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Serial analysis of hematological, biochemical, and immunological parameters alterations in regular healthy voluntary donors during plateletpheresis donation

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Abstract:

BACKGROUND AND OBJECTIVES: The long-term effect of regular plateletpheresis on donors has not been characterized. Hence, we planned to study the long-term alterations in hematological, biochemical, and immunological parameters in regular repeat platelet apheresis donors.

MATERIALS AND METHODS: Thirty-three healthy voluntary regular repeat apheresis donors presenting for platelet donation, fulfilling the requisite donor selection criteria, underwent sequential analysis of the hematological, biochemical, and immunological parameters over 1 year.

RESULTS: A total of 33 regular repeat donors were enrolled in the study; out of these, 22 could be followed up to 3 months, 12 up to 6 months, and 10 donors up to 12 months for their hematological, biochemical, and immunological parameters. Overall, there was no significant change in hematological profile except a rise in platelet count at 3 months ($P = 0.023$) with no significant difference at 6 and 12 months from the baseline. In addition, serum thrombopoietin levels at 3 months ($P = 0.010$) and serum erythropoietin at 6 months ($P = 0.01$) were significantly higher than baseline. Mean platelet volume was significantly higher from baseline at 12 months ($P = 0.00$). Serum protein, lymphocyte subpopulation, and serum ferritin did not show any significant change from baseline over 12 months of follow-up. However, there was a significant decline ($P = 0.00$) in serum calcium and an increase in serum magnesium from baseline ($P = 0.03$) at 12 months.

INTERPRETATIONS AND CONCLUSIONS: To conclude, apheresis platelet donation is a safe procedure. However, a complete hematological, biochemical, immunological profile and bone marrow density at regular intervals (3–6 months) are recommended to ensure the safety of regular repeat plateletpheresis donors.

Keywords:

Biochemical, hematological, immunological parameters, long-term donor safety, plateletpheresis

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Introduction

The long-term effect of regular plateletpheresis on donors has not been characterized. However, transient but significant decreases in platelet counts have been documented in donors undergoing single and serial short-term plateletpheresis collections. Apheresis platelet donors are precious in view of their commitment to undergoing the procedure willingly, which takes longer time (1.5–2 h) than that of whole blood donation (15–20 min). Because of the fact that the donor can donate platelets twice a week, therefore, it is essential to

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understand the long-term effects of plateletpheresis on these precious donors. They come forward voluntarily to support patients in emergency situations, such as the outbreaks of dengue and hemato-oncology patients on regular platelet transfusion support.

Since a complete hemogram is done before each donation, these parameters remained monitored. However, the long-term effects of repeat donation in the form of a decline in serum ferritin, serum proteins, and lymphocyte subpopulation go unmonitored and may at times compromise donor safety.

Another substantial effect that is expected in platelet donors is related to the infusion of citrate. The acute effects of citrate are well recognized. They are rapidly reversible because of their metabolism in the liver, kidneys, and muscles within minutes and other compensatory mechanisms, such as the release of parathyroid hormone, which mobilizes calcium from the reservoir in the bones, increases reabsorption of calcium in the kidney and enhances absorption of calcium in the small intestine.

Thus, donor safety assumes great importance in view of short-term citrate-related adverse effects and long-term effects on the overall health of the donor. However, there is not enough literature on the long-term effect of apheresis platelet donation in regular repeat donors, especially from our country. Therefore, in this current study, we evaluate the alterations in hematological, biochemical, and immunological parameters for 1 year and explore its importance in regular healthy donors who underwent plateletpheresis donation.

Materials and Methods

Study population

This study was conducted in the department of transfusion medicine in collaboration with the departments of biochemistry and immunopathology of a tertiary care hospital of North India, from March 2016 to February 2017. A convenient sample size of 33 voluntary healthy regular-repeat donors who consented for sequential sampling were enrolled in the study. All the enrolled donors had undergone a minimum of five platelet-apheresis donations, negative serological tests for HIV1/2, hepatitis B surface antigen, hepatitis C virus, Venereal Disease Research Laboratory, malaria, and fulfilling the requisite donor selection criteria[1] as per the drugs and cosmetics rule,[2] MoH and FW, GOI. The protocol of the study was approved by the institute ethics committee. Written consent of the donors was also taken after explaining the procedure in detail in their concerned language. The plateletpheresis procedures were done on cell separators based on the continuous flow centrifugation method available in the department (Amicus form Fresenius Kabi, Lake Zurich, IL 60047 USA and Trima Accel® from Terumo BCT Lakewood, CO 80215 USA). The mean platelet yield of collected apheresis product was $\geq 3 \times 10^{11}$ platelets fulfilling the quality control criteria.

