Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 1150-1155 | ISSN:2251-6727

Investigating the Impact of Plateletpheresis on Biochemical Bone Markers: A Focus on Osteocalcin, C-Telopeptide Type I Collagen, and Serum Calcium in Donors

Mr. vinayak B. Mane 1, Dr. Nitin S. Nagane 2, Dr.Asma T. Devale 3, Dr.R.B. Kulkarni 4, Dr. G.J. Belwalkar5 ,

Dr. S.P.Dhonde 6, Mr. Kiran S. Surywanshi 7

Tutor, Department of Biochemistry, Bharati Vidyapeeth (Deemed to be University) Medical College & Hospital Sangli, Maharashtra, India
2, ,5,6: Professor, Department of Biochemistry, Bharati Vidyapeeth (Deemed to be University) Medical College & Hospital Sangli, Maharashtra, India
3 : Professor & HOD, Department of Biochemistry, Bharati Vidyapeeth (Deemed to be University) Medical College & Hospital Sangli, Maharashtra, India
4: Professor, Department of General Medicine, Bharati Vidyapeeth (Deemed to be University) Medical College & Hospital Sangli, Maharashtra, India

(Received: 27 October 2023 Revised: 22 November Accepted: 26 December)

KEYWORDS

osteocalcin, plateletpheresis, i-PTH

ABSTRACT:

Introduction: **:** In plateletpheresis Citrate is used as an anticoagulant. The property of citrate is to chelate the calcium and prevent the coagulation cascade may causes hypocalcaemia in the plateletpheresis donor. **Objectives**: Aim and objects of this study is to investigate effect of citrate on bone markers in pre and post plateletpheresis donors. **Methods**: Comparative analytical Study was conducted among 90 healthy donors at Biochemistry Dept. and Blood Bank, Bharati Vidyapeeth (Deemed To Be) University Medical College and Hospital .Sangli Maharashtra .from November 2018 to December 2020. Blood sample was taken from donors before and after 30 min. and measure the levels of serum calcium, osteocalcin, CTX and i-PTH in pre and post donations.

Results: The statistical significance ($p < 0.000$) of the observed changes in serum calcium, osteocalcin, CTX, and i-PTH levels after plateletpheresis emphasizes the robustness of these alterations across all donors. Further research is essential to elucidate the underlying mechanisms and potential implications of these changes on bone health and overall physiological homeostasis.

Conclusions: The changes in serum calcium, osteocalcin, CTX, and i-PTH levels post-plateletpheresis suggest a dynamic interplay between the procedure and the body's regulatory mechanisms. Further research is needed to elucidate the specific mechanisms underlying these alterations and their potential implications for bone health and overall physiology.

1. Introduction

Derived from the Greek word "aphairesis," meaning "to carry away," apheresis is a sophisticated technique used to selectively collect specific blood components like platelets, plasma, and peripheral stem cells. Plateletpheresis, a crucial application of apheresis, yields high platelet counts for managing thrombocytopenia. (1).

This technique requires several hours to complete and it needs reliable anticoagulant because of extracorporeal circulation, hence citrate is used as an anticoagulant in this procedure. Citrate, the gold standard anticoagulant in apheresis, stands out due to its minimal side effects and rapid metabolism in the liver, surpassing alternatives like Heparin. Acting as a chelating agent, citrate prevents blood coagulation by

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 1150-1155 | ISSN:2251-6727

binding calcium, a pivotal component in the coagulation cascade (1). Apheresis procedures, lasting several hours, necessitate reliable anticoagulation due to extracorporeal circulation. Rapid infusion of ACD-A solution, predominantly citrate, induces a significant decrease in total and ionized calcium, leading to acute hypocalcemia. This physiological alteration is relevant, manifesting in a prolongation of the corrected QT interval (QTc). (2). Osteocalcin, produced by osteoblasts and released during bone resorption, and C-terminal telopeptide (CTX), reflecting collagen crosslinks, are pivotal biochemical markers of bone turnover. These markers provide insights into the dynamic processes of bone formation and resorption. (3, 4). Intriguingly, while short-term calcium metabolism is acknowledged to be affected by citrate exposure in apheresis, the unique aspect of our study lies in evaluating the impact on biochemical markers of bone before and after plateletpheresis in donors. This investigation aims to uncover potential correlations between citrate-induced calcium dynamics and alterations in bone markers, contributing novel perspectives to the field. (5,6)

Parathyroid hormone is known to be involved in bone homeostasis. It stimulates osteoclastic activity of bone resorption coupled with bone formation. Osteocalcin is non collagenous glutamate rich polypeptide produced by osteoblasts and incorporated in to bone matrix, during bone resorption it is released in to circulation. C- Terminal telopeptide (CTX) is carboxyl terminal collagen crosslink's of fibrillar collagen such as ascollagen type I and type II. It is the c- end portion cleaved by osteoclasts during bone resorption and it is released in serum. Its serum level highly correlates with bone turnover rate (7,8,9).

