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RESEARCH ARTICLE

CHANGES IN PRE AND POST DONATION PLATELET INDICES AND SERUM IONIZED CALCIUM LEVELS IN PLATELETPHERESIS DONORS

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ABSTRACT

Background: Apheresis is a procedure where blood is withdrawn from donor or a patient in anticoagulant solution and separated *ex vivo* into one or all components using automated cell separators. Single donor platelets (SDP) are prepared by plateletpheresis machine. In this, blood is withdrawn from a single donor and then the platelets are retained and remaining constituents are returned to donor.. SDP concentrate increases platelet count by 30,000 to 40,000/µL. **Methods:** The present study was conducted prospectively in the Department of Pathology in collaboration with Immunohematology and Blood Transfusion Department and Department of Biochemistry, Pt. B.D. Sharma Post Graduate Institute of Medical Sciences, Rohtak in 78 healthy plateletpheresis donors over a period of one year. All donors were male. **Results:** We observed statistically significant decrease in pre and post donation platelets (p value= 0.001), hemoglobin and hematocrit. A statistically significant increase was noted in pre and post donation PDW (p value=0.001). The decrease in serum ionized calcium levels before start of procedure, during and 30mins after the procedure were statistically significant (p value= 0.001, 0.001 and 0.04 respectively).However, non significant increase was noted in pre and post donation MPV and WBC values and decrease in RBC values. The adverse reactions were noted in 7.69% (6) donors which were due to anticoagulant used and managed conservatively by giving oral calcium tablet to donor. **Conclusion:** Plateletpheresis is a safe procedure for donors. The study concluded that appropriate counselling and selection of plateletpheresis donors should be done. Also, close monitoring and follow up of donors should be carried out to prevent any unfavourable event.

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INTRODUCTION

Apheresis is a Greek word 'APHAIRESIS' that means to 'separate' or 'remove'. In apheresis, blood is withdrawn from donor or a patient in anticoagulant solution and separated *ex vivo* into one or all components using automated cell separators.[1,2] Depending on the component that is being removed, apheresis is of different types like Plasmapheresis, Erythrocytapheresis, Plateletpheresis (thrombocytapheresis) Leukapheresis (leukocytes) and LDL apheresis.[3] Single donor platelets are prepared by plateletpheresis machine. In this, blood is withdrawn from a single donor and separated into various components and then the platelets are retained and remaining constituents are returned to donor, this method is known as single donor platelet (SDP).[1] SDP concentrate increases platelet count by 30,000 to 40,000/µL while random donor platelets raise the platelet count by 5,000-10,000/µL in an average sized adult.[4] Plateletpheresis units must contain 3.0 X 10^{11} or more platelets per unit in atleast 75% of units tested at maximal storage time. One unit of plateletpheresis is equal to 4-6 random donor platelets.[5,6] Single donor platelets are nowadays routinely required as an essential part of treatment for patients on chemotherapy, having bleeding disorders, undergoing open heart surgeries and organ transplantation.[7]

SDP provides several advantages over RDP (pooled random donor platelets) like higher platelet count per unit and large product volume leading to more platelets dose per bag, reduced need for leucoreduction , increased efficiency, decreased risk of exposure to multiple donors, hence decreased risk of infections and human leucocyte antigen (HLA) and human platelets antigen (HPA) alloimmunisation.[8]

Impact of citrate on body calcium: During apheresis, extracorporeal blood has a tendency to clot in the apheresis circuit. So, there is a need for anticoagulant to maintain the fluidity of the extravascular blood. Acid Citrate Dextrose (ACD) is the anticoagulant of choice.[9] Citrate chelates the free bioactive calcium in blood, thereby, preventing the later to participate in the coagulation cascade.[10,11] However, a 20% decrease in ionised calcium is usually tolerated by the donors but repeated platelet donation and prolonged plateletpheresis can result in citrate accumulation leading to hypocalcemia.[12] Normal ionized calcium levels range from 1.1-1.4 mmol/L (4.5-5.6 mg/dL). Ca^{2+} serves as a co-factor for phospholipid-dependent assembly of the tenase and prothrombinase complexes during homeostasis.

