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The relationship between frequency of donation to the calcium-sensing receptor (CaSR) and tartrate-resistant acid phosphatase 5b (TRACP 5b) levels in apheresis donors

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Abstract

Background: Apheresis donation, particularly platelet apheresis, exposes donors to citrate anticoagulants, which can cause hypocalcemia. This condition stimulates parathyroid gland activity and increases the risk of secondary hyperparathyroidism (SHPT), measurable through Calcium-Sensing Receptor (CaSR) levels. Additionally, hypocalcemia can trigger bone calcium release, elevating the risk of osteopenia and osteoporosis. This bone resorption can be detected early through Tartrate-Resistant Acid Phosphatase 5b (TRACP 5b) levels. This study aims to investigate the relationship between apheresis donation frequency and CaSR and TRACP 5b levels in donors.

Methods: This analytical study involved 57 male apheresis donors from the Blood Transfusion Service of Ngoerah General Hospital and the Indonesian Red Cross in Bali. CaSR and TRACP 5b levels were measured using the ELISA method. Statistical analysis was conducted using SPSS version 25.0 for Windows to determine the correlation between donation frequency and these biomarkers.

Results: All participants were male, with a mean age of 42 years (range: 18–62). Blood types were predominantly O (54.39%), followed by B (29.82%), A (10.53%), and AB (5.26%). The median donation frequency was 3 (range: 2–104). Median CaSR and TRACP 5b levels were 10.59 ng/mL

(0.43–39.01 ng/mL) and 4.83 U/L (1.0–16.80 U/L), respectively. Statistical analysis revealed no significant correlation between donation frequency and CaSR ($p = 0.439$) or TRACP 5b levels ($p = 0.084$).

Conclusion: There is no significant relationship between the frequency of apheresis donations and CaSR or TRACP 5b levels. These findings suggest that regular apheresis donation does not significantly impact parathyroid function or bone resorption markers in donors.

Keywords: Apheresis, CaSR, Blood Donation, TRACP 5b.

BACKGROUND

Based on blood donor collection methods, donations can be conducted manually through whole blood donation or using apheresis machines. One of the most common apheresis procedures is thrombocyte apheresis donation.[1]

The thrombocyte apheresis procedure involves distinct donor treatment, particularly through exposure to anticoagulants or citrate. This exposure often causes side effects and is a frequent concern among potential donors. In whole blood donations, such substances remain in the collection bag and do not enter the donor's circulation. Conversely, donors undergoing platelet apheresis come into direct contact with anticoagulants or citrate.[2] These substances enter the donor's bloodstream during each apheresis procedure, necessitating further investigation into citrate's effects on the body.

The entry of citrate into the bloodstream often causes short-term effects, which can be observed either during the apheresis procedure or shortly thereafter. These effects commonly manifest as symptoms of hypocalcemia, such as tingling or cramps in facial muscles, fingers, and toes, or, in some cases, seizures.[3] Numerous studies have explored the short-term side effects and changes associated with apheresis, yet research on long-term side effects in frequent apheresis donors remains scarce.

Repeated hypocalcemia is associated with hyperparathyroid conditions. Calcium Sensing Receptor (CaSR) is produced by principal cells located on the surface of the parathyroid glands and plays a predominant role in parathyroid function. Beyond the parathyroid glands, CaSR is expressed in osteoblasts, osteocytes, osteoclasts, and chondrocytes and is involved in the

development and maintenance of bones alongside calcium ions.[4] Measuring CaSR concentrations is critical to confirm increased parathyroid activity, not only in terms of secretion and synthesis but also in parathyroid cell proliferation. This parameter provides early detection of potential secondary hyperparathyroidism before it fully manifests.

The bone, as the primary calcium reservoir of the body, releases calcium to compensate for deficits in blood calcium levels. If this condition persists and is not offset by external calcium intake, the bone's calcium reserves will continuously diminish, ultimately leading to bone tissue degradation. Before reaching this terminal stage, molecular changes in bone tissue are accompanied by alterations in markers such as Tartrate-resistant Acid Phosphatase 5b (TRACP 5b), which is specifically secreted by osteoclasts in high concentrations in bone tissue and strongly correlates with the degree of bone resorption.[5]

Based on those mentioned above, this study aims to examine the relationship between donation frequency and CaSR and TRACP 5b levels in apheresis donors.

METHODS

This analytical study aimed to examine the correlation between the frequency of apheresis donations and the levels of Calcium-Sensing Receptor (CaSR) and Tartrate-Resistant Acid Phosphatase 5b (TRACP 5b) in individuals who donate through apheresis procedures. The study was carried out in several healthcare facilities, namely the Blood Transfusion Service at Ngoerah General Hospital, the Blood Transfusion Service of the Indonesian Red Cross in Bali Province, and the Clinical Pathology Laboratory within the Faculty of Medicine at Udayana University. These institutions were selected to provide a comprehensive and diverse donor population for analysis.

