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The alteration of parathyroid hormone level in Plateletpheresis donors

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Abstract: Generally apheresis procedures are safe and well tolerated. However, apheresis may cause several adverse effects in certain people. These include bradycardia, vomiting, hypotension or hypertension, twitching movements, paraesthesia, tingling, significant rash and severe itching. It has been reported that these symptoms appear due to citrate intoxication. Citrate is one of the components in ACD-A which used to prevent the normal process of coagulation or so called haemostasis. Citrate capable of binding with ionized calcium as this may lead to changes in level of parathyroid hormone (PTH). PTH plays an important role as a regulator of serum calcium, serum phosphate and vitamin D levels. Therefore any change in calcium level may affect the level of parathyroid hormone (PTH) as well as magnesium and vitamin D level. The objectives of this study include studying the alteration of PTH level in post-procedure of plateletpheresis. The mean of ionized PTH, ionized Ca, ionized Mg, and 25-OH-vitamin D was -8.707, 0.170, 0.017, and 1.713 respectively. The standard deviation of iPTH, iCa, iMg, and 25-OH vitamin D was 6.018, 0.102, 0.091, and 2.082 respectively. The p-value for iPTH, iCa, and 25-OH vitamin D were significant (<0.001) while iMg was not significant (0.326). The plateletpheresis procedure which use ACD-A as anticoagulant did cause significant increase of iPTH level. It also subsequently affects the level of 25-OH vitamin D. Further studies should be done to observe the long term effect of alteration of PTH as well as 25-OH vitamin D as this may cause problem for bone metabolism.

Key words: Plateletpheresis; Parathyroid hormone; Calcium; Magnesium; Anticoagulant

1. Introduction

Apheresis is a medical procedure in which blood is passed through an instrument which separates out the desired component and returns the remainder to the donor or patient. There are many kinds of apheresis procedure. These include extracorporeal photopheresis (ECP), erythrocytapheresis, filtration selective removal, immunoadsorption (IA), LDL apheresis, leukocytapheresis (LCP), therapeutic plasma exchange (TPE), plasmapheresis, plateletapheresis, RBC exchange, therapeutic apheresis (TA) (Szczepiorkowskiet al., 2010).

Basically there are two purposes for the apheresis procedure which are therapy and collection. The purpose of therapeutic apheresis is to remove a component of the patient's blood which contributes to a disease state. Collection on the other hand focuses on collecting the desired blood component from healthy donors and supplying it to patients. Generally these procedures are safe and well tolerated. However, apheresis may cause several adverse effects in certain people. Bradycardia, vomiting, hypotension or hypertension, twitching movements, paraesthesia, tingling, significant rash or severe itching or significant pain have developed in patients or donors who have undergone apheresis procedures (Yuan et al., 2010). These are considered as mild to moderate symptoms. Severe symptoms have been recorded when the donor fainted, had incontinence, muscle cramps, seizures, tetany, wheezing, laryngeal or tongue oedema, tingling persistent for more than 48 hours (Yuan et al., 2010). It has been reported that several symptoms like tetany, seizures, paraesthesia, and numbness of the limbs and around lips as well as nausea appear due to citrate intoxication (Mcleod et al., 1999). Citrate is used in the procedure as an anticoagulant and is known as ACD-A (Acid Citrate Dextrose Solution, Solution A). Most apheresis procedures use ACD-A as anticoagulant regardless of the component collected.

ACD-A is used to prevent the normal process of coagulation or so called haemostasis. The process of coagulation starts to occur once donor's blood flows in the collection tube. Coagulation cascade is one of the crucial sub-processes of haemostasis. Normally calcium is required in the formation of tissue factor (TF)/factor VII (FVII) complex, FVIIIa/FIXa complex and FXa/FVa complex (Hoffbrand & Moss, 2011). These complexes play a fundamental role in normal coagulation cascade. Rapid infusion of ACD-A solution which mainly constitutes citrate in apheresis procedure induces a fall in total and

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ionized calcium (Bolan et al., 2001). The presence of citrate in the solution will chelate calcium ion to interrupt the coagulation cascade. Since citrate forms complexes with calcium, it will lead to a decrease level of serum calcium (Kaplan, 2012). Thus a state of hypocalcemia may develop (Uhl & Kruskall, 1997).