Sample collection

Samples were taken at the time of enrolment (baseline), at 3 months, 6 months, and 12 months. For the evaluation of hematological, biochemical, and immunological parameters, a 10 ml venous sample was drawn from the dorsum of hand of the donor using a 21G sterile needle, with minimum stasis into sterile evacuated tubes (BD, Vacutainer, San Jose, CA). 2.5 ml sample was taken into purple top ethylenediaminetetraacetic acid tubes for hematological parameters, 2.5 ml sample was taken into red top plain tubes for biochemical parameters, and 5 ml sample was taken into red top plain 6 ml tubes for total IgG, IgM, and hormonal assessment. Hematological and biochemical parameters were analyzed within 1 h of collection. However, serum for total IgG, IgM, and hormonal assessment was stored in cryovials at −80°C till the analysis.

Hematological parameters

Hematological parameters, i.e., hemoglobin (Hb), total leukocyte count (TLC), hematocrit, and platelet count with mean platelet volume (MPV), were analyzed on an automated hematology analyzer (Make: SFRI, France). Serum ferritin was analyzed using the ELISA technique (Make: Ray Biotech, USA).

Biochemical parameters

Biochemical parameters, i.e., serum calcium, magnesium, and total proteins, were analyzed on an automated biochemistry analyzer (COBOS 8000, Make: Roche Diagnostics).

Immunological parameters

Donor lymphocyte subpopulation cellular profile, i.e., absolute CD3+ cells, CD4+ cells, CD8+ cells, CD19+ cells, and CD56+ cells, were analyzed using a panel of monoclonal anti-CD3 antibody (FITC-conjugated), anti-CD4 antibody (PE-Cy7 conjugated), anti-CD8 (APC-Cy7-conjugated), anti-CD19 (APC-conjugated), and anti-CD56 (PE-conjugated) on flow-cytometer, FACS Aria (Make: BD, Biosciences, San Jose, CA). Total IgG and IgM estimation was done, using a nephelometric assay (Make: MININEPH™, UK).

Hormonal assessment

Hormonal assessment, i.e., serum thrombopoietin, erythropoietin, interleukin-6 (IL-6), and stem cell factor, was analyzed using the ELISA technique (Make: Ray Biotech, USA).

Statistical analysis

Donor characteristics and cell counts were expressed with descriptive statistics such as mean ± standard deviation (SD) and ranges. Descriptive statistics were performed with Excel, Microsoft, and Redmond, WA. Significance tests for comparisons between baseline parameters and 3-month interval parameters were done with an independent *t*-test, using a commercial statistics program (IBM SPSS version 22, 2013, USA). $P < 0.05$ was considered significant.

Results

A total of 33 voluntary healthy regular repeat donors were enrolled in this study. Out of these 33 donors, 22 were followed up to 3 months, 11 up to 6 months, and 10 up to 12 months for their hematological, biochemical, immunological, and hormonal parameters. Characteristics of apheresis donors are described in Table 1.

Hematological parameters

The mean Hb, hematocrit, TLC, platelet count, and MPV were 14.8 g/dl, 41.2%, 7.1 × 10^3/µl, 202.9 × 10^3/µl, and 9.7 fl, respectively, in all the 33 donors at the time of enrollment. Out of these 33 donors, the hematological parameters of 22 donors were followed at 3 months, 11 donors at 6 months, and 10 donors at 12 months. Hb, hematocrit, and TLC were slightly decreased over 1 year, but not statistically significant as described in Table 2. However, there was a significant increase in platelet count at 3 months ($P = 0.02$) with no significant difference from baseline at 6 and 12 months. MPV was also increased significantly from baseline at 12 months ($P = 0.01$).