The participants in this study consisted of donors who had undergone apheresis donation at either the Ngoerah General Hospital Blood Transfusion Service or the Bali Provincial Indonesian Red Cross Blood Transfusion Service. To be eligible for participation, donors had to meet specific inclusion criteria. They were required to be between 18 and 60 years of age and to have satisfied all the standard eligibility requirements for apheresis donation as defined by the Blood Transfusion Service. Additionally, only donors who had completed at least two apheresis donations during the study period were considered for inclusion. This criterion ensured that

participants had a minimum level of exposure to the apheresis process, allowing for a more accurate assessment of how donation frequency might influence CaSR and TRACP 5b levels.

Conversely, certain exclusion criteria were established to maintain the integrity of the study results. Individuals diagnosed with medical conditions known to affect CaSR and TRACP 5b levels—such as kidney diseases, parathyroid gland disorders, or bone-related conditions—were excluded from the research. Furthermore, donors who were on regular calcium supplementation were not included, as this could interfere with the natural regulation of calcium in the body and consequently alter the study outcomes. Lastly, any individual who declined to participate or withdrew consent was also excluded from the study sample to uphold ethical research standards.

In order to accurately measure the concentrations of CaSR and TRACP 5b in participants, the study utilized the Enzyme-Linked Immunosorbent Assay (ELISA) method. This technique is widely recognized for its precision and reliability in detecting specific proteins and biomarkers in biological samples. By employing ELISA, the researchers could quantitatively assess the levels of CaSR and TRACP 5b, thereby enabling a thorough evaluation of the potential impact of apheresis donation frequency on parathyroid function and bone resorption activity. This methodological approach ensured that the data collected were both accurate and relevant to the study's objectives.

RESULTS

The study involved 57 male apheresis donors, with the majority (61.4%) aged 18–45 years and the rest (38.6%) aged 46–65 years (Table 1). Blood type O was the most prevalent (54.39%), followed by type B (29.82%), type A (10.53%), and type AB (5.26%), with all donors being Rhesus positive (100%) (Table 1).

Table 1. Characteristics of Research Subjects

Variable	Total (N=57)	Percentage (%)
Gender		
Male	57	100
Female	0	0
Age Group		
18–45 years	35	61.4
46–65 years	22	38.6
Blood Type		
O	31	54.39
A	6	10.53
B	17	29.82
AB		
Rhesus	3	5.26
Positive	57	100
Negative	0	0

Table 2 presents the data on donation frequency, Calcium-Sensing Receptor (CaSR) levels, and Tartrate-Resistant Acid Phosphatase 5b (TRACP 5b) levels among the 57 apheresis donors. The median donation frequency was 3 times, ranging from 2 to 104 donations. The median CaSR level was 10.59 ng/mL, with a wide range from 0.43 to 39.01 ng/mL, suggesting individual differences in parathyroid activity. Similarly, the median TRACP 5b level was 4.83 U/L, with values ranging from 1.0 to 16.80 U/L, reflecting varying degrees of bone resorption among donors (Table 2)

Table 2. Donation Frequency, CaSR Levels, and TRACP 5b Levels

Variable	Total (N=57)
Apheresis frequency	3 (2-104)
CaSR (ng/mL)	10.59 (0.43-39.01)
TRAPC 5b (U/L)	4.83 (1.0-16.80)

CaSR: Calcium-Sensing Receptor; TRACP 5b: Tartrate-Resistant Acid Phosphatase 5b

Table 3 displays the statistical analysis of the relationship between apheresis donation frequency and the levels of CaSR and TRACP 5b. The correlation coefficient (r) between donation frequency and CaSR levels was -0.104 with a p-value of 0.439, while the correlation with TRACP 5b levels was -0.231 with a p-value of 0.084. Both p-values are greater than 0.05, indicating no statistically significant relationship between donation frequency and either CaSR or TRACP 5b levels (Table 3). This suggests that the frequency of apheresis donations does not significantly impact parathyroid activity or bone resorption in donors.

Table 3. Relationship between Donation Frequency, CaSR Levels, and TRACP 5b Levels.