In the extracorporeal circuit, ionized Ca^{2+} levels can be reduced sufficiently (to 0.2-0.3 mmol/L) by citrate concentrations of 15-24 mmol/L and this can impair hemostasis thus can lead to an anticoagulant effect.[10] There has been variations in hematological parameters and biochemical parameters of plateletpheresis donors especially hemoglobin, hematocrit, platelet count, WBC count, and platelet distribution width (PDW) and ionized calcium (iCa) in various studies in past. So, the present study was planned to evaluate the changes in pre and post donation hematological and biochemical parameters.

MATERIAL AND METHODS

The present study was conducted prospectively in the Department of Pathology in collaboration with Immunohematology and Blood Transfusion Department and Department of Biochemistry, Pt. B.D. Sharma Post Graduate Institute of Medical Sciences, Rohtak in 78 healthy plateletpheresis donors over a period of one year.

Inclusion criteria: Donors who were apparently in good health, non reactive for HIV, HBsAg, HCV, VDRL, negative for Malaria and fit as per donor selection criteria of Director General of Health Services and Ministry of Health and Family Welfare, GOI and Drug and cosmetic Act, 1940, were selected for plateletpheresis. Donors having platelet count of $\geq 200 \times 10^9$/l were considered. An interval of at least 48 hours after platelet/plasma—apheresis was kept(not more than 2 times a week, limited to 24 in one year). Apheresis platelet donors were not be accepted for whole blood donation for 28 days from the last platelet donation provided reinfusion of red cell was completed in the last plateletpheresis donation. If the reinfusion of red cells was not completed then the donor was not be accepted for 90 days.

METHODS

Plateletpheresis procedure was performed on Multiprocedure COM.TEC apheresis machine of Fresenius Kabi. Whole blood samples were taken from the donors into EDTA tubes just before apheresis(baseline) and another whole blood sample was withdrawn after 30mins of donation. Pre and post donation values for Hb, hematocrit, platelet count, mean platelet volume, platelet distribution width, RBC count and WBC count were measured on Automatic Hematology Analyser BC 5800 Mindray. Similarly for serum ionized calcium measurement, whole blood sample was obtained under aseptic conditions in a plain vial from each apheresis donor just before the procedure, 30 mins after start of procedure and 30 mins after the end of procedure. Samples were centrifuged after collection and supernatant was used for determination of ionised calcium values on COMBILINE machine.

STATISTICAL ANALYSIS

The data was coded and entered into Microsoft Excel spreadsheet. Analysis was done using SPSS version 20 (IBM SPSS Statistics Inc., Chicago, Illinois, USA) Windows software program. Descriptive statistics included computation of percentages, means and standard deviations. The paired t-test (for quantitative data to compare before and after observations) was applied. The chi square test was used for quantitative data comparison of all clinical indicators. Level of significance was set at $P \leq 0.05$.

RESULTS

The present study was undertaken in the Department of Pathology, Immunohematology and Blood Transfusion and Biochemistry, PGIMS Rohtak over a period of one year. This study was done to evaluate the changes in pre and post donation platelet indices and serum ionized calcium in plateletpheresis donors. The study included 78 plateletpheresis donors. For hematological parameters, pre and post donation blood samples were taken and analyzed on Automatic Hematology Analyzer BC 5800 Mindray and for biochemical parameters, besides pre and post donation samples, an additional sample was also taken 30 mins after start of the procedure and these samples were analysed on Combiline machine. The age of the donors varied from 18 to 54 years with mean age of 28.23years. Maximum numbers of donors were in age group of 21-30 years followed by donors in age group of 31-40 years. All donors were males and majority of them were first time donors having blood group O + followed by A+ > B+ >AB+ >AB- > O-. The mean of weight, height and BMI was 77.60, 1.72 and 26.1 respectively. There was significant decline in post-donation values of platelets, hemoglobin, hematocrit, whereas it was non-significant in post donation MPV,WBC and RBC. A statistically significant increase in PDW post donation was also seen. The changes in pre and post donation values are shown in Table 1. There was a significant decrease in serum ionized calcium significant at different intervals as shown in Table 2. The change (%) and p-value between pre and during plateletpheresis were 17.31% and 0.001 respectively, between pre and post plateletpheresis were 11.76% and 0.001 respectively and between during and post plateletpheresis were 6.12% and 0.04 respectively. The correlations among different parameters can be seen in the table 3 below:

Post-donation platelet showed significant positive correlation with pre-donation platelet, weight and BMI. It means post-donation platelets increased with increasing weight, BMI and vice versa. In our study, no adverse events were noted in majority of donors (92.3%) and in only 7.7% donors adverse events were seen such as tingling sensation (3.8%),twitching of foot in two donors (2.6%) and perioral numbness in one donor (1.3%).

DISCUSSION

The cell separators can yield high dose plateletpheresis that may result in upto four units of apheresis platelets for transfusion to patients. Post plateletpheresis, variations have been seen in hematological and biochemical values in donors. So, it becomes important to observe the changes in pre and post donation hematological and biochemical parameters due to the safety concerns of platelet donors. Different results have been seen in various studies; in most of the studies there was post donation decrease in hemoglobin concentration, hematocrit, platelet count, RBC count, WBC count whereas a variable result in Mean Platelet Volume has been seen. But changes in levels of serum ionized calcium have also been explored less in literature. During our study period of one year, various haematological and biochemical parameters of 78 plateletpheresis donors were studied. The plateletpheresis procedures were performed on Fresenius Kabi COM.TEC. Hematological parameters and biochemical parameters were analysed on Automatic Hematological Analyser BC 5800 Mindray were analysed on COMBILINE machine respectively.

In present study, the age of donors ranged from 18-54 years with mean age of 28.23 ± 8.27 (SD).All donors were male which was in concordance with the study conducted by Suresh *et al.*[2], Sahoo *et al.*[1], Sharma *et al.*[5],Garg *et al.*[11],Farahat *et al.*[12],Patidar *et al.*[13] and Bassi *et al.*[14]. This could be because of the lack of awareness, low Hb concentration, low weight and poor venous access among females. In the current study, donors from all blood groups were seen. Maximum donors had O+ (34.6%) blood group and only one donor had O- blood group (1.3%). Our study is in concordance with studies conducted by Suresh *et al.*[2] and Bassi *et al.*[14] in which maximum donors were of O+ blood group 53.3% and 35.6% respectively. 84.6% of donors were first time donors followed by 12.8% donors who donated for the second time. In our study, there was statistically significant decrease in mean of pre and post-donation platelet count of plateletpheresis donors was 2.69± 0.49 and 2.08±0.48 respectively (p-value = 0.001). The similar findings were found in the studies conducted by Das *et al.*[15], Mahmood *et al.*[16],Suresh *et al.*[2],Garg *et al.*[17], Khurshid *et al.*[7], Sharma *et al.*[5], Sahoo *et al.*[1], Farahat *et al.*[12] and Patidar *et al.*[13].The reduction in the platelet count could be because this was the object of the procedure and it is expected that some cellular loss would have been there.

Table 1. Comparison of pre and post donation hematological values.

Variables	Pre donation values (Mean ±SD)	Post donation values (Mean ±SD)	Change (%)	Statistical significance (p-value)
Platelet count(lac/cumm)	2.69±0.49	2.08±0.48	23.42	0.001
MPV(fl)	9.029 ±1.59	9.07 ±1.53	0.55	0.69
PDW (%)	16.053± 0.48	16.22 ±0.58	1.05	0.001
Hemoglobin(g/dl)	14.86 ±1.06	14.45±1.09	2.75	0.001
Hematocrit (%)	47.02±3.97	45.68 ± 3.93	2.84	0.001
WBC (X 10^9/L)	7.707±2.01	7.87± 2.02	2.07	0.31
RBC (X 10^{12}/L)	5.19 ±0.52	5.16 ±0.608	0.57	0.48

Table 2. Serum ionised calcium levels at different durations.