Biochemical parameters

Baseline biochemical parameters were assessed in 33 donors, repeated in 22 donors at 3 months, 11 donors at 6 months, and 10 donors at 12 months of follow-up as described in Table 3. The mean baseline serum calcium was 9.3 mg/dl, serum magnesium was 2.1 mg/dl, and serum protein was 7.3 g/dl at the time of enrollment. The average serum calcium decreased over 1 year and was statistically significantly low at 12 months ($P = 0.01$). However, the serum magnesium was increased during the study's tenure and was found statistically increased at 12 months ($P = 0.03$). In addition, the serum protein remained constant during 1 year, as described in Table 3.

Table 1: Plateletpheresis donor characteristics

Donor characteristics	Mean±SD (*n*=33)
Age (years)	36.54±11.21
Weight (kg)	81.69±9.97
Height (cm)	172.45±7.24
BMI (kg/cm^2)	27.43±2.52
Number of whole blood donations	23.68±23.99
Number of SDAP donations	21.78±21.22

BMI=Body mass index, SD=Standard deviation, SDAP=Single Donor Apheresis Platelet

Immunological parameters

Out of the total 33 donors enrolled in the study, total IgM and IgG levels could be repeated in 22 donors at 3 months and then in 9 donors at 6 months and 4 donors at 12 months described in Table 4. Total IgM and IgG levels decreased over 12 months of follow up, but not statistically significant [Table 4].

Donor lymphocyte subpopulation cellular profile assessment, i.e., absolute CD3+ cells, CD4+ cells, CD8+ cells, CD19+ cells, and CD56+ cells levels, was assessed in all 33 donors at baseline, 22 donors at 3 months, and 8 donors at 6 months and 12 months interval. Absolute CD3+ cells, CD4+ cells, CD8+ cells, and CD19+ cells decreased slightly; however, absolute CD56+ cells increased, as described in Table 5. In addition, the donor lymphocyte subpopulation cellular profile did not change significantly during the period of study [Table 5].

Hormonal/cytokine assessment

Out of the total 33 donors enrolled in the study, the hormonal assessment could be repeated in 22 donors at 3 months and then in 11 donors at 6 months and 10 donors at 12 months, as described in Table 6. We observed that there was an increasing trend of serum erythropoietin levels up to 6 months and the increase was statistically significant ($P = 0.00$), but returned at baseline levels at 12 months. Serum thrombopoietin was also significantly increased ($P = 0.01$) at 3-month intervals but could not be observed further due to technical reasons. IL-6 and stem cell factors remain constant during the period of study [Table 6].

Discussion

The effect of long-term regular plateletpheresis on donor safety is an important aspect for retaining donors and managing an emergency pool of these precious donors. They come forward voluntarily to support our patients in an emergency, such as the outbreaks of dengue and hemato-oncology patients on regular platelet transfusion support. In this present study, we enrolled 33 regular repeat plateletpheresis donors at our center to assess the changes in hematological, biochemical, and immunological parameters at 3-month interval over 1 year.

All the enrolled donors donated apheresis platelets with a mean donation as 21 and most of them kept donating throughout the study period. They had a continuous

Table 2: Change in hematological parameters at different intervals in follow-up

Parameters	Mean±SD								
	Baseline (*n*=33)	Baseline versus 3 months (*n*=22)			Baseline versus 6 months (*n*=11)			Baseline versus 12 months (*n*=10)		
		Baseline	3 months	*P*	Baseline	6 months	*P*	Baseline	12 months	*P*
Hb (g/L)	148.00±12.00	150.00±12.00	151.80±15.00	0.53	149.00±10.00	149.10±16.00	0.96	140.10±11.20	139.20±9.00	0.79
Hematocrit (proportion of 1)	0.41±0.03	0.41±0.03	0.42±0.05	0.17	0.40±0.03	0.42±0.04	0.28	0.40±0.02	0.41±0.05	0.40
TLC ($\times10^9$/L)	7.10±1.75	7.05±1.80	6.70±1.42	0.25	6.85±1.65	6.34±1.10	0.18	6.51±1.32	5.96±1.17	0.22
Platelet count ($\times10^9$/L)	202.90±64.50	189.64±41.42	216.36±63.20	0.023	187.91±29.64	227.73±77.50	0.13	209.30±50.12	224.50±77.23	0.54
MPV (fL)	9.7±1.1	9.94±1.15	10.54±1.06	0.07	9.52±1.14	10.33±0.92	0.06	9.71±1.10	10.96±0.85	0.01
Serum ferritin (µg/L)	40.30±27.80	39.75±25.17	38.85±25.19	0.849	40.95±17.51	43.40±23.90	0.759	50.86±35.78	33.04±19.98	0.082