Variable	r	p
Donation frequency		
CaSR (ng/ml)	-0.104	0.439
TRAPC 5b (U/L)	-0.231	0.084

CaSR: Calcium-Sensing Receptor; TRACP 5b: Tartrate-Resistant Acid Phosphatase 5b; r: coefficient correlation; *Statistically significant if p-value less than 0.05

DISCUSSION

This study exclusively involved male donors, with no participation from female donors. Generally, women are less likely to become apheresis donors due to several factors. Beyond the challenge of meeting donation eligibility criteria, plasma collection from female donors is often minimized compared to male donors. Women who have experienced pregnancy are more prone to developing alloantibodies, resulting from fetal antigen exposure. These alloantibodies may target Human Leukocyte Antigen (HLA) or Human Neutrophil Antigen (HNA), both of which can elevate the risk of Transfusion-Related Acute Lung Injury (TRALI). These antibodies can activate leukocytes attached to the pulmonary endothelium, potentially causing endothelial damage and pulmonary edema. Due to the frequent presence of HLA and HNA antibodies in previously pregnant women, plasma products are predominantly sourced from male donors. Therefore, thorough screening of female donors regarding their pregnancy history is crucial to minimize the risk of TRALI reactions.[6,7]

Supporting this observation, a previous study involving 53,089 apheresis donors reported a male-to-female ratio of 2.6:1, with most donors having blood type O Rhesus positive, followed by types A, B, and AB.[8] The low participation of female donors can be linked to both physiological

factors—such as menstruation, pregnancy, and breastfeeding—and psychological concerns, including fear of anemia, needles, disease transmission, and anxiety about the blood donation process.[8-10]

The age eligibility for apheresis donors is set between 18 and 60 years, although donations are permitted up to 65 years for healthy, regular donors. In this study, the majority of participants were aged between 18 and 45 years, with fewer donors above 45 years, likely due to health-related disqualifications.

Regarding blood type distribution, most donors in this study had blood type O, followed by types B, A, and AB. This pattern aligns with Indonesia's general population blood type distribution, where blood type O dominates (38.04%), followed by type B (28.43%), type A (25.02%), and type AB (8.51%). In addition, all participants were Rhesus-positive, consistent with the predominantly Rhesus-positive blood type distribution in the general population.

The majority of participants had donated blood more than three times, with some donating over 100 times. Donors with lower donation frequencies were generally younger and newer to regular apheresis, whereas those with higher donation frequencies were typically older, long-term donors. The frequency of donations is influenced by the ability to donate thrombocyte apheresis as often as every two weeks, unlike whole blood donations, which require at least a two-month interval. A previous study indicates that repeated thrombocyte apheresis does not significantly impact platelet counts in donors.[11] Although a temporary drop in platelet count occurs immediately after the procedure, platelet levels typically recover within 48 hours.[12]

The Calcium-Sensing Receptor (CaSR), produced by principal cells in the parathyroid gland and in smaller amounts by skeletal muscle and osteoclasts, is essential for regulating parathyroid gland function. It plays a significant role in hormone secretion, hormone synthesis, and cell proliferation. Increased parathyroid activity, marked by elevated CaSR secretion, is commonly observed in hyperparathyroidism, which can lead to secondary osteoporosis characterized by heightened bone turnover and reduced bone mineral density (BMD).[13]

Serum TRACP 5b levels serve as a vital indicator of bone resorption, making it a key marker for assessing bone metabolism and managing osteoporosis.[14,15] In addition to measuring osteoclast activity, TRACP 5b levels can estimate osteoclast numbers, where higher levels signify a reduction in osteoclast quantity. Research in China has demonstrated a strong correlation

between TRACP 5b levels and osteoclast numbers, with immunoassay-based TRACP 5b testing proving more efficient and reliable than traditional microscopic counting methods.[16]

The absence of significant findings in this study may be attributed to several factors. First, the calcium bound by citrate during the apheresis process may be sufficiently replenished by the donor's internal calcium reserves. Second, the standard practice of providing calcium supplements before and during the apheresis procedure could effectively mitigate calcium depletion.

Supporting this, a study by Bialkowski et al. revealed significant differences in bone mineral homeostasis between frequent apheresis donors and non-donors due to citrate exposure.[2] However, no significant differences in bone density were identified, suggesting that current apheresis guidelines effectively safeguard bone health in regular male donors, even with high-frequency donations.[2]

This study has several limitations. The exclusive participation of male donors limits the generalizability of the findings to female donors. Additionally, the sample size was relatively small, potentially reducing the statistical power to detect subtle associations between apheresis frequency and changes in CaSR and TRACP 5b levels. Furthermore, this study did not account for lifestyle factors, dietary calcium intake, or physical activity, all of which could influence parathyroid function and bone metabolism.

CONCLUSION

This study concludes that there is no significant relationship between donation frequency and CaSR or TRACP 5b levels in apheresis donors. These findings can serve as a reference to educate donors, indicating that the use of citrate during apheresis procedures does not pose a significant risk for long-term parathyroid or bone disorders.

Ethics Consideration This study was conducted following the ethical standards set by the Research Ethics Committee, Faculty of Medicine, Universitas Udayana. Informed consent was obtained from all participants prior to data collection.

Conflict of Interest The authors declare that there are no conflicts of interest related to this study.

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Authors' Contributions

N.K.M designed the study, collected and analyzed the data, and drafted the manuscript. S.H supervised the research process and contributed to the data analysis and manuscript revision. Both authors reviewed and approved the final version of the manuscript.

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