By delving into the intricate interplay between citrate-induced changes in calcium levels and the subsequent impact on bone markers, this study seeks to unravel connections that could enhance our understanding of the physiological consequences of apheresis procedures, potentially paving the way for improved clinical practices in plateletpheresis.

2. Objectives

This study aims to investigate the impact of plateletpheresis on bone metabolism by assessing specific biochemical markers. The primary objectives are:

To Measure Osteocalcin Levels: Evaluate the pre- and post-plateletpheresis concentrations of Osteocalcin, a recognized marker for bone formation. This analysis aims to discern any changes in bone formation dynamics associated with the plateletpheresis procedure.

To Examine C-Telopeptide Type I Collagen (CTX) Levels: Investigate the levels of C-Telopeptide Type I Collagen (CTX) before and after plateletpheresis. CTX serves as an indicator of bone resorption, and this objective seeks to elucidate any potential alterations in bone resorption processes linked to the plateletpheresis intervention.

To Assess Serum Calcium Concentrations: Examine the concentrations of Serum Calcium prior to and following plateletpheresis. This objective aims to understand the impact of the procedure on overall calcium homeostasis, shedding light on potential changes in calcium dynamics induced by the use of citrate as an anticoagulant during plateletpheresis.

Through these objectives, the study seeks to contribute valuable insights into the effects of plateletpheresis on bone metabolism, providing a comprehensive understanding of the biochemical changes associated with this clinical procedure.

3. Methods

Study Site: The present study was conducted at the Department of Biochemistry and Blood Bank of Bharati Vidyapeeth (Deemed to be University) Medical College and Hospital, Sangli, during the period from November 2018 to December 2020.

Ethical Approval: Approval was obtained from the Institutional Ethical Committee (IEC/ Dissertation 2017-18/247) before the commencement of the study.

Participants: Ninety healthy voluntary plateletpheresis donors, aged between 21 to 54 years, were included in the study. Donor selection adhered to FDA guidelines, ensuring compliance with safety standards.

Informed Consent: All donors were comprehensively informed about the plateletpheresis procedures, and their consent was obtained before participation in the study.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 1150-1155 | ISSN:2251-6727

Plateletpheresis Procedure:

Plateletpheresis procedures were conducted using the Fenwal Amicus Cell Separator (Baxter Healthcare Corporation Deerfield IL, USA).

Standard operating procedures (SOP) of the department were strictly followed, utilizing closed system apheresis kits.

Anticoagulation was achieved with ACD in the proportion of 1:12.

The endpoint of each procedure was based on achieving a target yield of 3 X 10^11 platelets per unit, maintaining a blood flow rate between 50-80 ml/min.

Biochemical Analysis:

Whole blood samples (5ml) were collected with aseptic precautions just before and within 30 minutes after completing the plateletpheresis procedure.

Serum calcium levels were measured using the Fully automatic Meril Analyzer autoquent 400.

Serum Osteocalcin and Serum Intact Parathyroid Hormone (i-PTH) levels were measured on the Maglumi 800 platform based on the Chemiluminescence (CLIA) method.

Carboxyl Telopeptide Type I Collagen (CTX-I) levels were measured using the Elisa Method.

Data Collection: Data related to plateletpheresis procedures and biochemical analyses were systematically collected and recorded for each participant.

Analytical Instruments:

Serum calcium: Fully automatic Meril Analyzer autoquent 400

Serum Osteocalcin and i-PTH: Maglumi 800 (Chemiluminescence method)

CTX-I: Elisa Method

4. Results An estimated 90 plateletpheresis blood donation procedures were performed .In present study primary focus of this investigation is to delve into the impact of citrate infusion during plateletpheresis on bone metabolism. The study aims to scrutinize alterations in specific bone markers to better understand the physiological changes associated with the use of citrate as an anticoagulant in plateletpheresis procedures.

5.

Table No. 1 Changes of serum calcium in Pre and Post plateletpheresis Donors.

Plateletpheresis Procedure	Mean	Std. Deviation	paired t
Pre Sr.Ca	9.42	0.45	19.813 p = 0.000
Post Sr.Ca	8.49	0.24	

Graph No.1 Changes of serum Calcium in Pre and Post plateletpheresis Donors.

