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Effect of plateletpheresis on postdonation serum thrombopoietin levels and its correlation with platelet counts in healthy voluntary donors

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Abstract:

BACKGROUND: Thrombopoietin (TPO) is regulated by a feedback mechanism between megakaryocytes and platelets. This is important in plateletpheresis donors to compensate for donation-associated platelet loss.

AIMS AND OBJECTIVES: The aim and objective of this study were to investigate changes in serum TPO levels in healthy plateletpheresis donors and its correlation with platelet recovery pattern.

MATERIALS AND METHODS: Out of 50 plateletpheresis donors recruited in the study over 1 year, only 29 completed follow-up and were further analyzed. Plateletpheresis procedures were performed on two types of cell separators (TRIMA ACCEL[®], Terumo BCT Lakewood Colorado and AMICUS[®], Fresenius Kabi, Germany). Platelet parameters were estimated pre- and post-platelet donation, at 3rd- and 5th-day postdonation. Serum TPO levels were determined using quantitative sandwich enzyme-linked immunosorbent assay technique (Raybiotech, USA) as per the protocol of the manufacturer.

RESULTS: The majority of donors (72%) in our study were first-time donors. The baseline platelet count was $226 \pm 44 \times 10^3/\mu\text{l}$ with a significant decline (30%; $P < 0.001$) in postdonation phase and remained below baseline on the 3rd and 5th day. The serum TPO levels increased significantly ($P < 0.001$) from a baseline of 227.81 (interquartile range [IQR]: 176.06) pg/ml to 269.94 (IQR: 110.68) pg/ml postdonation and remained elevated from baseline levels on the 3rd and 5th day. An inverse relation was observed between change in serum TPO levels and platelet count during postdonation phase which was not statistically significant ($P > 0.05$).

CONCLUSION: Serum TPO levels increase significantly post plateletpheresis donation corresponding to decrease in platelet counts showing that TPO plays a vital role in compensatory mechanism after platelet loss.

Keywords:

Platelet counts, plateletpheresis, thrombopoietin

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Introduction

Plateletpheresis donors are either volunteer donors, patient's relatives, or human leukocyte antigen or platelet antigen-matched donors. The donor's platelet count determination before the

donation is one of the major suitability criteria for plateletpheresis. The donor's baseline platelet count helps in deciding the target yield. Donors with higher platelet counts are particularly valuable because in that case, one is more likely to collect a concentrate that can be divided into more than one platelet transfusion dose and helps in better patient and inventory management. It is important to know about

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the regulatory mechanisms involved in the recovery of platelet count postdonation and the time required for the same. So that appropriate gap can be given between two successive platelet donations, especially in regular repeat apheresis donors.

Megakaryocytopoiesis is regulated by various cytokines, and thrombopoietin (TPO) is a key cytokine. It promotes the proliferation and differentiation of committed megakaryocytic precursor cells by exerting its action through binding to the *mpl* receptor. The expression of *mpl* receptors on both megakaryocytes and platelets suggests that serum TPO is regulated by a feedback mechanism between platelet-producing cells and platelets in circulation.^[1] As TPO is the physiologic regulator of platelet production, the study of serum TPO levels in healthy plateletpheresis donors may provide an insight into the megakaryocytopoietic activity of a donor after plateletpheresis donation. Keeping this in view, we planned to investigate changes in serum TPO levels in healthy voluntary donors at different time points after platelet donation and its relation with platelet counts.

Materials and Methods

Study design

A prospective observational study was conducted over 1 year.

Study setting

The study was conducted in the apheresis section of the department of transfusion medicine at a tertiary care center in North India.

Study subjects

Healthy plateletpheresis donors who fulfilled criteria for plateletpheresis as per the Directorate General Health Services (DGHS), Ministry of Health and Family Welfare, Government of India.^[2]

Sample size

A convenient sample of 50 voluntary plateletpheresis donors were recruited in the study.

