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Quantitative Analysis in Terms of Counts of Activated Platelet by Flow Cytometry at Tertiary Health Care Centre of Central India

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ABSTRACT

Platelet transfusions are the primary therapy for thrombocytopenia due to various causes. Since traditional function evaluations have limitations, research into platelet function has drawn interest beyond mere counting. This study aims to conduct quantitative analysis in terms of counts of activated platelet by flow cytometry. The current study, which had a cross-sectional design, was carried out at the MGM Medical College and M.Y. Hospital in Indore's transfusion medicine department. A total of 105 study participants were enrolled in this investigation. Activated platelet counts were quantitatively analyzed by flow cytometry in various platelet products. Platelet counts in the platelet concentrate category ranged from 1177.704±132.40 103 mL^{-1} on day 1 to 1173.224±131.79 103 mL^{-1} on day 4. Day 1 platelet count in the platelet rich plasma category was 496.3198± 18.96 103/ml, whereas day 4 platelet count was 492.12±18.87 103 mL^{-1}. Researchers discovered that platelet counts were highest on days 1 and 4 when they came from a single donor. The difference observed in mean platelet counts in SDP (Single donor platelet) was 6.09×10^3 μL^{-1} whereas this difference in mean platelet counts in PC was 4.48×10^3 μL^{-1}. The difference noted was 4.19×10^3 μL^{-1} for mean platelet counts in Platelet rich plasma. Conclusion: According to this research, platelet activity declines from day 1 to day 4, yet they are still well conserved at the conclusion of that period. Based on our findings, SDP is preferable to both PC and PRP in terms of platelet count. Activated platelets in various platelet products may be analyzed qualitatively with the use of flow cytometry.

INTRODUCTION

Since platelets were first identified in 1881, there has been continuous and accelerating progress in our basic understanding of platelet function and its utilization in various bleeding disorders[1].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).[2] Despite the variations, researchers are increasingly interested in how changes in platelets impact the process of clotting[3].

Platelet transfusions are the primary therapy for thrombocytopenia due to various causes. Thrombocytopenia may be due to qualitative defect, i.e. defect in platelet function or quantitative defect, i.e. decreased platelet count which can be seen in various hemato-oncological patients either due to primary disease or chemotherapy[4]. 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[5].

High counts of platelets are required for optimal accuracy and precision when counting platelets. 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. Since traditional function evaluations have limitations, research into platelet function has drawn interest beyond mere counting[6]. Flow cytometry in combination with monoclonal antibodies to activation-dependent epitopes allow for single-cell-level analysis of platelet activation in whole blood. The cytometer will be the superior instrument in really unusual circumstances (such as in individuals with huge platelets, as found in those with coronary artery disease) because it can differentiate platelets that are part of the red cell population. 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 counts of activated platelet by flow cytometry from the Indore region of the Madhya Pradesh state.

MATERIALS AND METHODS

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 >18yrs age and <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 <12.5 gm dL^{-1}.

The following steps made up the flow cytometry procedure. Sample Preparation Before using flow cytometry, the cells to be studied must first be made into a single-cell suspension. It is important to separate clumped cultured cells or cells found in solid organs in order to establish a single cell solution prior to conducting an analysis. The next step is mechanical filtration, which keeps equipment clean and produces flow readings that are more precise. Before being analyzed by a flow cytometer the produced cells are treated with either unlabeled or fluorescently conjugated antibodies in test tubes or micro titer plates. Before the cells can be processed by the flow cytometers, they must first be suspended as single cells. It is important to separate clumped cultured cells or cells found in solid organs in order to establish a single cell solution prior to conducting an analysis. The next step is mechanical filtration, which keeps equipment clean and produces flow readings that are more precise. After being exposed to either unlabeled or fluorescently conjugated antibodies in test tubes or microtiter plates the resulting cells are then analyzed by a flow cytometer. After the sample is prepared, antibodies that are fluorescein-conjugated and that target several cell surface markers are added to the cells. Staining from the inside of cells, either directly or indirectly, is a potential method. An indirect stain is

produced when cells are exposed to an antibody that has been directly linked to a fluorophore. In indirect staining, the secondary antibody coupled to a fluorophore can identify the primary antibody. With intracellular staining, cytoplasmic or nuclear antigens can now be directly quantified. Antibodies are stained onto the cells while they are still in the permeabilization solution to accomplish this. Flowing Examples. With the assistance of control samples the voltages of the detectors are first calibrated. After the cytometer's flow rates have been adjusted, the sample is run.

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

Young adults (18-30) made up 42 of the study's participants, or 40%. 35 participants, whose ages ranged from 31-40, fit within this group. 25 people, or 23.8%, were between the ages of 41 and 50, while 3 were between the ages of 51 and 60. Twenty-one women made up 20% of the study population, while eighty-four men made up 80% of it (Table 1).