The alteration of calcium level may subsequently lead to changes the level of parathyroid hormone (PTH). A study has reported that PTH plays an important role as a regulator of serum calcium, serum phosphate and vitamin D levels (Silver et al., 1999). Apart of that, Favus M.J., Bushinsky D.A., and Jr J.L. had stated that balance of calcium, magnesium and phosphate are mediated by PTH and $1,25(OH)_2D_3$. Therefore, any change in calcium level may subsequently affect the level of parathyroid hormone (PTH) as well as magnesium and vitamin D level. Any imbalance of PTH is important to be determined because continuation of any related biochemical may cause parathyroid gland to become autonomous. This has been shown to have occurred in secondary and tertiary hyperthyroidism (Terris et al., 2009). Besides, persistent increase in PTH may give negative impact on bone mineralization (Wood et al., 2005). Hypomagnesemia may also tend to develop in accordance with the increase level of PTH. It is crucial therefore to investigate the alteration of body chemicals due to apheresis procedure as it may lead to several pathological conditions.

There have been some studies that have documented citrate-induced apheresis procedure is capable of giving changes to the level of PTH (Bolan et al., 2001). The data were limited only to changes during the apheresis procedure and the level of PTH was above the normal range at the end of the procedure. This study is conducted to assess whether there is a change in PTH level in post-procedure. The level of calcium, magnesium and vitamin D will be analysed together as it is closely related to the changes of PTH level (Silver et al., 1999; Favus et al., 2006). These data will be useful as predictive value of biochemical derangement triggered by reduction of calcium. Besides that, data of biochemical derangement especially PTH in plateletpheresis donor have never been recorded on local population. Subjects that will be recruited in this study will be larger compared to the previous study done by Bolan et al. in 2001. This is done to enhance the significance of the result obtained. If abnormal levels of PTH in post-procedure were established, so as to minimize side effects to the donors, steps could be taken to address the mild inefficiency of the procedure. The aim of this research is to study the alteration of PTH level in an immediate post-procedure of plateletpheresis.

2. Literature review

2.1. Adverse effect

Instead of its benefit, apheresis process may also bring adverse effect. This has been proved by several studies. The adverse effect seems to appear in all stages of life. In 2007, Michon et al. investigated the complications of apheresis in children and concluded that the rate of complications in children was much higher than in adults. Like adults, children expressed both non-severe as well as severe adverse effects. The non-severe adverse effects include paraesthesia, mild hypertension or hypotension, catheter-related complications and transfusion-related complications. The adverse effects of apheresis can also be seen in therapeutic type of apheresis. Regarding these matter, Ishihara T et al. in 2010 proved that different types of therapeutic apheresis will yield different complication. Yuan et al. in 2010 reported that by using Trima Accel (TA; Caridian BCT), out of 15,763 procedures, 59 (0.37%) developed adverse effects. McLeod *et al.* in 1999 reported a larger number with 242 adverse events from 163 procedures. Citrate toxicity was reported by Crocco I et al. in 2009 in 189 out of 50,072 of apheresis donation and severe adverse reactions occurred in 10 out of 240,596 of the procedure.

2.2. Vasovagal complication

Bueno J.L. et al. in 2005 stated that the most frequent vasovagal reactions include pallor, dizziness, weakness, bradycardia and hypotension. These reactions were believed to be varying depending on frequency of donation, weight, race, and youth (Newman, 2002 & 2003; Newman et al., 2003; Trouern-Trend et al., 1999). Younger donors and first time donors were reported to have more incidence rate of vasovagal reaction (Trouern-Trend et al., 1999; Kasprisin et al., 1992; Oosaka & Kojima, 1999). Oosaka M and Kojima K in 1999 reported that there was significant difference in sex of vasovagal reaction among Japanese apheresis donors. Women seem to have higher rate of vasovagal incidence (4.04%) compared to men donors (1.24%). Tomita T et al. in 2002 obtained quite similar results, where women have higher vasovagal reaction especially those who are over 45 years old with repeating cycles of apheresis.

