MAK HILL Publications Research Journal of Medical Sciences

Research Article
doi: 10.59218/makrjms.2024.1.119.123

OPEN ACCESS

Key Words
Platelet count, platelet rich plasma, flow cytometry, platelet products

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Received: 15 October 2023
Accepted: 29 October 2023
Published: 30 October 2023

Citation: Gourav Silawat, Ashutosh Singh, Sachin Sharma, Sanjay Silawat and Aman Shakya, 2023. Quantitative Analysis (Volume and PH) of Activated Platelet in Different Platelet Products by Flow Cytometry From Indore Region of Madhya Pradesh. Res. J. Med. Sci., 18: 119-123, doi: 10.59218/makrjms.2024.1.119.123

Quantitative Analysis (Volume and PH) of Activated Platelet in Different Platelet Products by Flow Cytometry From Indore Region of Madhya Pradesh

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ABSTRACT
Platelets are vital to regular hemostasis as well as pathological bleeding and clotting in humans. The cytometer's subsequent counts may be trusted to be reliable because of this first count's precision. Clinical investigations involving heart disease and other platelet-activation illnesses currently make use of flow cytometry. This study aims to conduct quantitative analysis in terms of volume and pH of activated platelet in different platelet products by flow cytometry at tertiary health care center of central India. The present study was cross-sectional in design, conducted at department of transfusion medicine, MGM Medical College and M.Y Hospital, Indore. In this study a total number of 105 study participants were included. Quantitative analysis (volume and pH) of activated platelet in different platelet products by flow cytometry was performed. The percentage of active platelets on day 1 in the single donor platelet category was 78.8±4.5 while on day 4 it was 74.23±4.8. On days 1 and 4 the percentage of activated platelets was highest in platelets from a single donor. pH on day 1 in platelet rich plasma category was 6.51±0.13 while pH on day 4 was 6.22±0.11. There was decrease in numerical values of pH in each category on day 4 as compared with day 1. Volume and pH of activated platelet in different platelet products declines from day 1-4 day yet they are still well conserved at the conclusion of that period. Storage conditions determine whether the platelet products may be utilized within 5 days. Activated platelets in various platelet products may be analyzed qualitatively with the use of flow cytometry. Clotting, platelets, activated platelet, flow cytometry.

INTRODUCTION

Platelets are vital to regular hemostasis as well as pathological bleeding and clotting in humans. Platelet's interactions with other cells also contribute to inflammation, its related illnesses and the development of atherosclerosis (such as white blood cells, endothelial cells or smooth muscle cells)[1]. Despite the variations, researchers are increasingly interested in how changes in platelets impact the process of clotting[2]. Two types of platelet concentrates are available for transfusion one which is the co-product of normal blood donation i.e. random donor platelets (RDP), (platelet rich plasma-platelet concentrate (PRP-PC) and buffy coat poor-platelet concentrate (BC-PC) and the other is single donor platelets (SDPs), (apheresis-PC,) collected from voluntary thrombocytapheresis donors with the help of an automated cell separator.

Traditional cytometers provide just an approximation of cell counts, not a precise count. In recent efforts to build absolute counting methods, blood's erythrocyte count or fluorescent beads of a known concentration have been utilized as reference standards[3]. Since traditional function evaluations have limitations, research into platelet function has drawn interest beyond mere counting. Flow cytometry in combination with monoclonal antibodies to activation-dependent epitopes allow for single-cell level analysis of platelet activation in whole blood. Microparticles and platelet aggregates, two additional activation markers, may be detected and identified[4-5].

The most precise technique of counting the platelets in a person's blood is with the use of a machine that analyzes their blood. The cytometer's subsequent counts may be trusted to be reliable because of this first count's precision[6]. Clinical investigations involving heart disease and other platelet-activation illnesses currently make use of flow cytometry[7]. We conducted this study due to the dearth of data research on this topic. Aim of this study was to conduct quantitative analysis in terms of Volume and pH of activated platelet in different platelet products by flow cytometry at tertiary health care center of central India.

MATERIALS AND METHODOS

The present study was cross-sectional in design, conducted at department of transfusion medicine a tertiary care teaching institute (MGM Medical College and M.Y. Hospital) situated in Indore region of Madhya Pradesh state for a duration of one year. In this study, a total number of 105 study participants were included. Study participants were divided into three categories with each category consisting of 35 participants namely, category 1 single donor platelet, category 2 platelet concentrate and category 3 platelet rich plasma. Study methods used were quantitative analysis of functional by flow-cytometer and quantitative platelet analysis by hematology analyzer.