Single donor platelet counts ranged from 1258.696 149.09 103 mL on day 1 to 1252.569 149.04 103 mL on day 4. Platelet counts in the platelet concentrate category ranged from 1177.704±132.40 103 mL^{-1} on day 1 to 1173.224±131.79 103 mL^{-1} on day 4. Day 1 platelet count in the platelet rich plasma category was 496.3198±18.96 103 mL^{-1}, whereas day 4 platelet count was 492.12±18.87 103 mL^{-1}. Researchers discovered that platelet counts were highest on days 1 and 4 when they came from a single donor. The statistically significant difference was p 0.05 (Table 2).

The difference observed in mean platelet counts in SDP (Single donor platelet) was 6.09×10^3 μL^{-1} whereas this difference in mean platelet counts in PC was 4.48×10^3 μL^{-1}. The difference noted was 4.19×10^3 μL^{-1} for mean platelet counts in Platelet rich plasma. The statistically significant difference was p 0.05 (Table 3).

Table 1: Distribution of study participants according to age group

Age group (years)	No of patients	Percentage
18-30	42	40.0
31-40	35	33.4
41-50	25	23.8
51-65	3	2.8

DISCUSSIONS

The platelet count in this trial was 1258.696 149.09 103 mL^{-1} on day 1 in the single donor platelet category and 1252.569 149.04 103 mL^{-1} on day 4. The platelet count in the platelet concentrate group was 1177.704132.40 103 mL^{-1} on day 1 and it dropped to 1173.224131.79 103 mL^{-1} on day 4. The platelet rich plasma group had a platelet count of 496.319818.96 103 mL^{-1} on day 1 and a platelet count of 492.1218.87 103 mL^{-1} on day 4. On day 1 and day 4, the highest platelet counts were seen in single donor platelets. The statistically significant difference ($p<0.05$) was there. There was no statistically significant difference in the platelet counts on day 1 and day 4 for any of the groups.

Similarly, in another investigation by Singh *et al*.[7] The platelet counts for PRP-PC, BC-PC and apheresis-PC were all 3.2-2.97 1010/unit on average, with respective ranges of 0.6-16.4 1010/unit, 1.22-8.9 1011/unit and 3.2-16.2 1010/unit. The average platelet count of PRP-PC and BC-PC was not different statistically. We also evaluated the platelet count conformance rate of platelet-poor plasma (PRP-PC), bone marrow (BC-PC), and apheresis (PC) units. There was no statistically significant difference between the two groups in terms of the percentage of PRP-PC units that had platelet counts >5.5 x 1010/unit (78%) against BC-PC units (83.9%, 52/62), however, 21.8% (14/64) of PRP-PC units and 16.1% (10/62) of BC-PC units did. When comparing apheresis-PC to PRP-PC/BC-PC units, a substantial difference was found. Eighteen of twenty apheresis-PC units (90%) met the quality control norms for platelet counts, defined as >3 1011/unit (p 0.01). All three types of PCs passed the standard for quality control in terms of platelet count (i.e. 75% of tested units should satisfy the specified quality control standards of platelet count), however a higher number of apheresis-PC units did so.

Similarly, Fijnheer *et al*.[8] reported 15% higher platelet yield in PRP-PC than BC-PC units. Hirosue *et al*[12]. 51 also reported higher platelet count in PRP-PC units than BC-PC. Murphy *et al*.[13] 54 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*.[9] 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

Table 2: Platelet count at day one and at day 4

Platelet products	Platelet count 10^3 mL^{-1} at day one	Platelet count 10^3 mL^{-1} at day four	p-value
Single donor platelet (SDP)	1258.696 ± 149.09	1252.569 ±149.04	p 0.05
Platelet concentrate (PC) 1177.704 ±132.40	1173.224± 131.79	P 0.05	
Platelet rich plasma (PRP)	496.3198 ± 18.96	492.12± 18.87	p 0.05

Table 3: Difference in parameters with respect to platelet count at day one and at day 4

Parameters	Difference	p-value
Mean platelet counts in SDP	6.09×10^3 μL^{-1}	p 0.05
Mean platelet counts in PC	4.48×10^3 μL^{-1}	p 0.05
Mean platelet counts in PRP	4.19×10^3 μL^{-1}	p 0.05

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. During the preparation of PCs there is deterioration of platelet function manifested by abnormal shape changes, aggregation and secretory response. The main cause of deterioration of platelet function during preparation is lesions associated with the preparative manipulation and storage. In a study reported by Bode *et al*.[9] in BC-PC units after 48 hrs of storage the shape change, aggregation, and secretory responses were at the same levels as those in PRP-PCs units immediately after preparation.[10] However, the difference between PRP-PC and BC-PC units became less significant after 4 days of storage. The results of this study are consistent with another study in which after 5 days of storage the author did not find any difference in either type of PCs regarding morphological changes as well as in vivo survival.[11] Fijnheer *et al*.[8] reported 15% higher platelet yield in PRP-PC than BC-PC units. Hirosue *et al*.[12] also reported higher platelet count in PRP-PC units than BC-PC. Murphy *et al*.[13,14] found that the platelet recovery was higher in patients who received PRP-PC (60-70%) than those with BC-PC (40-60%) transfusion.

CONCLUSION

According to this research, platelet activity declines from day 1 to day 4, yet they are still well conserved at the conclusion of that period. Based on our findings, SDP is preferable to both PC and PRP in terms of platelet count and activity. 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.

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