ORIGINAL ARTICLE

# Donors' Calcium Level and Bone Density after Frequent and Regular Plateletpheresis Blood Donation

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## ABSTRACT

**Introduction:** Citrate is commonly used as an anti¬coagulant during plateletpheresis procedure. The calcium chelating property of citrate may cause hypocalcaemia when the anticoagulated blood are returned to the donor's circulation after selective removal of platelet. This study aims at investigating how regular plateletpheresis affects calcium level and bone density in the donors. **Methods:** A cross-sectional study was conducted among healthy donors at National Blood Centre, Kuala Lumpur, from 15th January till 31st March 2016. Donors were divided into two groups based on the frequency of plateletpheresis donation: low frequency group - donors who had donated less than 20 times, high frequency group - donors who had donated more than 50 times. Dual emission X-ray absorptiometry (DEXA) scan was performed to assess bone density. Pre-donation blood sampling was taken for albumin level. Calcium and magnesium levels were measured before and after donation. **Results:** Fifty donors participated in this study where the median age of participants was 35.0 years for low frequency and 45.2 years for high frequency group. There was no significant difference in the corrected calcium for both groups before and after plateletpheresis. However, the magnesium levels were significantly reduced in both arms ($P<0.05$). The albumin level and DEXA scan showed no significant differences between the groups. **Conclusion:** This study did not show any significant difference in calcium levels, albumin and bone density; but donors' magnesium levels were reduced in low and high frequency donors after plateletpheresis.

**Keywords:** Plateletpheresis, National Blood Centre, Dual emission X-ray absorptiometry, Magnesium and calcium, Bone density

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## INTRODUCTION

Plateletpheresis is a process where thrombocytes or platelet are selectively collected from whole blood and the other blood components are returned to the donor. In this procedure, blood is directed from the vein into a cell separator machine. An anti-coagulant such as citrate is used to prevent clotting by reducing the ionised calcium levels in the blood. After platelet is collected, the anti-coagulated blood is returned to the donors. Apheresis blood products are superior compared to non-apheresis products since apheresis blood products reduce patients' exposure to multiple donors and the products are of higher quality, consistent and standardised. Other advantages include the products are matched between donors and patients and thus can reduce reactions, leading to high donors' acceptance and in turn lead to safety enhancement for the patient (1).

Most cell separator machines use Anticoagulant Citrate Dextrose-Solution A which largely contains sodium citrate. The recommended ratio of citrate to whole blood is 1:9. Citrated blood gets diluted in the extracellular fluid and also into the intravascular space after the remaining blood components are returned to the donor. Citrate metabolism occurs in the liver, kidney and muscle, thus releases the bound calcium into the circulation in order to maintain calcium homeostasis.

In the state of hypocalcaemia, the parathyroid glands release parathyroid hormone (PTH) which acts on the bones to mobilise ionised calcium out of the skeletal stores and increases calcium absorption by the kidneys. PTH reaches its maximum level within half an hour after completion of apheresis even though the calcium level still decreases (2). Another mechanism to compensate hypocalcaemia state is to mobilise ionised calcium which is bound to serum albumin. Ionised calcium is the physiologically active form of calcium and involved in many cellular processess in the body. The normal ionised calcium levels range from 1.1 to 1.4 mmol/L and this does not vary with albumin concentration.

The commonest adverse effect of apheresis is citrate toxicity (3) and this is manifested as hypocalcemic symptoms such as circumoral numbness, sensation of buzzing or vibrating, tingling, or feeling cold; which are not uncommon symptoms experienced by most plateletpheresis blood donors (4). Severe cases of hypocalcaemia may present with muscle cramps, generalised shivering, nausea, vomiting and seizure. Although citrate also chelates magnesium, symptoms of hypomagnesaemia are uncommonly seen in apheresis blood donors (5). Magnesium levels falls more acutely than calcium during citrate infusion and hypomagnesaemia has a longer period of recovery. Ionised magnesium may decrease up to 30% to 40% in that situation (6). Symptoms of hypomagnesaemia are similar with hypocalcaemia.

Amrein et al studied bone resorption in 102 plateletpheresis donors with an average of 85 apheresis procedures. In this study, they demonstrated significantly lower bone density at the lumbar spine in the plateletpheresis blood donors compared to non-blood donor controls (7). The bound calcium-citrate complexes compromised the acid-base balance and calcium homeostasis and ultimately alter the bone density.