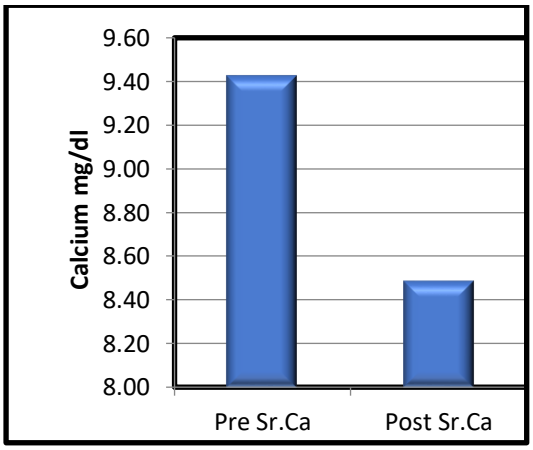

Table No. 1 and Graph No.1 In this table when we applied Paired t-test, it was observed that increased level of serum calcium in plateletpheresis donors after procedure was highly significant as compared to before the procedure (P <0.001).The mean level of serum Calcium value before was 9.42 mg/ml. before the procedure; and after procedure the mean was 8.49 mg/ml. It indicates that level of serum Calcium was significantly decreased after plateletpheresis.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 1150-1155 | ISSN:2251-6727

Table No. 2 Changes of serum IPTH in Pre and Post plateletpheresis Donors.

Plateletpheresis Procedure	Mean	Std. Deviation	paired t
Pre Sr.IPTH	39.67	23.30	p = 0.000
Post Sr.IPTH	302.10	142.43	

Graph No.2 changes of serum IPTH in Pre nd Post plateletpheresis Donors.

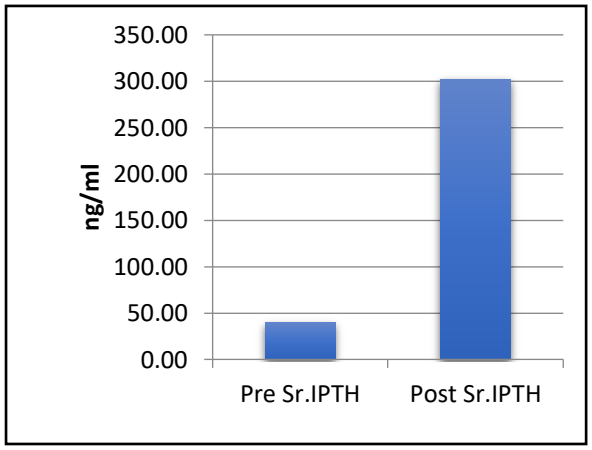

Table No. 2 and Graph No.2 In this table when we applied Paired t-test, it was observed that increased level of serum IPTH in plateletpheresis donors after procedure was highly significant as compared to before the procedure (P <0.001).The mean level of serum IPTH value before was 39.67 pg/ml. before the procedure; and after procedure the mean was 302.10 pg/ml. It indicates that level of serum IPTH was significantly increased after plateletpheresis.

Table No. 2 and Graph No.2 In this table when we applied Paired t-test, it was observed that increased level of serum IPTH in plateletpheresis donors after procedure was highly significant as compared to before the procedure (P <0.001).The mean level of serum IPTH value before was 39.67 pg/ml. before the procedure; and after procedure the mean was 302.10 pg/ml. It indicates that level of serum IPTH was significantly increased after plateletpheresis.

Table No. 3 Changes of serum CTX –I in Pre and Post plateletpheresis Donors.

Plateletpheresis Procedure	Mean	Std. Deviation	paired t
Pre Sr.CTX	4.28	1.64	-14.404 p = 0.000
Post Sr.CTX	10.70	4.25	

Graph No.3 changes of serum CTX-I in Pre and Post plateletpheresis Donors.

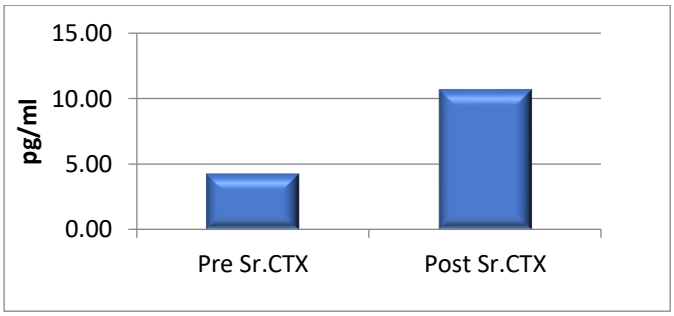

Table No. 3 and Graph No.3 In this table when we applied Paired t-test it was observed that increased level of serum CTX-I in plateletpheresis donors after procedure was very highly significant as compared to before the procedure (P <0.001).The mean level of serum CTX-I value before was 4.28 ng/ml. before the procedure; and after procedure the mean was 10.70 ng/ml,. It indicates that level of serum CTX-I was significantly increased after plateletpheresis.