Ethics approval

The study was approved by the Institutional Ethics Committee before enrollment of the individuals. Participants were recruited after a written informed consent.

Study procedures

Plateletpheresis procedures were performed on two types of cell separators (TRIMA ACCEL[®], Terumo BCT Lakewood, Colorado and AMICUS[®], Fresenius Kabi, Germany). The mean platelet yield of collected platelet

product was $\geq 3 \times 10^{11}$ platelets, fulfilling the requisite quality control criteria.^[2]

Before the procedure, the donor was briefed about the procedure and a written informed consent was obtained. Antecubital veins on both sides were assessed for their suitability for phlebotomy. Sampling was done from the dorsum of the hand for blood grouping and transfusion transmissible infections screening of the donor. For the evaluation of serum TPO levels and platelet counts, 10-ml blood was drawn (2 ml in EDTA vacutainer and an 8 ml of blood in plain vacutainer) before platelet donation, at the end of the donation (apheresis), and at the 3rd- and 5th- postdonation day. At each time point, platelet parameters, including platelet count, mean platelet volume (MPV), and platelet distribution width (PDW), were estimated using ORION 60TM hematology analyzer.

Serum TPO levels were determined using quantitative sandwich enzyme-linked immunosorbent assay technique (Raybiotech, USA) on stored serum samples, as per the protocol of the manufacturer.

Statistical analysis

The means/median of serum TPO, platelet counts, and other hematological parameters were evaluated using the paired *t*-test or Wilcoxon signed-rank test (as per the normality of data) for comparison between the two time points. The Friedman's two-way analysis of variance or ANOVA test (as per the normality of data) was used to compare the parameters at three or more time points postplateletpheresis. Correlation between the two variables was analyzed using the Spearman's correlation.

Results

Of the total 50 voluntary apheresis donors who underwent plateletpheresis, 29 donors could complete follow-up until day 5 postdonation. The mean age of the donors was 31 ± 8 years, there were no female donors in the study. Out of 29 donors, 21 (72%) were first-time donors. The baseline donor platelet count was $226 \pm 44 \times 10^3/\mu\text{l}$. The baseline demographic and hematological parameters of 29 donors who were analyzed are summarized in Table 1.

Changes in platelet parameters

Overall, the mean platelet counts remained significantly low during the postdonation study period [Table 2 and Figure 1]. On *post hoc* analysis to assess the difference in mean platelet counts at different time points, there was a significant decline in mean platelet count (30%, $P < 0.001$) in immediate postprocedure period. The platelet counts remained significantly below the pre-procedure (baseline) value on the 3rd day ($P < 0.001$) and 5th day ($P = 0.002$). There was a

significant increase ($P < 0.001$) in mean platelet counts on day 5 as compared to immediate postdonation period.

Table 1: Demographic and hematological parameters of plateletpheresis donors (n=29)

Parameters	Values (mean±SD)
Age (years)	31±8
First-time donors, n (%)	21 (72)
Repeat donors, n (%)	8 (28)
Hb (g/dl)	14.94±1.23
Hct (%)	39.78±3.07
TLC (×1000/μl)	7.45±1.43
Platelet count (×1000/μl)	226.41±44.06
MPV (fl)	9.70±0.83
PDW (%)	12.86±1.86
Serum TPO level (median with IQR) (pg/ml)	227.81 (176.06)

SD=Standard deviation, IQR=Interquartile range, TPO=Thrombopoietin, Hb=Hemoglobin, Hct=Hematocrit, TLC=Total leukocyte count, MPV=Mean platelet volume, PDW=Platelet distribution width

However, the counts on day 5 were still below the predonation level. The unit (delta) change (decrease from baseline) in platelet counts among the three different time points after the donation was statistically significant ($P = 0.000$) as shown in Table 2. There was no significant change in MPV and PDW from baseline to day 5, and no correlation of MPV and PDW was observed with platelet count [Table 2].