		Mean	Std. Dev	Change (%)	p-value
Pair 1	Pre	1.19	0.11	17.31	0.001
	During	0.98	0.27		
Pair 2	Pre	1.19	0.11	11.76	0.001
	Post	1.04	0.09		
Pair 3	During	0.98	0.27	6.12	0.04
	Post	1.04	0.09		

Pre: Baseline value; During: At 30 mins after start of the procedure; Post: At 30 mins post procedure

Table 3. Correlations among different parameters

Parameters		Pre-donation (lac/cumm)	Post-donation Platelets count (lac/cumm)	WT(kg)	BMI	Platelet products (lac/cumm)
Pre-donation PLT(lac/cumm)	Pearson Correlation	1	.684**	.121	.047	.025
	p value		.000	.289	.681	.831
Post-donation PLT(lac/cumm)	Pearson Correlation	.684**	1	.371**	.321**	-.032
	p value	.000		.001	.004	.782
Weight(kg)	Pearson Correlation	.121	.371**	1	.908**	-.041
	p value	.289	.001		.000	.723
BMI	Pearson Correlation	.047	.321**	.908**	1	-.026
	p value	.681	.004	.000		.819
Platelet products (lac/cumm)	Pearson Correlation	.025	-.032	-.041	-.026	1
	p value	.831	.782	.723	.819	

Table 4. Changes in pre and post-donation platelet indices in various studies and present study

Study	Pre donation platelet (N x10^9)	Post donation platelet (N x10^9)	Pre donation MPV(fL)	Post donation MPV(fL)	Pre donation PDW (%)	Post donation PDW (%)
Das et al[15], 2009	213.7±53.16 (150-467)	150.7±46.77 (79-413)	8.6±1.04 (6.3-12.9)	8.7±1.06 (6.2-14.0)	14.4±2.27 (9.8-18.1)	14.3±2.39 (9.2-18.0)
Mahmood et al[16], 2011	264.0±39.8 (189-359)	193.4±28.9 (141-269)	10.0±0.8 (7.7-12.3)	9.7±0.8 (7.7-12.3)	12.3±1.6 (8.7-17.2)	11.8±1.5 (8.5-17.3)
Suresh et al[2], 2014	280.34±54.55 (208-589)	175.58±44.56 (100-295)	8.61±0.77 (6.8-10.9)	8.72±0.83 (6.8-12.0)	15.96±1.38 (3.3-16.8)	16.27±0.39 (15.1-17.8)
Farahat et al[12], 2016	288.1±41.8	176.9±8.8	10.5±1.4	10.6±1.4	12.9±1.7	12.7±1.9
Garg et al[17], 2017	267.32±56.39 (165-450)	202.90±45.69 (108-312)	9.24±3.34 (7.90-9.24)	8.63±0.60 (7.79-8.63)	NA	NA
Khurshid et al[7], 2020	225.91±48.24 (161-330)	169.33±43.20 (113-278)	11±1.58 (7-13)	12.18±4.53 (4.4-20)	NA	NA
Present study	**269±0.49 (2.0-4.4)**	**208±0.48 (1.08-4.2)**	**9.029±1.59 (6-14.7)**	**9.07±1.53 (5.9-14.3)**	**16.05±0.48 (15-17.2)**	**16.22±0.58 (15.1-18.3)**

Table 5. Comparison of pre and post-donation hematological parameters of plateletpheresis donors with other studies