2.3. Hypocalcemia

The complications that occur during the procedure of peripheral blood progenitor cell harvesting have been described by Kishimoto et al. in 2002. The study emphasized specifically on the use of ACD-A as anticoagulant. The intake of isotonic drinks containing calcium was recommended in the suggestion to minimize the effect of hypocalcaemia during apheresis. ACD-A was determined to be the main factor that causes the complication since it contains citrate.

In 2001, Bolan et al. stated that the use of citrate causes ionized calcium (iCa) and ionized magnesium (iMg) concentrations to decrease markedly by 33 percent and 39 percent respectively at a citrate infusion rate of 1.6 mg per kg per minute. In 1994, Mokrzyeki M et al. had shown that ACD-A which

mainly constitutes citrate, will complex calcium and subsequently result in hypocalcemia. Through retrospective observation, Kaplan in 2012 suggested that the citrate toxicity can be controlled by administration of oral calcium tablets during procedure. In addition, Bolan et al. in 2003 suggested that 2g of calcium tablet should be given to any donor who has a history of uncomfortable citrate-related effects. However the effectiveness of oral calcium administration was limited only by symptom recovery. Citrate intoxication might also become severe if the citrate infusion line disengages from its rotary pump (Uhl & Kruskall, 1997). Lee G and Arepally G.M. in 2012 classified mild symptoms of hypocalcemia as headaches, nervousness, irritability, flushing, shivering, nausea, vomiting, chest discomfort, and abdominal cramping. On top of that, Bolan C.D. *et al.* found that these symptoms may appear when ionized calcium level falls 33% below baseline.

2.4. Citrate toxicity

Citrate is the anticoagulant that has been used since 1914 (Crookston & Novak, 2010). It is likely to be chosen compared to heparin due to its cost effectiveness, safeness, and rapid systemic clearance (Lee & Arepally, 2012). Citrate plays it role as an anticoagulant by chelating Ca^{2+} and Mg^{2+}. This may lead to sequestration of these ions from its normal function. Calcium exists in the body normal condition in the form of albumin complex (40%), complexes with small endogenous anions (13%), and in ionized form (47%). Ionized calcium is also known as free calcium and it is physiologically active and important in haemostasis process (Strauss, R.G., 1996). Acid-Citrate-Dextrose Formula A (ACD-A) and ACD-B are the most commonly used citrate formulations (Kishimoto et al., 2002). In 2010, Crookston K.P. and Novak D.J. stated that anticoagulant which contains higher citrate concentration is rarely used as anticoagulant in apheresis procedure. The infusion rate of citrate is calculated by using total blood volume (TBV) and it is controlled by an instrument to maintain the citrate delivery rates between 1.0 and 1.8 mg/kg/min (Weinstein, 1996). The reduction of ionized calcium by 10-15%, 15-25%, and 20-35% were proved by Hester *et al*. in 1983 to have associated with the infusion rate of anticoagulant at 0.8, 1.0 and 1.2 ml ACD-A/min/L TBV. Strauss R.G in 1996 reported that 0.1 mmol/L of ionized calcium was lowered with the increment of 0.5-0.6 mmol/L of plasma citrate. In relation to that, reduction of ionized calcium level may also cause reduction in protein bound calcium level by 35%. This happened because ionized calcium is dissociated from albumin to maintain homeostasis (Toffaletti, 1983). Ionized calcium which falls below 4.4 mg/dL (1.1 mmol/L) is seen to have associated with symptom of tetany while level below than 3.2 mg/dL (0.8 mmol/L) is seen to have associated with fatal arrhythmias (Urban et al., 1988). The severity of symptom is depending on the rate of citrate infusion rate, the level of declining ionized calcium, and the hepatic metabolism of citrate. Exogenous citrate in the body is metabolized through Kreb's cycle (Lee & Arepally, 2012). As a result, bound calcium and three bicarbonate molecules are released, three hydrogen ions are consumed. Thus this may subsequently lead to blood alkalinity. On top of that, this process constitutes about 80% to 82% metabolization of exogenous citrate while the remaining 18 to 20% is excreted out of body through urination (Flanigan et al., 1996).