MPV=Mean platelet volume, TLC=Total leukocyte count, Hb=Hemoglobin, SD=Standard deviation

Table 3: Change in biochemical parameters at different intervals in follow-up

Parameters	Mean±SD								
	Baseline (*n*=33)	Baseline versus 3 months (*n*=22)			Baseline versus 6 months (*n*=11)			Baseline versus 12 months (*n*=10)		
		Baseline	3 months	*P*	Baseline	6 months	*P*	Baseline	12 months	*P*
Serum calcium (mmol/L)	2.33±0.15	2.36±0.17	2.38±0.23	0.68	2.35±0.17	2.23±0.20	0.06	2.34±0.13	2.04±0.25	0.01
Serum magnesium (mmol/L)	0.86±0.12	0.89±0.14	0.86±0.07	0.22	0.88±0.15	0.84±0.13	0.45	0.87±0.12	0.96±0.12	0.03
Serum protein (g/L)	73.00±3.00	74.10±3.20	74.10±4.10	0.94	72.90±2.90	70.10±6.70	0.11	72.60±3.20	72.80±5.50	0.92

SD=Standard deviation

Table 4: Change in immunoglobulin levels at different intervals in follow-up

Parameters	Mean±SD								
	Baseline (*n*=33)	Baseline versus 3 months (*n*=22)			Baseline versus 6 months (*n*=9)			Baseline versus 12 months (*n*=4)		
		Baseline	3 months	*P*	Baseline	6 months	*P*	Baseline	12 months	*P*
Total IgG (g/L)	14.5±2.5	14.4±2.7	12.8±3.4	0.09	14.2±2.7	12.5±2.8	0.26	14.6±0.7	13.7±2.2	0.40
Total IgM (g/L)	0.98±0.47	0.9±0.35	0.88±0.42	0.70	0.92±0.34	1.5±2.5	0.50	1.0±0.34	0.7±0.33	0.30

Ig=Immunoglobulin, SD=Standard deviation

Table 5: Change in donor lymphocyte subpopulation cellular profile at different intervals in follow-up

Parameters	Mean±SD								
	Baseline (*n*=33)	Baseline versus 3 months (*n*=15)			Baseline versus 6 months (*n*=8)			Baseline versus 12 months (*n*=8)		
		Baseline	3 months	*P*	Baseline	6 months	*P*	Baseline	12 months	*P*
Absolute CD3+ cells ($\times10^3$/µL)	4.6±1.3	4.21±0.92	4.16±0.70	0.83	3.96±0.75	3.98±0.80	0.92	4.05±0.89	3.18±1.16	0.41
Absolute CD4+ cells ($\times10^3$/µL)	2.9±0.8	2.69±0.63	2.77±0.29	0.66	2.53±0.52	2.60±0.82	0.75	2.45±0.43	2.47±0.67	0.92
Absolute CD8+ cells ($\times10^3$/µL)	2±0.6	1.96±0.56	1.86±0.58	0.40	1.85±0.57	1.80±0.58	0.78	2.01±0.58	1.95±0.52	0.74
Absolute CD19+ cells/µL	700±300	605.69±401.22	698.69±322.18	0.10	569.87±346.37	643.12±261.33	0.35	535.25±306.78	625.0±430.52	0.37
Absolute CD56+ cells/µL	1400±600	1479.84±737.09	1393.84±466.82	0.55	1673.37±806.85	1674.87±495.20	0.99	1629.75±775.86	1528±492.40	0.59

SD=Standard deviation

donation of whole blood and platelet apheresis during the period of the study. However, out of these 33 donors, 22 donors were followed at 3 months, 11 donors at 6 months, and 10 donors at 12 months. In addition, in our study, all the donors were male, and the average age of all the donors was 36.54 years, and the body mass index was 27.43 kg/cm^2, which was more than the normal range compared to their respective ages.