Study	Pre donation Hb(g/dl) (Mean±SD) (Range)	Post donation Hb(g/dl) (Mean±SD) (Range)	Pre-donation Hct(%) (Mean±SD) (Range)	Post-donation Hct(%) (Mean±SD) (Range)	Pre-donation WBC (N x 10^{11}) Mean±SD) (Range)	Post-donation WBC (Nx10^{11}) (Mean±SD) (Range)
Das et al[15],2009	13.9±1.08 (12.2-17.2)	12.6±4.74 (10.5-16.3)	40.8±4.01 (31.8-54.1)	38.9±3.41 (28.5-49.2)	7.5±1.79 (3.8-15.1)	6.8±3.06 (2.5-14.6)
Mahmood et al[16],2011	14.9±0.9 (12.4-17.0)	14.7±1.0 (12.5-17.2)	44.6±2.5 (38.8-51.8)	44.1±2.6 (37.8-53.1)	7.1±1.4 (4.5-12.2)	7.5±1.6 (4.4-13.8)
Suresh et al[2],2014	14.8±1.097 (12.6-17.3)	14.5±1.4 (10.6-17.3)	43.29±6.62 (36.6-98.3)	41.64±4.96 (30.1-68.5)	8.28±1.88 (4.6-14)	6.95±1.76 (2.02-11.13)
Farahat et al[12],2016	14.7±1.3	14.3±1.3	44.1±3.4	42.9±3.9	7.7±2.3	7.2±2.3
Garg et al[17],2017	14.13±1.06 (12-16.20)	13.61±1.04 (11.50-15.70)	43.91±3.04 (37-50)	42.99±3.12 (35-49)	7.40±1.42 (5.80-7.40)	6.20±1.39 (4.30-6.20)
Khurshid et al[7],2020	15.3±1.33 (12.3-18.2)	14.7±1.47 (11.2-16.7)	45.09±3.57 (36.1-55.1)	42.31±3.94 (37.37-52.47)	7.20±1.63 (4.0-11.9)	5.50±1.89 (2.6-9.7)
Present study	14.86±1.06 (12.5-17.0)	14.45±1.09 (12.0-16.6)	47.02±3.97 (37.5-55.8)	45.68±3.93 (36.1-55.8)	7.707±2.01 (3.9-13.45)	7.87±2.02 (4.5-13.51)

Also biomaterials of the apheresis can lead to activation of intrinsic coagulation pathway which could aggravate platelet adhesion and activation, forming clots and thereby decreasing the platelet count.[18] Clinical thrombocytopenia can have ill effects to the donors but in the present study, no donor had showed significant decrease in platelet count which could lead to clinical thrombocytopenia. This could be due to standard operating procedure of department under which the donors having less than 2 lac/cumm pre-donation platelet count were selected. A statistically non-significant increase of 0.55% was found between pre and post donation mean MPV value (p-value=0.69). Similar results were seen in studies conducted by Das *et al.*[15], Suresh *et al.*[2],Khurshid *et al.*[7] and Farahat *et al.*[12] whereas the findings were not in concordance with study conducted Mahmood *et al.*[16] where authors noted post-donation decrease in MPV which could be because of splenic sequestration of activated platelets. This activation of platelets could be after passing through nozzles of the apheresis kit. The spleen sequesters the activated and big platelets, which is reflected in temporary decrease in MPV.[18]

The mean and standard deviation of pre and post donation PDW in platepheresis donors were 16.05±0.48 and 16.22±0.58 in our study with a p-value 0.001, which was statistically significant It was similar to results in study conducted by Suresh *et al.*[2], and in contrast to results of noted by Das *et al.*[15], Mahmood *et al.*[16],Farahat *et al.*[12] who observed decrease in post-donation PDW values. In our study, the increase in post donation MPV and PDW could be because during activation, platelets undergo morphological changes including pseudopodia formation. With the increasing numbers and sizes of pseudopodia a significant increase of MPV and PDW is observed.[19]