In accordance to citrate toxicity, Lee G. and Arepally G.M. stated that intravenous (IV) calcium supplementation can be used to control the moderate to severe symptomatic hypocalcemia. There are two types of IV formulations; calcium gluconate (CaGluc) and calcium chloride ($CaCl_2$). Broner C.W et al. in 1990 proved that calcium chloride has better outcome in restoring calcium level.

2.5. Other related biochemical

Biochemical properties other than calcium that may be altered due to citrate intoxication include parathyroid hormone (PTH), vitamin D, and magnesium. The reduction in level of calcium may alter the level of PTH. This has been proved by Silver *et al.* in 1999. They explained the molecular basis of any changes of calcium level and the interactions with levels of PTH as well as vitamin D. Ionized calcium level will be reverted back to normal by increasing the PTH level which in turn will mobilize calcium from skeletal stores (Silberstein et al., 1986). However Bolan C.D. et al. in 2001 found that the level of intact PTH may elevate limitedly within the first 30 minutes of the collection process. This could explain the development of symptoms despite regulatory mechanism does exist. The effect of citrate infusion which result in changes of serum PTH level, ionized calcium level, urinary calcium excretion level and bone markers serum level have been discussed by Chen Y. et al. in 2009. In their study, a total of 10 male plateletpheresis donors who fulfilled the European guidelines were recruited. Donors were divided into two groups; citrate infusion, and saline infusion. Blood was collected at 20 minutes before, at the start, in the middle, and at the end of apheresis process. The results indicated that infusion of citrate significantly caused an increase in serum levels of bone markers with $P<0.0001$. It also caused significant increase in serum PTH and urinary calcium excretion while decreased in serum ionized calcium. In relation to this, prolong exposure of bone to iPTH results in bone resorption. It obviously played by vitamin D which crucial in regulatory mechanism of calcium level.

Infusion of citrate may also affect the level of magnesium. Like calcium, magnesium has the capability to bind with citrate (Winters, 2006). Citrate may decrease the level of magnesium by 30

to 40% through the process of plateletpheresis (Bolan et al., 2001; Mercan, 1997). In fact, Mercan D. et al. in 1997 reported that the reduction rate of ionized magnesium occurs more rapidly than the reduction rate of ionized calcium. The similar symptom of hypocalcaemia like muscle spasms, muscle weakness, decreased vascular tone, and impaired cardiac contractility may appear in hypomagnesaemia (Winters, 2006). Besides hypomagnesaemia takes longer recovery time compared to calcium (Bolan et al., 2001; Bolan et al., 2003). Symptoms of hypomagnesaemia are not subsided with calcium supplementation but require administration of magnesium sulphate. This has been proved to reduce symptom of paraesthesia (Olson et al., 1977).

3. Methodology

This cross sectional study was done with the aim to look into the possibility of related hormone to become imbalance due to plateletpheresis procedure. Blood samples were taken from plateletpheresis donor at Hospital Universiti Sains Malaysia after informed consent was obtained. The laboratory work was done at endocrine laboratory in Hospital USM and some of the tests were outsourced to private laboratory.

A total of 30 plateletpheresis donors were required in this study. All donors which were selected in this study passed the donor selection process. The general acceptance criteria include age; 18 to 60 years old, weight; ≥55 kg, hemoglobin level; ≥12.5 g/dL, adequate sleep before donation; >5 hours, healthy, and had taken at least a light meal before donation, had undergone normal blood donation beforehand at least twice in 12 months. Donors who did not comply with the criteria provided by National Blood Centre were not eligible to donate and participate in the study.

Prior to donation, informed consent was obtained from all eligible donors. They were explained about the purpose of the study, benefit for the community, and any possible adverse effect that might happen during and after the procedure. Details of the donor were taken. These include age, gender, ethnic, weight and frequency of previous donation.