In the local population of blood transfusion donors in Malaysia, the long-term effects of regular plateletpheresis donation on calcium and magnesium levels and bone density have not been studied. This study aims to provide a local data which may improve blood transfusion services involving plateletpheresis donation.

## MATERIALS AND METHODS

### Subjects, sample calculation and study design

A prospective cross-sectional study was conducted among healthy blood donors at the National Blood Centre, Kuala Lumpur. The sample size was calculated using a computer software (PowerSampleSize) with alpha of 0.05, sigma of 3, delta of 1.28 and a power of 0.8, according to a study by Bolan et al, 2003. Therefore, the total sample was 50 subjects, with 20% drop-out rate. Donors were categorised into two groups based on their plateletpheresis donation, whereby low frequency group comprised of donors who had donated less than 20 times, and the high frequency group comprised of donors who had donated more than 50 times. The values of 20 and 50 were arbitrary cut off points to represent low and high frequencies for convenient sampling of the study population. The bone scan was performed at the Radiology Department, Hospital Kuala Lumpur (HKL). Ethical consideration was fully addressed; this study was approved by Medical Research and Ethic Committee, Ministry of Health Malaysia and Human Ethics Committee, Universiti Sains Malaysia.

### Study Measurements

Pre-donation blood samples were removed from the sampling pouch before the blood entered the apheresis machine. A post-procedural sample was taken the same way. Both samples were sent to Pathology Laboratory, HKL for measurement of serum calcium level, serum magnesium, and albumin level. For bone density measurement, a non-invasive technique to measure bone mineral density was performed using a DEXA scan. A central DEXA scan was used to measure bone mineral density of the central skeletons at the levels of L1 to L4 and the hip, as previously described by Blake at al (8).

### Statistics

Data analyis was performed using Statistical Package for the Social Sciences (SPSS) version 19.0 (IBM Corporation). Descriptive statistics was presented as mean and SD for numerical variables; and N % for categorical variables. Data with non-normal distribution were analysed using non-parametric statistical tests. Pre- and post-procedure measurements were analysed using Wilcoxon signed rank test to compare two related samples, Mann-Whitney test was used to determine the difference of median in two groups, and Chi Square test to analyse on association between two categorical variables. P value of $<0.05$ was considered as significant.

## RESULTS

The median age of study subjects was 35.0 years and 45.2 years for low frequency group and high frequency group respectively. Male participants formed the majority of study subjects in both arms (96.0%) and subjects in the high frequency group were siginificantly older (P=0.006). There was no significant difference in gender and race, P=1.000 and P=0.413, respectively.

Table I showed the levels of corrected calcium and magnesium at baseline and after the procedure in both groups of plateletpheresis donors. Corrected calcium level in the low frequency group showed a slight reduction post-plateletpheresis, (P=0.653). Baseline values and after plateletpheresis showed no significant difference in corrected calcium levels in the high frequency groups, P=0.132.

However, there was a significant reduction ($P<0.05$) in magnesium levels for both frequency groups pre- and post-plateletpheresis. In the low frequency group, median magnesium level reduced from 0.86 mmol/L to 0.83 mmol/L post procedure. In the high frequency group, median level was 0.85 mmol/L at baseline and reduced to 0.83 mmol/L post procedure. Magnesium level was more affected by plateletpheresis in both groups of the study than calcium level.

Albumin level and bone density were measured in low and high frequency groups, and the results are as

**Table 1:** Corrected calcium and magnesium levels at baseline and after plateletpheresis procedure in low and high frequency groups.

| | Low (Median IQR) | | | High (Median IQR) | | |
|---|---|---|---|---|---|---|
| | **Baseline** | **Post** | ***P value** | **Baseline** | **Post** | ***P values** |
| **Calcium** (mmol/L) | 2.30 (2.20,2.30) | 2.20 (2.20,2.30) | 0.653 | 2.30 (2.20,2.30) | 2.30 (2.20,2.30) | 0.132 |
| **Magnesium** (mmol/L) | 0.86 (0.80,0.88) | 0.83 (0.79,0.85) | <0.001 | 0.85 (0.81,0.89) | 0.83 (0.79,0.87) | 0.023 |

*Wilcoxon Signed Rank Test, significant if p-value <0.05
Normal range for Calcium: 2.20 -2.65 mmol/L, Magnesium : male: 0.73 – 1.06 mmol/L, female : 0.77 – 1.03 mmol/L.

displayed in Table II. Albumin levels were 43 g/L and 46 g/L in the low and high frequency group respectively (P=1.67), which showed no significant difference between the two groups. Therefore the albumin level has no significant effect on the measurements of calcium or magnesium. Bone mineral density status measured using DEXA scan showed no significant difference. However, three subjects had osteopenia; one in the low frequency group and two in the high frequency group.