Changes in serum thrombopoietin levels

Corresponding to decrease in platelet counts, median serum TPO levels increased significantly ($P < 0.01$) from a baseline of 227.81 (interquartile range [IQR]: 176.06) to 269.94 (IQR: 110.68) pg/ml postdonation. Further, it did not change significantly on day 5, although it decreased significantly on day 3 when compared to postdonation values [Figure 1 and Table 2]. Overall, the serum TPO levels remained above the baseline till day 5.

Table 2: Comparison of serum thrombopoietin, platelet counts (including delta change from baseline), and other hematological parameters at different time points of platelet donation (n=29)

Parameters	Time points	Mean±SD/median (IQR)	P^s	$P^®$
Platelet count (×1000/μl)	Pre	226.41±44.06	Reference	0.000 ^{##}
	Post	160.37±35.22	<0.001*	
	Day 3	181.00 (67.75)	<0.001**	
	Day 5	206.20±49.00	0.002*	
	Pre-post	62.50 (32.50)	0.000 ^{##}	
Delta change in platelet count (×1000/μl) (median with IQR)	Pre-day 3	40.50 (40.50)		
	Pre-day 5	22.50 (49.50)		
	Pre	9.70±0.83	Reference	0.657 [#]
MPV (fl)	Post	9.48±0.91	NS	
	Day 3	9.76±1.00	NS	
	Day 5	9.73±0.91	NS	
	Pre	12.86±1.86	Reference	0.768 [#]
	Post	12.75±1.72	NS	
PDW (%)	Day 3	13.15±1.92	NS	
	Day 5	13.21±2.20	NS	
	Pre	227.81 (176.06)	Reference	0.000 ^{##}
	Post	269.94 (110.68)	0.000**	
	Day 3	226.99 (95.51)	0.166**	
TPO (pg/ml)	Day 5	267.25 (88.11)	0.000**	
	Pre-post	-26.78 (34.77)	0.005 ^{##}	
	Pre-day 3	-2.87 (51.54)		
	Pre-day 5	-31.63 (56.16)		
	Pre	14.94±1.23	Reference	0.623 [#]
Hb (g/dl)	Post	14.93±1.53	NS	
	Day 3	14.53±1.32	NS	
	Day 5	14.84±1.25	NS	
	Pre	39.78±3.07	Reference	0.868 [#]
	Post	39.73±3.70	NS	
Hct (%)	Day 3	39.12±4.54	NS	
	Day 5	39.83±2.92	NS	
	Pre	7.45±1.43	Reference	0.786 [#]
	Post	7.15±1.29	NS	
	Day 3	7.47±1.51	NS	
TLC (×1000/μl)	Day 5	7.31±1.05	NS	

**Wilcoxon signed-rank test; *Paired t-test; ##Friedman's test; #ANOVA test, P^s difference of mean/median when compared to reference value. $P^®$ difference of mean/median when compared at different time points. "Reference:" All preprocedure values were taken as reference against which other time points were compared. ANOVA=Analysis of variance, SD=Standard deviation, IQR=Interquartile range, TPO=Thrombopoietin, MPV=Mean platelet volume, PDW=Platelet distribution width, Hb=Hemoglobin, Hct=Hematocrit, TLC=Total leukocyte count, NS=Not significant

The unit (delta) change (increase from baseline) in serum TPO levels among the three different time points after the donation was statistically significant ($P = 0.005$) as shown in Table 2.

Correlation between platelet counts and serum thrombopoietin levels

An inverse relation was observed between serum TPO and platelet count as shown in Figure 2. However, the correlation was not statistically significant at any

time point after the donation ($R^2 = 0.001$, $P = 0.87$ for postdonation; $R^2 = 0.052$, $P = 0.23$ for day 3; and $R^2 = 0.046$, $P = 0.26$ for day 5).