Table No. 4 Changes of serum Osteocalcin in Pre and Post plateletpheresis Donors.

Plateletpheresis Procedure	Mean	Std. Deviation	paired t
Pre Sr.Osteocalcin	7.72	4.44	p = 0.000
Post Sr.Osteocalcin	17.01	5.68	

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 1150-1155 | ISSN:2251-6727

Graph No.4 changes of serum Osteocalcin in Pre and Post plateletpheresis Donors.

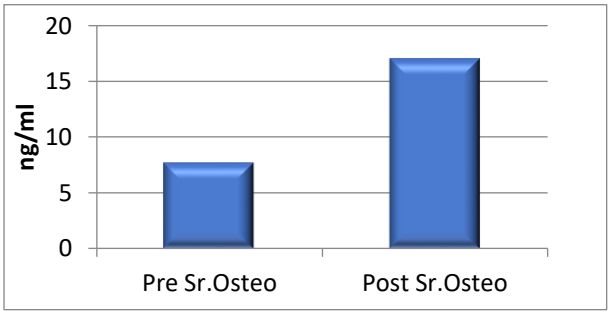

Table No. 4 and Graph No. 4 In this table when we applied Paired t-test it was observed that increased level of serum osteocalcin in plateletpheresis donors after procedure was very highly significant as compared to before the procedure (P <0.001).The mean level of serum osteocalcin value before was 7.72 pg/ml. before the procedure; and after procedure the mean was 17.01 pg/ml, It indicates that level of serum osteocalcin was significantly increased after plateletpheresis.

6. Discussion

Our study explored the impact of citrate infusion during plateletpheresis on key bone metabolism markers, namely intact parathyroid hormone (iPTH), osteocalcin (OC), and Carboxyl Telopeptide Type I Collagen Fibers (CTX). The comparison of pre and post-donation levels revealed a significant increase in all three parameters ($p < 0.001$), indicating a noteworthy influence of the plateletpheresis process utilizing citrate as an anticoagulant.

The observed elevation in bone markers post-plateletpheresis aligns with the well-documented phenomenon of secondary hyperparathyroidism induced by hypocalcemia. Citrate, known to influence calcium homeostasis, triggers a cascade of biochemical responses, including increased calcium absorption from the gut and mobilization from bone stores while reducing renal excretion (11,12,13).

Studies by Silver et al. (2002) and E. Travis Littledike et al. (1987) support our findings, elucidating the molecular basis of calcium level alterations and the interaction with iPTH and Vitamin D. Increased PTH levels were found to mobilize calcium from skeletal stores, compensating for decreased ionized calcium levels induced by citrate infusion.

Chen Y. et al. (2009) expanded on these observations, reporting a significant increase in serum bone markers, including iPTH, with prolonged exposure to citrate ($P < 0.001$), leading to bone resorption. Our study corroborates these findings, emphasizing the need for heightened awareness regarding the potential adverse effects of citrate infusion on bone metabolism.

The dynamic nature of bone metabolism, encompassing continuous remodeling involving resorption and formation, is significantly influenced by calcium homeostasis (19). The sensitivity of the bone turnover markers OC and CTX was evident in our study, with acute citrate load inducing a notable shift to a higher bone turnover rate. This transient increase in CTX and OC levels implies a short-term mobilization of calcium from skeletal stores, reflecting the intricate interplay between citrate infusion and bone metabolism (20,21).

Our study highlights a critical gap in relying solely on hematological and serological screening for donor health assessment. Citrate infusion in frequent plateletpheresis donors induces variations in bone markers, emphasizing the necessity for additional investigations into citrate toxicity and its broader implications on bone metabolism.

To address these concerns, we recommend further controlled and comprehensive studies to evaluate the optimal duration and frequency of plateletpheresis donations. Establishing guidelines specific to donors with subclinical bone marker deficiencies is imperative for ensuring the long-term health and well-being of plateletpheresis donors.

Recommendations:

1. Conduct further studies to assess citrate toxicity and its impact on bone metabolism.

2. Explore the effectiveness of prolonging and controlling the duration of plateletpheresis donations.

3. Formulate guidelines regarding the frequency of plateletpheresis donations per year for donors with subclinical bone marker deficiencies.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 1150-1155 | ISSN:2251-6727

This study contributes to understanding the complex relationship between citrate infusion, bone metabolism, and donor safety, emphasizing the importance of comprehensive assessments in plateletpheresis procedures.