Inclusion criteria:

- Platelet products (Platelet concentrate, platelet rich plasma, Single donor platelet, Pooled platelet concentrate.) separated in blood bank.
- Platelets obtained from both the genders of $\geq$18 years age and $\leq$60 year age groups.

Exclusion criteria:

- Clotted sample
- Unpreserved sample.
- Refrigerated sample.
- Discarded bags.
- Expired products.
- HIV positive, hepatitis positive, venereal disease positive donors.
- Donors with hemoglobin $\leq$12.5 gm dL^{-1}.

The process of flow cytometry consisted of the following steps.

Sample preparation: The cells to be analyzed must first be prepared into a single-cell suspension before being put through the flow cytometry. Before performing an investigation, it is necessary to dissociate clumped cultured cells or cells found in solid organs to create a single cell suspension. After that, mechanical filtering is performed to keep instruments clean and provide more accurate flow readings. The resultant cells are treated with either unlabeled or fluorescently conjugated antibodies in test tubes or micro titer plates before being examined by a flow cytometer. There must be a single-cell suspension of the cells to be analyzed before they can be processed through the flow cytometers. Before performing an investigation, it is necessary to dissociate clumped cultured cells or cells found in solid organs to create a single cell suspension. After that, mechanical filtering is performed to keep instruments clean and provide more accurate flow readings. The resultant cells are then examined by a flow cytometer after being treated with either unlabeled or fluorescently conjugated antibodies in test tubes or microtiter plates.

Antibody staining: After the sample is ready, fluorochrome-conjugated antibodies targeting distinct cell surface indicators are added to the cells. Direct, indirect or intracellular staining are all viable options for this. Cells are treated with an antibody that has been directly coupled to a fluorophore, resulting in an

indirect stain. The primary antibody is detected by the fluorophore-conjugated secondary antibody in indirect staining. Direct quantification of cytoplasmic or nuclear antigens is now possible using intracellular staining. This is achieved by staining antibodies onto the cells while they are still in the permeabilization solution.

Running Samples: The detecto's voltages are first calibrated with the help of control samples. The sample is run after the flow rates in the cytometer are adjusted. The investigation was started after receiving ethical approval from the institute. The data collected was coded appropriately on MS Excel spreadsheet. Microsoft Excel was used to input data. The average and standard deviation were used to describe continuous data. Percentages and proportions were used to represent the categorical data. Chi-square tests and other appropriate significance tests were used where appropriate. Data was checked for any potential errors. Statistical software was used for analyzing the data.

RESULTS

Seventy-seven people (73.4 per) of the study population were young adults (Less than 40 years of age). Remaining participants (n = 28-26.6%) ranged equal or more than 40 years of age. Gender wise male subjects (n = 84-80%) outnumbered females (n = 21-20%) (Table 1).

The percentage of active platelets on day 1 in the single donor platelet category was 78.8±4.5, while on day 4 it was 74.23±4.8. On day one, platelet activation percentages in the platelet concentrate group were 59.5±5.1 and by day four, they had dropped to 51.61±4.9. On day 1, platelet rich plasma had a percentage of active platelets of 59.5±5.1 but by day 4, that number had dropped to 51.61±4.9. Results showed that on days 1 and 4 the percentage of activated platelets was highest in platelets from a single donor (Table 2). pH on day 1 in single donor platelet category was 6.58±0.13 while pH on day 4 was 6.27±0.19. pH on day 1 in platelet concentrate category was 6.57±0.15 while pH on day 4 was 6.28±0.19. pH on day 1in platelet rich plasma category was 6.51±0.13 while pH on day 4 was 6.22±0.11. There was decrease in numerical values of pH in each category on day 4 as compared with day 1 (Table 3).

Table 1: Distribution of study participants according to age group and gender

Age group (years)	Number of patients	Percentage
Less than 40	77	73.4
Equal or more than 40	28	26.6
Gender		
Male	84	80
Female	21	20

DISCUSSION

Similarly a total of 146 platelet concentrates was studied by Singh *et al*.[1] 20 whereby I platelet rich plasma platelet concentrate (PRP-PC), (ii) buffy coat poor-platelet concentrate (BC-PC) and (iii) single donor platelets (apheresis-PC) were all made from random donor platelets (n = 42 each).

A similar pattern of ages was found in research by Fijnheer *et al*.[8] and Mallhi *et al*.[9] study participants had a mean age of 34.452 years and mean age of 33.328 years, respectively. Most research participants were between the age of 20 and 30 with the youngest participant being under the age of 50.