Changes in other hematological parameters

There was no significant change observed in other hematological parameters such as hemoglobin, hematocrit, and total leukocyte count from baseline to day 5 postdonation as shown in Table 2.

Discussion

In this study, we assessed changes in the platelet counts and serum TPO levels in volunteer plateletpheresis donors after platelet donation and their correlation. The majority of donors in our study were first-time donors (72%) with mean baseline (predonation) platelet count as $226 \pm 44 \times 10^3/\mu\text{l}$ which declined in postdonation phase and remained below baseline on day 3 and 5, corresponding to which serum TPO levels increased from a median baseline of 227.81 (176.06) pg/ml to 269.94 (110.68) pg/ml postdonation and remained elevated on day 3 and 5. The above results suggest an inverse relation of serum TPO with platelet count recovery; however, it was not statistically significant.

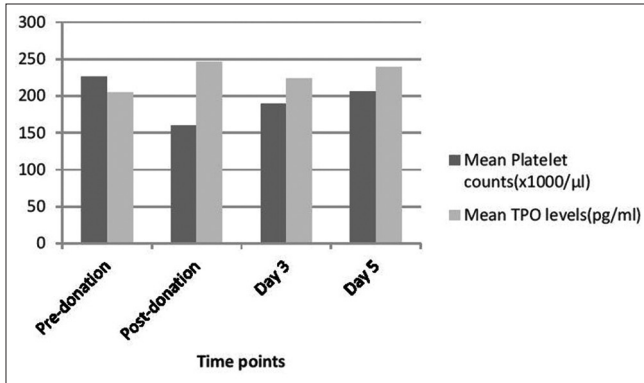


Figure 1: Mean platelet count and serum thrombopoietin levels at different time points of plateletpheresis