There was statistically significant decrease of 2.75% and 2.8% in post-donation Hb and Hct values respectively. As shown in the below table, our study was in concordance with studies conducted by Das *et al.*[15], Mahmood *et al.*[16],Suresh *et al.*[2],Farahat *et al.*[12], Garg *et al.*[17], Khurshid *et al.*[7], Sharma *et al.*[5] and Patidar *et al.*[13] also noted significant decrease in post-donation Hb and Hct values in their studies. The decrease in Hb and Hct could be the result of infusions of anticoagulant solutions and 0.9% normal saline during the procedure or it could be due to blood loss in residual volume of apheresis kit.[2] In studies conducted by Mahmood*et al.*[16] and Patidar *et al.*[13], a non significant increase in post-donation WBC counts was observed, concomittent with our study. Our results were in contrast to the findings noted by Suresh *et al.*[2],Sharma *et al.*[5], Khurshid *et al.*[7] and Farahat *et al.*[12] as shown in the table above. The reason for increase in WBC count could not be stated clearly in literature and our study. A non-significant decrease of 0.57 % was seen in pre donation and post donation RBC values (p-value =0.48) which was in concordance with the results noted by Suresh *et al.*[2] and Farahat *et al.*[12] whereas in other study by Sahoo *et al.*[1], authors reported that RBC counts increased in most cases. This decrease in post-donation RBC values could be due to the blood loss in void volume of apheresis kit at the end of procedure, mechanical hemolysis due to squeezing of blood tubes by device pumps and hemodilution due to infusion of saline and citrate solution during the procedure.[17]

Significant changes in serum ionised calcium levels were noticed at three time intervals i.e. 30 mins before start of procedure, during and 30 mins after procedure. The p-value came out to be 0.001, 0.001 and 0.04 at these intervals. Also there was a decrease in serum calcium value from baseline in the samples drawn at 30 min after start of procedure that rose towards baseline value in post procedural sample. Our results were in concordance with the results reported by Das *et al.*[19], Patidar *et al.*[13] and Farahat *et al.*[12] .However, post procedural calcium levels were found close to their initial baseline levels. The drop in ionised calcium could be due to use of anticoagulant (ACD) that causes reversible chelation of calcium and increase in calcium levels could be due to increased parathyroid hormone.[6] Six donors (7.69%) experienced few adverse events during our platepheresis procedures like tingling, twitching of foot and perioral numbness. All these were mild in nature and managed by giving one oral calcium tablet to the donor. There were no vascular injuries, vasovagal reactions, fault in kit/ equipement or technical aberrations in our study in contrast to study by Bassi *et al.*[14], where 13 adverse events were noted out of which 8 were donor related, 3 were due to kit/ equipment related and only 2 were due to technical aberrations. The adverse reactions in our study were found to be lower as compared to the studies conducted by Garg *et al.*[11](15%) and Patidar *et al.*[13](18%) and higher than the studies by Sahoo *et al.*[1](0.7%),Bassi *et al.*[14](6.1%) and Philip *et al.*[20] (2.6%). Most adverse reactions were due to ACD infusion to donors. The rate of citrate reactions in donors depends on various factors like the type of anticoagulant used (ACD-A having more reactions than ACD-B), the rate of infusion of ACD, the amount of citrate infused and donor serum albumin levels prior to start of procedure.[21] However, in study conducted by Sahoo *et al.*[1], calcium tablet of 1g was given to all donors few minutes before procedure, therefore only one adverse event was noted with one donor.

CONCLUSION

Plateletpheresis is a safe procedure for donors. Though a significant decrease in post procedural hematological and biochemical parameters was noticed but no significant clinical manifestation was observed. However, hematological parameters of the donors need be tested repeatedly at intervals after the procedure which could be useful for deciding subsequent donations. The study concluded that appropriate counselling and selection of plateletpheresis donors should be done. Also, close monitoring and follow up of donors should be carried out to prevent any unfavourable event.

CONFLICTS OF INTEREST: There are no conflicts of interest.

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