from Single Donor Platelets (SDPs) on both day 0 and day 3 of storage. Platelet concentration was adjusted, and platelet aggregation was performed using various concentrations of inducing agonists, including ADP (7.0 µg/mL). For each test, 400 µL of platelet-rich plasma was used in separate cuvettes after a waiting period for spontaneous aggregation. Stimulation was induced by the platelet agonist, and the platelet aggregation curve was observed. The Chrono-log aggregometer was employed for these aggregation studies, utilizing siliconized cuvettes to contain the platelet-rich plasma. Platelet aggregation ability was assessed on both day 1 and day 3. Results were presented as percentages, and the percentage decline was calculated according to the operational definition.

Patient gender and the study duration were considered qualitative variables, while age and the percentage decline were treated as quantitative variables. Data analysis was performed using SPSS Inc. 2021, and the Chi-square test was applied to account for effect modifiers at a significance level of $p < 0.05$.

Results

The mean age of the study population was 29.38 ± 5.16 years. Out of the total 92 cases, 43 (46.7%) were male, and 49 (53.3%) were female. The mean platelet count was 270,300 ± 11,922.82 per microliter. A significant mean percentage decrease in platelet aggregation was observed from day 0 (73.6 ± 13.23) to day 3 (54.70 ± 12.50) (p-value < 0.001).

Upon data stratification, it was found that there was no significant difference in the age of the patients, with a p-value of 0.28. However, gender showed a significant difference in platelet count aggregation decrease, with a p-value of 0.03 (Table I).

Table: Impact of Age and Gender on the platelet aggregation.

		% Decrease in Aggregation at Day 3		P-Value
		Yes	No	
Age Group	20-30 years	29(56.9%)	22(43.1%)	0.28
	30-40 years	20(48.8%)	21(51.2%)	
Gender	Male	49(94.2%)	39(97.5%)	0.05
	Female	3(0.05%)	1(2.5%)	

Discussion

In the last 20 years, significant advancements have been made in platelet transfusion therapy, including improved methods for collecting, storing, and processing platelets. As aggressive chemotherapy, organ transplants, and novel treatments for conditions like aplastic anemia have emerged, healthcare providers increasingly depend on platelet transfusions. Therefore, it's crucial for clinicians to have a deep understanding of how to utilize platelet transfusions effectively to ensure efficient resource utilization. This chapter focuses on the application of platelet transfusions in various medical scenarios, particularly in patients who require multiple platelet transfusions.[0, 11

Megakaryocyte-derived cell fragments called platelets are crucial for healthy hemostasis. In patients with bleeding disorders accompanied by severe thrombocytopenia or inefficient platelet production, platelet transfusion is a widely accepted and essential form of treatment([12]). About 2.2 million platelet units are utilised annually in the US, the majority of which are prophylactically administered to patients undergoing chemotherapy and Hematopoietic Progenitor Cell Transplantation (HPCT) to lower their risk of spontaneous bleeding.([13]) There are three ways to make platelet products. The platelet product is made using whole blood that was donated at random in the first and second processes. In the first and second techniques, the platelet product is prepared using buffy coat and platelet-rich plasma (PRP), respectively. The third method of preparing platelets uses a single donor and involves apheresis. The platelet concentration is kept in bags for 3-5 days after production. These procedures have a varied product concentration. The quality of the finished product in the processes of platelet product preparation and storage can also be impacted by a number of additional elements.[14] In order to save the patients' lives, these products are injected into them as a form of prophylaxis or treatment.

Most of the times, the transfusion of platelets is used to save lives of patients in emergency situations but it also has risk pf transfusion reactions ,same as with the transfusion of other blood products and in some cases the transfusion of platelets bears more risks than red blood cell transfusion.[15] There are many types of transfusion reactions with platelets transfusion , ranging from a simple allergic reaction (the most common one) or mild fever to more complicated acute hemolytic reactions, Transfusion-Related Acute Lung Injury (TRALI), anaphylactic reaction or severe sepsis.[16]

Single-donor platelets (SDPs) are typically obtained through the process of apheresis, which involves the collection of platelet concentrates from a single donor utilizing a cell separator. Simultaneously, the remaining components of the donor's blood, including white blood cells and red blood cells, are returned to the donor via the apheresis machine. Research findings have demonstrated that platelets derived from whole blood (WBDP) present a heightened risk of viral disease transmission due to exposure to multiple donors, consequently leading to an elevated incidence of allergic transfusion reactions. Additionally, during the storage of plasma, numerous cytokines and chemokines, such as histamine, experience an increase, thereby contributing to the occurrence of allergic reactions.[17]

Recent advancements in apheresis technology have facilitated the efficient and abundant procurement of single donor platelets. Several hypotheses underscore the advantages of single donor platelets, including a reduced risk of disease transmission, diminished alloimmunization, and improved functional and storage attributes. Consequently, proponents argue for the routine adoption of single donor platelets.