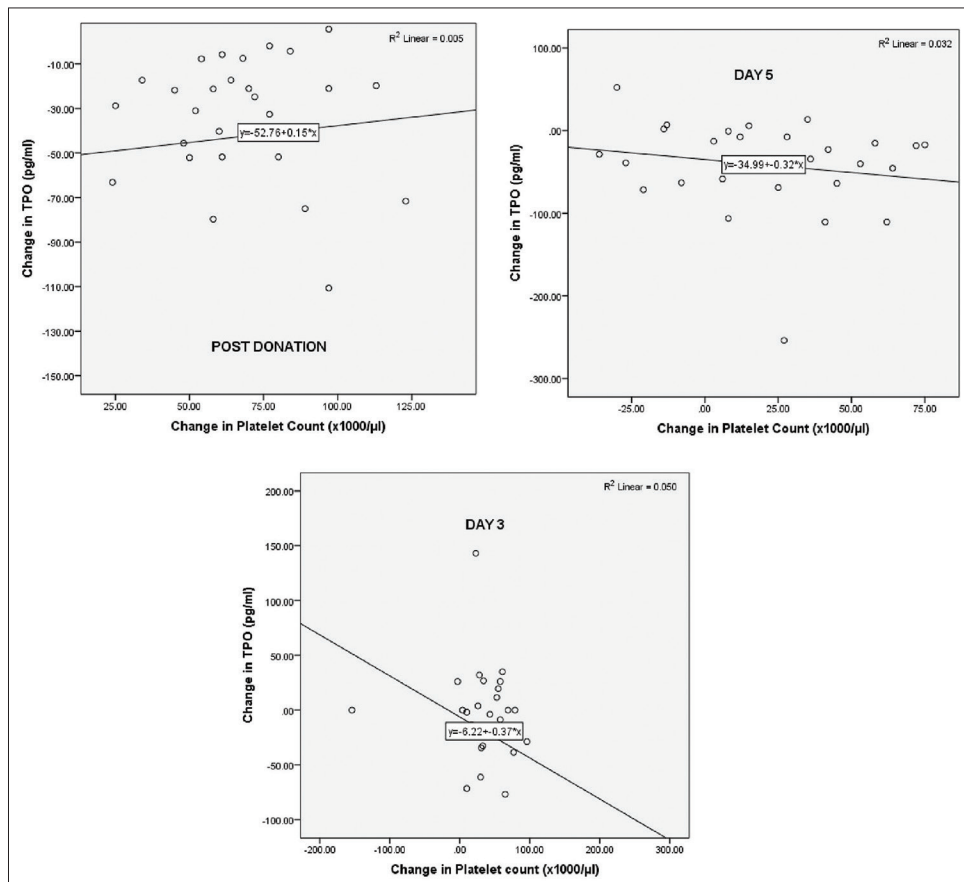


Figure 2: Correlation of (delta) change in serum thrombopoietin and platelet counts at postdonation, day 3, and day 5

We observed a 30% decline in postprocedure platelet count after the donation of a standard apheresis platelet dose, and similar observations have been reported previously by Lasky *et al.*,^[3] Tendulkar and Rajadhyaksha,^[4] Kalish *et al.*,^[5] and Sahoo *et al.*^[6] Whereas Chaudhary^[7] had observed a higher drop in postprocedure platelet count by 50%–55% than in our study. We followed the platelet count on day 3 and 5 of the procedure and found that at day 5, platelet count was below baseline whereas Wagner *et al.*^[8] followed platelet counts on day 1, 4, and 7 and observed that platelet count reached preapheresis levels by day 7 in their study. Thus, on the basis of our study's observation of having platelet counts less than baseline till day 5, it is important to increase the donation interval in regular repeat apheresis donors at least for 7 days as against the current recommendation for 3 days as per DGHS, Ministry of Health and Family Welfare, Government of India.^[2]

In this study, the peak serum TPO level was observed immediately postdonation and did not reach the predonation level till day 5 indicating a compensatory megakaryopoietic drive postdonation. In previous studies by Dettke *et al.*^[9] and Wagner *et al.*,^[8] the peak TPO levels were observed after 24-h postdonation and remained above predonation levels till day 4 and day 7, respectively. Another study by Weisbach *et al.*^[10] reported a short-term significant rise in serum TPO levels at the end of platelet donation and on day 1 of donation. The results of the above studies along with observations of our study suggest that loss of platelets during plateletpheresis affects the mechanism of thrombopoiesis at the level of progenitor cells, possibly through alterations in serum TPO concentrations. Weisbach *et al.*^[10] also suggested that there is a decrease in serum stem cell factor levels during apheresis due to its consumption by early hematopoietic progenitors to stimulate megakaryocytopoiesis.

In addition, we observed that the serum TPO levels shared an inverse correlation with the platelet counts which was not statistically significant. This is in concordance with the observations of Schrezenmeier *et al.*^[11] and further with the findings of Tacke *et al.*^[12] who studied the serum levels of TPO, erythropoietin, interleukin (IL)-6, and IL-11 in plateletpheresis donors and did not observe any correlation of these cytokines with platelet counts or with the frequency of platelet donations in the last 6 months. The authors concluded that platelet donation does not lead to a persisting increase of thrombopoietic cytokines. In this study, baseline TPO levels were not achieved till day 5 as observed by Dettke *et al.*^[9] who also did not observe any significant correlation between serum TPO levels and absolute platelet counts. Further, the authors observed that female donors showed a delayed

normalization of platelet count and TPO levels.^[9] In this study, we did not have female donors to comment on differences based on gender.