Two types of machine were used for this purpose; Trima Accel by Terumo BCT and Haemonetics. This study emphasizes more on the standard execution of plateletpheresis process. Therefore these machines were chosen because they were widely used in most of the collection centre across the country. Nevertheless, the concentration of ACD-A used by both machines was approximately the same. Prior to apheresis process, venipuncture was made on cubital fossa after skin was decontaminated. Labeled EDTA tube and plain tube were used. 10 ml of blood was drawn using 10cc syringe through the pouch bag. It was done once the blood flew into the tubing system. 2 ml of blood was transferred into EDTA tube while the remaining 8 ml was transferred into plain tube. The first two tubes were labeled as 'pre'. Standard procedure of plateletpheresis was executed. This process took about 60 to 80 minutes depending on the amount of platelet harvested. Any complication was observed carefully during the yield process. Besides adverse reactions that appear during and immediately after the procedure was recorded. After the yield platelet reached desired amount, process was halted and another 10 ml of blood was drawn into syringe and subsequently transferred into EDTA and plain tubes respectively and were labeled as 'post'. The similar procedure was done for the other 29 subjects.

Specimens that were delayed for analysis were stored overnight before analyzed. It's were stored at 4 degree Celsius. EDTA tubes were sent to endocrine laboratory within Hospital USM for parathyroid hormone analysis. The automation used for this analysis was Cobas 600 E411 and the reagent used was manufactured by Roche Diagnostic. Plain tubes were sent to private laboratory for calcium, 25-OH vitamin D, and magnesium analysis. The automation used for these analyses was Abbott Architect and the reagent used was manufactured by Abbott Laboratories. All data were kept for analysis. SPSS software was used to analyze the data.

4. Result

The studies were evaluated from 30 plateletpheresis donors. All donors have been assessed for eligibility to be recruited in the study. All subjects were male from Hospital Universiti Sains Malaysia. Subjects consist of Malay and Chinese while no Indian and other ethnic group were recruited. The subjects' ages were between 25 to 55 years old. The subjects' weights were between 62 to 93 kilograms. The frequencies of previous donation of all subjects were between 2 to 19 times. Results for pre-donation and post-donation were collected and recorded. Mean and standard deviation of four parameters were calculated. The p-value was calculated using SPSS software to compare mean of two groups (pre and post-donation). Paired t-test was used and results have been tabulated.

4.1. Distribution of subjects by ethnicity

Fig. 1 shows the distribution of race among donor who had participated in the study. From a total of 30 subjects, 25 (83.3%) subjects were Malay while 5 (16.7%) subjects were Chinese. There was no any other race involved other than Malay and Chinese.

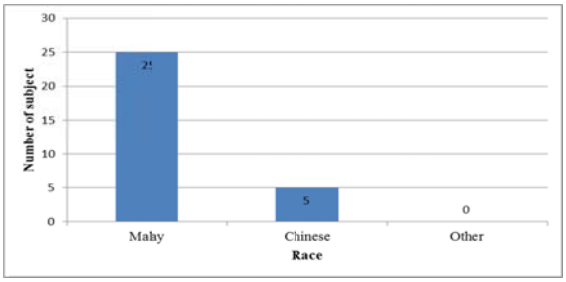

Fig. 1: Distribution of subjects by ethnicity

4.2. Distribution of subjects by range of age

Fig. 2 shows the distribution of subjects by range of age. From a total of 30 subjects, 2 (6.7%) of them were in the range of 18 to 30 years old, 10 (33.3%) of them were in the range of 31 to 40 years old, and 18 (60%) of them were in the range of 41 to 60 years old.