Table 6: Change in hormonal/cytokine assessment at different intervals in follow-up

Parameters	Mean±SD									
	Baseline (*n*=33)	Baseline versus 3 months (*n*=22)			Baseline versus 6 months (*n*=11)			Baseline versus 12 months (*n*=10)		
		Baseline	3 months	*P*	Baseline	6 months	*n*	Baseline	12 months	*n*
Serum thrombopoietin (pg/mL)	85.2±56.8	70.50±35.28	100.63±45.99	0.010	ND	ND	ND	ND	ND	ND
Serum erythropoietin (mIU/mL)	39.3±28.7	45.7±29.8	34.5±29.6	0.209	36.6±24.7	70.7±26.4	0.004	44.2±28.7	44.5±33.0	0.984
Serum IL-6 (pg/mL)	5.3±4.0	4.7±2.9	6.1±6.0	0.227	5.3±3.9	7.2±7.5	0.333	4.6±3.3	5.4±2.8	0.621
Serum SCF (pg/mL)	28±12	28.18±9.94	31.86±17.58	0.409	26.45±8.07	24.66±10.26	0.603	28.26±10.00	34.35±12.1	0.275

ND=Not determined, SD=Standard deviation, SCF=Stem cell factor, IL=Interleukin

In 2005, FDA published a draft guidance[3] on automated platelet collections, in which it was stated that a plateletpheresis donor should not undergo more than 24 platelet donations in 12 months. However, this was questioned by Page *et al*. in 2010,[4] as they observed that plateletpheresis donors lose up to 100 ml of blood at each donation and regular repeat donors donating at the maximum allowed frequency, i.e., 24 times in a year, will lose 2400 ml of blood equivalent to 4–5 whole blood donation and will become prone to iron deficiency. They also observed that the minimum number of donations (whole blood and platelets) before any subject showed depleted iron stores was 14, and there was a strong correlation between the frequency of plateletpheresis and iron depletion. About 63.9% of male donors donating at the 2-week interval were having serum ferritin concentrations below the normal range. However, in our study, we observed that Hb and hematocrit remain constant, and serum ferritin slightly decreased during the study period, but not statistically significant. However, serum erythropoietin levels were significantly increased ($P = 0.004$) over 6 months. Therefore, it may be predicted that Hb and hematocrit may remain constant in our study due to the production of more red cells from bone marrow in response to increased serum erythropoietin. The rise in Hb and Hct was not significant as expected with a significant rise in serum erythropoietin, this could be due to whole blood donations done by the enrolled donors during the period of follow-up.

National Blood Services in UK[4] recommended to change the policy of maximum allowed donation of 24 in a year and advised that a volunteer should not donate platelets more than 15 times in a year, thus supporting the observation made by Page *et al*.[4] Furthermore, Castro *et al*.[5] highlighted that plateletpheresis donors incur a loss of Hb equivalent to 1 unit of whole blood after 10 plateletpheresis donations. However, Katz *et al*.[6] in their study concluded that these restrictions are not required to prevent thrombocytopenia in frequent platelet donors as it would adversely impact the supply of apheresis platelets as they did not find any clinically significant decrease in platelet counts in donors donating multiple platelet components up to 24 times a year. However, in our study, we observed that platelet count was in increasing trend during the study period; however, statistical significance was observed only at 3-month interval ($P = 0.023$). Furthermore, serum thrombopoietin also increased significantly ($P = 0.010$) at 3-month interval; however, serial levels were not reported after the 3 months because of the technical issues as values were not detected within the limits of kits, so could not be commented.