In an Indian study by Singh *et al.*,^[13] where baseline levels of serum TPO were measured in aplastic anemia patients and were compared to healthy blood donors taken as controls in the study, the authors observed median TPO levels and platelet count as 121.1 pg/ml and $193 \times 10^9/L$, respectively, which was less than our predonation values. Further, the authors found no statistically significant correlation between platelet count and serum TPO levels as observed in our study. The inverse correlation between platelet count and serum TPO was also supported by Chaudhary R *et al.*^[7] as well as by Kuter and Rosenberg^[14] who developed a model of nonimmune thrombocytopenia in rabbits by administering subcutaneous busulfan. They observed a decrease in elevated levels of TPO when platelets were transfused into these rabbits close to the time of their platelet count nadir. In addition, platelets were observed to remove TPO from thrombocytopenic plasma *in vitro*. These results confirm that TPO is the cytokine for megakaryocytopoiesis, and platelet count may directly play a role in regulating its circulating levels. In another study, Kuter *et al.*^[15] investigated an interesting therapeutic role of recombinant human TPO in healthy donors for increasing the yield of platelets during apheresis donations. In this blinded, two-cycle, crossover study, 59 platelet donors were randomized to receive a single subcutaneous injection of recombinant TPO 15 days before the donation. It was observed that donors treated with placebo had significantly lower median peak platelet count when compared to the donors who received recombinant TPO, resulting in a collection of median threefold more platelets.

Kalish *et al.*,^[5] in their study, postulated rapid-response mechanisms which function with different effectiveness in plateletpheresis donors to maintain the platelet count during the donation. Furthermore, Lee and Schiffer proposed that platelets are rapidly mobilized from the spleen during platelet donation to maintain their levels in the circulation.^[16] The authors further suggested that these mechanisms are activated rapidly as suggested by the finding of smaller decrease in platelet count than expected on the basis of selected platelet yield during the donation. Furthermore, slower-acting mechanisms work to restore platelets in circulation as proven by the finding that platelet counts return to baseline levels about 4 days after the donation.^[9] The activation of above-mentioned mechanisms in restoring platelet counts may explain the variation in serum TPO levels at different time intervals postdonation which was reported in previous studies^[8-10] and also in our study.

In this study, we did not observe a significant change in MPV in our plateletpheresis donors. As the majority of our donors were first-time donors donating standard platelet dose, which suggested that there was no significant stress to the marrow to release reticulated platelets as compensation to the platelet loss occurred during standard dose plateletpheresis procedure. Similar findings were reported regarding MPV in a study by Stohlawetz *et al.*^[17]

As the serum TPO is part of a compensatory hematologic response required to replenish the collection associated platelet loss, it may serve as a marker of the megakaryocytopoietic capacity of a plateletpheresis donor. It would further influence the consideration of a donor for repeat plateletpheresis and the interval between two consecutive donations. This would further help to develop a strategy for maintaining a registry of plateletpheresis donors and further aids in optimizing the donation interval in case of rare blood group donors and donors donating double yield platelets.

Our study is relevant as there is scanty published Indian data regarding compensatory mechanisms of restoring platelet counts in Indian plateletpheresis donors who volunteer repeatedly for platelet donations during emergencies and dengue epidemics when there is frequent and continuous need of plateletpheresis products for transfusion purposes (lifesaving in many) and requiring donors to donate on more frequent basis. The limited studies assessing TPO levels in Western population of plateletpheresis donors have reported variable results.

The dropout rate in our study has limited the analysis of data to 29 donors only against the 50 donors enrolled in the study. Moreover, the results of our study cannot be generalized to repeat regular donors as the majority of our plateletpheresis donors were first-time donors.

Conclusion

The results of our study have shown that serum TPO levels increase significantly postplateletpheresis donation corresponding to decrease in platelet counts. However, the serum TPO levels may not be the only compensatory mechanism involved in the platelet recovery after donation. More studies may be required to provide further insights into the compensatory mechanisms involved in platelet restoration after plateletpheresis donation in healthy donors. Through our study, we are generating a need for conducting further studies to recognize the effect of other thrombopoietic cytokines in regular repeat donors. This is important to formulate appropriate strategies for platelet collection and

recommendation for interval between two consecutive donations for donor safety.

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Conflicts of interest

There are no conflicts of interest.

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