A similar distribution of sexes was found in a research by Hiroshue *et al*.[10] and Singh *et al*.[1] This mostly included guys as subjects. The proportion of activated platelets on day 1 was 78.8±4.5 in this research within the single donor platelet category, while the percentage on day 4 was 74.23±4.8. Platelet rich plasma had a higher proportion of activated platelets on day 1 (59.5±5.1) than platelet concentrate (51.61±4.9) and platelet concentrate (59.5±5.1) than platelet rich plasma (51.61±4.9). A single-donor platelet us proportion of activated platelets was highest on days 1 and 4. The statistically significant difference (p0.05) was there. The proportion of active platelets dropped across the board by Day 4 compared to day 1. These results were statistically significant (p 0.05). Research by revealed similar results. Matzdorff *et al*.[11] included incubation of agonists with whole blood and PRP (ADP, collagen, thrombin). The aggregation of platelets and microparticles as well as the expression of CD62p, were investigated. The Flow-Count-Fluorospheres (R) were included into the calculation of absolute concentrations. Platelets with a CD62 p-positivity increased following activation. "The total number of platelets decreased which number of CD62 p-positive platelets decreased fact that the number of CD62 p-positive platelets did not decrease as much as the number of CD62 p-negative platelets may account for the rise in the relative

Table 2: Percentage of activated platelet day 1 and day 4

Platelet products	% of activated platelet day 1 (Mean±SD)	% of activated platelet day 4 (Mean±SD)	p-value
Single donor platelet	78.8±4.5	74.23±4.8	p-0.05
Platelet concentrate	59.5±5.1	51.61±4.9	p-0.05
Platelet rich plasma	39.09±4.4	28.40±3.5	p-0.05

Table 3: pH value at day one and day four.

Platelet products	pH at day one (Mean±SD)	pH at day 4 (Mean±SD)	p-value
Single donor platelet	6.58±0.13	6.27±0.19	p-0.05
Platelet concentrate	6.57±0.15	6.28±0.19	p-0.05
Platelet rich plasma	6.51±0.13	6.22±0.11	p-0.05

fraction of CD62 p-positive platelets. Microparticle counts went up and down by similar percentages and absolute numbers. The proportion and total number of platelet aggregates increased. These results suggest that flow cytometry alone is insufficient for identifying activated platelets and highlight the requirement for assessing absolute concentrations.

Pieterz *et al*.[12] where the average pH was between 6-5-7 and no discernible variation was seen depending on the stabilizer employed in the plastic platelet storage bags and the storage circumstances, the pH might drop significantly over time. Significant loss of viability is linked to the increased platelet glycolysis that causes a pH drop to around 6.0 in plasma-stored PC. Most unstimulated, newly formed platelets have a discoid shape with few protrusion. PCs maintained at 20-24°C were first shown to undergo a progressive disc-to-sphere change over time. Incubation at 37°C in fresh plasma may reverse some of these alterations. These shifts in quality are also seen during PC storage, however the pH drop experienced by first-generation containers is a key extra variable. Platelet volume falls by around 10% over the course of three days if the pH does not drop to less than 6.8.

Murphy *et al*.[13] PRP-PC transfusion resulted in a greater increase in platelet count (60-70%), compared to BC-PC transfusion (40-60%), for the patient. After being transformed into PCs, platelets lose part of their original functionality, changing shape, clumping together, and secreting abnormal compounds. Platelets lose their effectiveness mostly because to lesions brought on by their processing and storage. Bode *et al*.[14] found that after 48 hrs of storage, there was no significant difference between BC-PC units and PRP-PCs units with respect to shape change, aggregation or secretory responses. There was a considerable reduction in the gap between PRP-PC and BC-PC units after 4 days of storage. The results of the current research are consistent with those of a previous one that also demonstrated no difference in morphological changes or in vivo survival between the two kinds of PCs after 5 days of storage.

CONCLUSION

Based on findings of this research, volume and pH of activated platelet in different platelet products declines from day 1-4 day yet they are still well conserved at the conclusion of that period. Storage conditions determine whether the platelet products may be utilized within 5 days. Activated platelets in various platelet products may be analyzed qualitatively with the use of flow cytometry.

ACKNOWLEDGMENT

The authors would like to thank Department of Transfusion Medicine, MGM Medical College and M.Y Hospital for all kind of support provided to carry out this research. Authors express the gratitude to everyone who supported them to conduct this investigation.

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