In contrast, a retrospective study published in 2001[7] described a significant and sustained decrease in platelet count for all donation frequency categories. The frequency of donation correlated directly with a reduction in platelet count for all but the highest-frequency (24 times in a year) donation group. A mean decrease of 40,000/µl from baseline was observed in the frequent-donor subgroup, and a total of 84 donors (9%) were deferred due to low platelet counts. It was also predicted that in our study, the increasing trend of platelet count was due to the hormonal effect of serum thrombopoietin. However, in other published literature, the platelet count was not correlated with serum thrombopoietin levels during the period of study.

As in automated platelet donations, around 200–300 ml plasma is collected per donation to suspend the harvested platelets, and although the product is leukoreduced, there is still a loss of white blood cells (WBCs). Hence, a fall in serum protein, immunoglobulins, and WBC count changes are expected in regular repeat platelet donors. There are different reports from the literature; Lewis *et al*.[8] observed no significant change in lymphocyte subsets in platelet donors than whole blood donors. However, Koepke *et al*.[9] found a transient decrease in B cells, but there was no change in the absolute number of T cells in repeat plateletpheresis donors. In contrast, Matsui *et al*.[10] observed a significant decrease in T4, T8, T4/T8 ratio, and IgG levels in platelet donors. Furthermore, there was a significant increase in B cells and monocytes. However, in our study, absolute CD3+ cells, CD4+ cells, CD8+ cells, CD19+ cells were slightly decreased, and absolute CD56+ cells were in an increasing trend during

the period of study, but not statistically significant. Moreover, the total IgM and IgG levels showed a decline over 12-month follow-up, but it was also not statistically significant.

Another important effect that is expected in platelet donors is related to the infusion of citrate. The acute effects of citrate are well recognized and are rapidly reversible because of its metabolism in the liver, kidneys, and muscles within minutes and other compensatory mechanisms, such as the release of parathyroid hormone, which mobilizes calcium from the reservoir in the bones, increases reabsorption of calcium in the kidney and enhances absorption of calcium in the small intestine. Except for the short-term calcium metabolism disturbance due to citrate exposure during apheresis procedures, there is a lack of evidence of the long-term effects of repeated apheresis procedures on calcium balance and bone mineral density (BMD). In a cross-sectional study by Amrein *et al*.[11] on regular plateletpheresis donors, a small but statistically significant association of citrate effect was reported with lumbar BMD. In contrast, only serum concentrations of calcium, magnesium, and total protein were observed in our study. We observed a significant fall in serum calcium at 12-month interval (P = 0.01), whereas serum magnesium increased significantly at 12-month interval (P = 0.03) from baseline but was within the normal range, this could be explained by intake of a diet rich in magnesium such as green vegetables as advised to the donors at the time of donation. However, total protein remained constant.

Based on our search, there was no published literature on the serial analysis of hematological, biochemical, and immunological parameter alterations in regular healthy voluntary donors during plateletpheresis donation in India. However, in a prospective study by Suresh *et al*.[12] from south India, 90 healthy plateletpheresis volunteered donors were studied. They compared the pre- and postplateletpheresis donor hematological profile such as Hb, hematocrit, platelet count, mean corpuscular volume, mean corpuscular Hb, mean corpuscular Hb concentration, platelet distribution width (PDW), red blood cell count, WBC count, and MPV and found a significant decrease in postdonation Hb, hematocrit, platelet count, TLC, and PDW with a slight rise in MPV. However, none of them developed clinical manifestations of anemia or thrombocytopenia. However, these donors were not followed up to comment on the long-term effects of plateletpheresis.

Conclusion

In the present scenario, only hematology counts are done before each donation, these parameters remained monitored, and a donor can be deferred temporarily and again enrolled back once he fulfills the criteria of platelet donation. However, the long-term effects of repeat donation in the form of a decline in serum ferritin, serum proteins, and lymphocyte subpopulation go unmonitored and compromise donor safety. Thus, our study acquires a unique importance.

To conclude, apheresis platelet donation is a safe procedure. However, regular repeat plateletpheresis donors undergo variable amounts of citrate infusion during the procedures so, a complete hematological, biochemical, and immunological profile at regular intervals (3–6 months) along with BMD is recommended in such donors to ensure their safety.

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Conflicts of interest

There are no conflicts of interest.

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