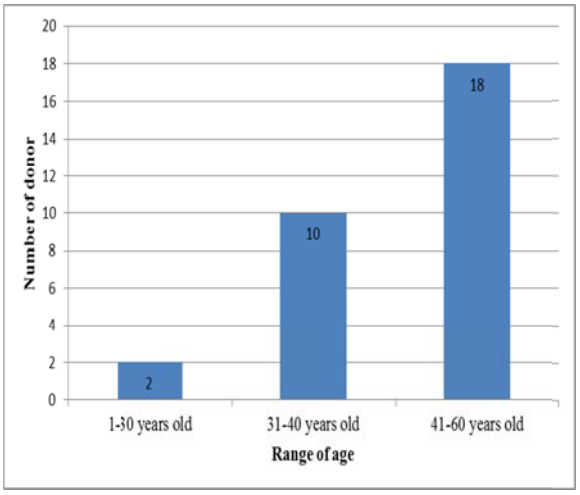

Fig. 2: Distribution of subjects by range of age

4.3. Distribution of subjects by range of weight

Fig. 3 shows the distribution of subjects by range of weight. From a total of 30 subjects, 7 (23.3%) had weight in the range of 61-70 kg, 10 (33.3%) had weight in the range of 71-80 kg, 12 (40%) had weight in the range of 81-90 kg, and 1 (3.3%) had weight in the range of 91-100 kg. The lowest weight recorded is 62 kg while the highest weight is 93 kg.

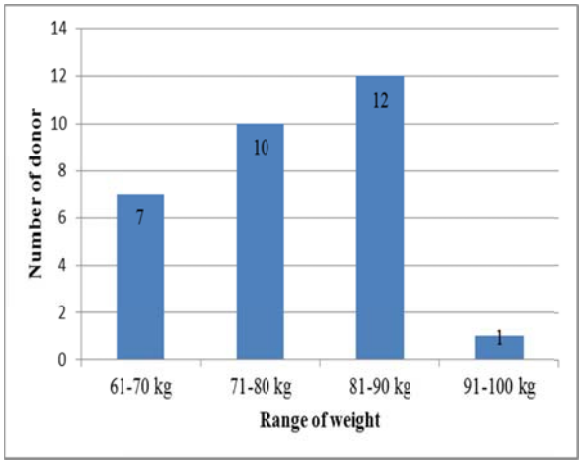

Fig. 3: Distribution of subjects by range of weight

4.4. Distribution of subjects by range of donation frequency

Fig. 4 shows the distribution of subjects by range of donation frequency. From a total of 30 subjects, 9 (30%) had donated in the range of 1 to 5 times, 8 (26.7%) had donated in the range of 6 to 10 times, 8 (26.7%) had donated in the range of 11 to 15 times, and 5 (16.7%) had donated in the range of 16 to 20 times. The lowest number of donation frequency is 2 times while the highest number is 19 times.

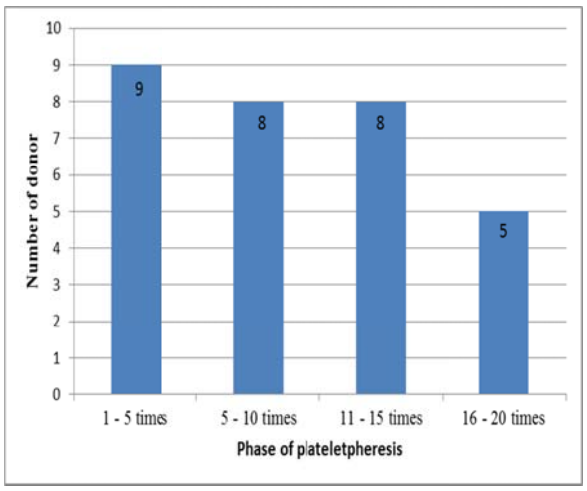

Fig. 4: Distribution of subjects by range of donation frequency

4.5. Distribution of subject by adverse reaction

Fig. 5 shows the number of subjects that had adverse reaction during and immediate after the plateletpheresis process. From 30 subjects, only 4 (13.3%) of them had adverse reaction while the remaining 26 subjects had no symptoms. Among 4 who had adverse reaction, 3 (10%) appear with paraesthesia, while 1 (3.3%) subject had twitching movement.

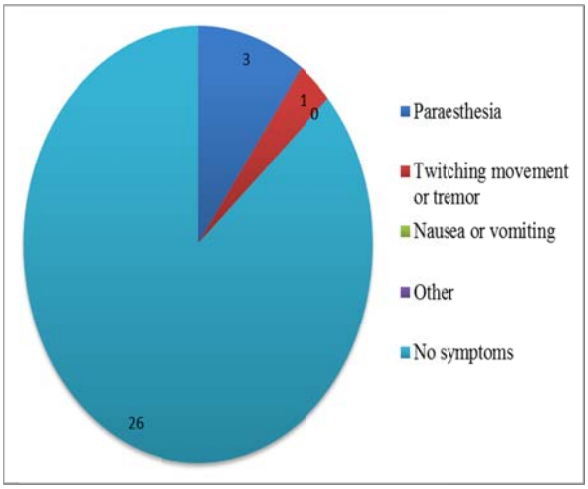

Fig. 5: Adverse reaction that appear during and immediate after plateletpheresis process.