**Table II:** Albumin level and DEXA scan between low and high frequency groups.

| | **Low** (Median IQR) | **High** (Median IQR) | ***P values** |
|---|---|---|---|
| [a]**Albumin (g/L)** | 45.00 (43.00,46.00) | 43.00 (42.00,46.00) | 0.167 |
| [b]**DEXA scan:** | | | |
| Normal | 24 (96.0) | 23 (92.0) | 1.000 |
| Osteopenia | 1 (4.0) | 2 (8.0) | |

[a]Mann-Whitney test, [b]Chi-square test, *Significant if p-value <0.05
Normal range for albumin level is 34 to 54 g/L

## DISCUSSION

In the current study, the effect of citrate on donors' calcium level was not demonstrated in both low and high frequency groups. This attributes to the short half-life of citrate in circulation which is approximately 36 minutes. Exogenous citrate from the apheresis procedure is physiologically metabolised in the body within 24 hours (9). This is supported by a previous study by Bolan et al, where they demonstrated declining levels of calcium after 30 minutes of citrate infusion, then reached the lowest level after 90 minutes and started to return to baseline after 120 minutes. The calcium level had normalised after 24 hours post-plateletpheresis (10). The same time-dependent decrease of ionised calcium after apheresis was similarly observed in a study in Austria (7).

None of the donors in this study developed adverse symptoms of citrate anti-coagulant. However, other studies on citrate toxicity effects using similar multiple cell separators machines e.g. Cobe Spectra, Haemonetics, Baxter Amicus and Trima, had reported increased incidence of hypocalcaemia with increased amount of citrate usage, rate of infusion, longer duration of the procedure and female subjects were more at risk to develop severe symptoms of hypocalcaemia (4, 11).

This study demonstrated a significant reduction (P<0.05) in magnesium level in both low and high frequency groups pre- and post-plateletpheresis. Magnesium level was measured as a surrogate marker for calcium. Therefore, the magnesium level reflects similar pattern of calcium, or even more rapidly than calcium. This finding is in agreement with a study by Das et al, in which a significant acute reduction of ionised magnesium level from the baseline measurement was also observed among plateletpheresis donors (5). Similarly, a study among healthy apheresis donors in a tertiary care centre in North India demonstrated significant decrease in calcium and magnesium levels in donors during the procedure which normalised to baseline 30 minutes after completion of apheresis (12).

In the current practise, citrate toxicity is measured by ionised calcium levels while neglecting the importance of hypomagnesaemia which may manifest as a cause of citrate toxicity. Further investigations is necessary to investigate the role of magnesium and whether magnesium supplements would improve the adverse effects of apheresis.

Regular and frequent plateletpheresis may cause secondary hyperparathyroidism due to hypocalcaemia. PTH regulates calcium in the body by increasing its absorption from the gut, mobilising calcium from the bones and reducing its renal excretion. However, we found no significant difference in bone density among the study subjects in both groups. This finding is similar to a study done by Boot et al on 20 post-menopausal apheresis donors (13). It is evident that short half life of citrate causes a transient hypocalcaemia effect in blood circulation and does not cause a significant reduction in bone density among the platletpheresis blood donors.

In a larger study by Grau et al, they compared a wider range of frequency of donation and a longer exposure window period to citrate. This study had assured there was no long term risk of fracture associated with

frequent apheresis donations (14). However, in our study one donor from the low frequency group and two donors from the high frequency group had osteopenia. Two of them were post-menopausal women and another affected donor was a 55 year-old male with no significant medical history. Aging is a physiological process known to cause osteopenia in both men and women, and menopause is a confounding factor for many health issues in women. Calcium and magnesium supplements may have a role in select cases to prevent injurious effects of apheresis in high risk blood donors.

## CONCLUSION

This study did not show significant effects of regular plateletpheresis on calcium level in regular plateletpheresis donors involved in low or high frequency donations. However, there was a significant reduction on the magnesium level. The increasing demand for apheresis products should instigate the Blood Transfusion Service to improve surveillance for potential citrate toxicity. Bone density scans may be selectively performed on high risk osteopenia cases.

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