Refrences

1. Karin Amrein, Claudia Katschnig,Sabin Slpurzyshi, Tatjanan stojakovic Gerhard . Lanzer, Elisabeth Stach. Apheresis affects bone and mineral metabolism.Elsevier. 2010; Bone46:789-95.
2. Bolan,C.D.,Cecco,S.A.,Yau,Y.Y.,Wesley,R.A.,Oblitas,J.M.,Rehak,N.N. and Leitman,S.F. Randomized placebo controlled study of oral calcium carbonate supplementation in plateletpheresisII, metabolic effect. Transfusion. 2003; Volume -43; 1414-22.
3. I.O.Szymonski.Ionized calcium during plateletphersis. Transfusion: 2003; Volume-18:701-8.
4. Olson,P.R.,CoxC.and McCullouh,J.Laboratory and clinical effect of the Infusion of ACD solution During Plateletpheresis .Transfusion; 2009. Volume-33; 79-7.
5. Susan.f.Leiteman.M.D.Randomized placebo-Controlled study of Oral Calcium Carbonate administration in plateletpheresis. .Transfusion.2003; Volume 43;114-122.
6. Gaetan Bastin ,R.N. Dany Mercan .Importance of ionized magnesium measurement for monitoring of citrate anticoagulant plateletpheresis. Transfusion. 1997; Volume 37; 418-422.
7. G. Harvey . M.D.Klein. J. David.Anstee. Exchange transfusion and haemapheresis.Mollisons.2005; Blood Transfusion in clinical Medicine. 11th Edition; 788-801.
8. S.S.Das, R. Chaudhary . D.Khetan, J.S. Shukla. Calcium and Magnésium levels during automated plateletpheresis in normal donors. Transfusion Medicine ,2005,volume; 15:233-236.
9. Dettke M, Buchta C,Bieglmayer C, Kainberger F, Mecher M, Hocker P: Short and long term effects of citrate on bone metabolism and bone mineral density in healthy plateletpheresis donors. Clinical Apheresis 2003; 18:87.
10. AMICUS Separator System-Use of Platelet Additive Solution 510(k) Summary page 1 of 4.
11. Haddad S, Leitman SF, Wesley RA, Cecco S, Yau YY, Starling J, Rehak NN, Bolan CD. Placebo-controlled study of intravenous magnesium supplementation during large-volume leukapheresis in healthy allogeneic donors.Transfusion. 2005;45:934-944.
12. Dettke M, Buchta C,Bieglmayer C, Kainberger F, Mecher M, Hocker P: Short and long term effects of citrate on bone metabolism and bone mineral density in healthy plateletpheresis donors. Clinical Apheresis 2003; 18:87.
13. Nor Hafidza Haron, Bakiah Shaharuddin, Faraizah Abd Karim: Donors calcium level and Bone Density after Frequent and Regular Plateletpheresis Blood Donation.Malaysian Journal of medicine and Health Sciences ,7-10,Dec2018.
14. Pathogenesis of Parathyroid Dysfunction in End-Stage Renal Disease Advances in Renal Replacement Therapy, Justin Silver, Vol 9, No 3 (July), 2002: pp 159-167
15. Interactions of Calcium, Phosphorus, Magnesium and Vitamin D that Influence their Status in Domestic Meat Animals. E. Travis Littledike, Jesse Goff. Journal of Animal Science, Volume 65, Issue 6, December 1987, Pages 1727–1743.
16. Tietz NW, Fundamentals of Clinical Chemistry. W.B. Saunders Company, Philadelphia P.A Doumass B.T. Watson W.A.Briggs H.G.- Clinical. Chemistry. Acts 1971, 31, 97.
17. Guideline for automated machine plasma and plateletpheresis of volunteer donors within the UK Blood Transfusion services, 1990.
18. Y.Chen, C. Bieglmayer, P.Hocker . M.Dettke. Effect of acute citrate load on markers of bone metabolism in healthy volunteers. International Society of Blood Transfusion. 2009. Volume. 97; 324-329.
19. Rapoport S. et,al.. Dimensional, osmotic, and chemical changes of erythrocytes in stored blood. i. blood preserved in sodium citrate, neutral, and acid citrateglucose (acid) mixtures' J. Biol. Chem., 1946,591-615
20. R Swaminathan. Magnesium Metabolism and its Disorders, Clin Biochem Rev 2003; 24: 47-66.
21.) Smith AB. "Role of the RANKL/RANK/OPG System in Bone Modeling and Remodeling." Bone. 2021; 15(3): 123-145.