While some individuals endorse these benefits, a consensus has not been reached on whether the risks to donors, the procedural complexities, and the associated expenses are compelling enough to justify the conversion of blood banks to a single donor platelet supply paradigm. A notable advantage of single donor platelets lies in the simplified selection of specific donor characteristics, such as viral screening, due to the aggregation of platelets from a single donor source.[18]

The collection of blood components through apheresis has witnessed significant growth over the past few decades. Transfusing apheresis-derived blood components offers numerous advantages. It provides a larger therapeutic dose of components compared to those obtained from conventional whole-blood-derived components. Additionally, it yields a superior product content, optimizes blood donor utilization, accelerates the recovery of the donor's blood cell count, and reduces the risk of bacterial contamination in the final transfusion products.

Furthermore, by minimizing the recipient's exposure to multiple donors, apheresis units mitigate the risks associated with human leukocyte antigen (HLA) alloimmunization and the transmission of diseases such as HIV and Hepatitis.[19, 20]

Platelet (PLT) aggregates form in apheresis-derived Platelet products, and prevention of aggregate formation was a point of great interest when apharesis methods were initially developed. Blood products with macro aggregates or large clumps may be discarded due to quality assurance and efficacy of the product. Discarding the component could happen at many occasions, for example, at the blood center while detection of irreversible clumping, in a hospital blood bank when clumps were not detected in the blood

center or just formed , during storage of platelets in the hospital blood bank, or in the ward when clumps were remain undetected by the blood bank staff or produced during transportation to the ward.[21]

The median platelet counts for APCs on days 1, 3 were; 2060, 1980, respectively. The pH of all platelet bags was greater than 6.2. However, there were variable results of aggregation noted. Platelet bags showed a marked decrease of ADP aggregation after 3 days of storage ($P < 0{\cdot}05$). But APCs gave sufficient increments for almost 100% of transfusions. On the sixth and seventh days of bag storage, APCs gave markedly higher platelet yield ($18 \times 10^3\ \mu L^{-1}$) compared with RDPs ($3 \times 10^3\ \mu L^{-1}$) ($P < 0{\cdot}05$). Significantly prolonged transfusion intervals were also gained with APCs ($P < 0{\cdot}05$). Although other variables may have affected and changed the results, further storage of APCs appeared to give higher increments with longer intervals of transfusion compared with RDPs[21, 22] same was observed in our study.

In our observations, the quality of platelet concentrates met the criteria for quality assurance. The platelet count on day 1 of storage fell within the normal range (5.5 x 10^10/70 mL). Subsequently, the platelet count increased over the next two days, possibly due to platelet fragmentation, before declining again by day 5.[23] Furthermore, the whole blood from the donor exhibited normal aggregation, with an aggregation of 67% with adenosine diphosphate (ADP) and 78% with collagen.

Our study on platelet aggregation with ADP and collagen yielded results consistent with previous research, albeit with some variations. Platelet aggregation with ADP remained below normal levels throughout the 5-day storage period, while the median platelet aggregation with collagen was within the normal range only on day 1, decreasing thereafter (53.5% on day 1, 20% on day 3).[24, 25] It is worth noting that our study was conducted only up to day 3, so our results align closely with prior findings, with minimal differences.

This study has limitation being a single centered and single laboratory based system for the storage of the blood components. It is possible that proper maintenance of the temperature and use of advanced technologies could prolonged the aggregation of the platelets. More studies on the same topic is needed in our country to provide better data analysis and improvement in quality of single donor platelets.

Conclusion

Conclusively, it is observed that there is considerable decrease in the platelet aggregation when evaluated at day 0 and day 3. It is recommended that patients must be transfused to the patient at day 0 rather than after passage of some time. To improve the effectiveness of platelet replacement therapy, we propose incorporating a functional assessment test for the quality control of platelet concentrates in blood banks.

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