4.6. Distribution of subjects by Parathyroid hormone status in pre and post-plateletpheresis donation.

Fig. 6 shows distribution of subject by PTH status in pre and post-plateletpheresis donation. The normal range for iPTH is 1.6 to 6.9 pmol/L. Therefore any result below 1.6 pmol/L is considered as low and any result that exceeds 6.9 pmol/L is considered as high. In pre-plateletpheresis phase, from a total of 30 subjects, 27 (90%) subjects had normal level of PTH, while 3 (10%) had high level of

PTH. In contrast to that, in post-plateletpheresis phase, 26 (86.7%) subjects had high level of PTH and 4 (13.3%) had normal level of PTH.

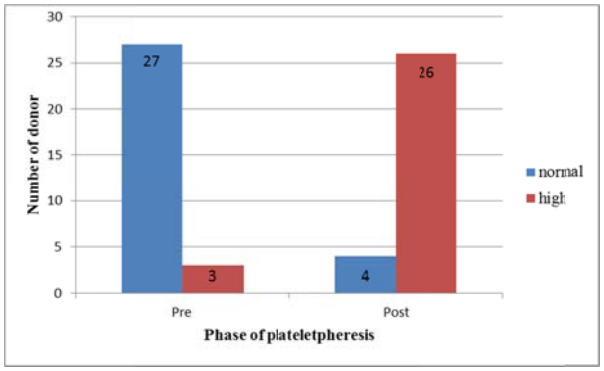

Fig. 6: Distribution of subjects by PTH status in pre and post-plateletpheresis donation

4.7. Distribution of subjects by calcium status in pre and post-plateletpheresis donation

Fig. 7 shows the distribution of subjects by calcium status in pre and post-plateletpheresis donation. The normal range for ionized calcium is 2.1 to 2.5 mmol/L. Therefore any result lower than 2.1 mmol/L is considered as low, and any result that exceeds 2.5 mmol/L is considered as high. In pre-plateletpheresis donation, the levels of ionized calcium for all subjects fall within normal range. In post-plateletpheresis donation, from 30 subjects, 2(6.7%) subjects had low level of ionized calcium and 28 (93.3%) subjects were still normal.

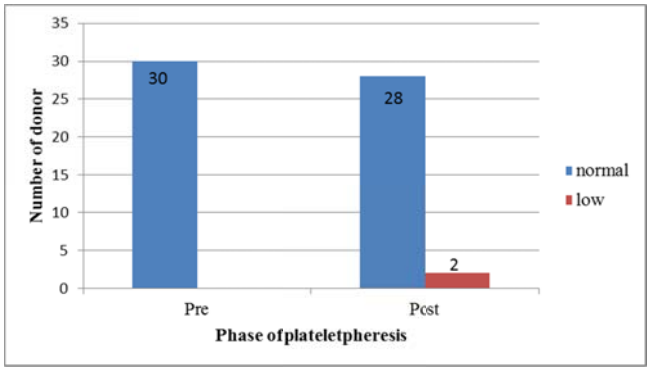

Fig. 7: Distribution of subjects by calcium status in pre and post-plateletpheresis donation

4.8. Distribution of subjects by magnesium status in pre and post-plateletpheresis donation

Fig. 8 shows the distribution of subjects by magnesium status in pre and post-plateletpheresis donation. The normal range for ionized magnesium is 0.66 to 1.07 mmol/L. Therefore any result lower than 0.66 mmol/L is considered as low and any result that exceeds 1.07 mmol/L are considered as high. All subjects had normal magnesium level in pre-plateletpheresis donation. In post-plateletpheresis donation from a total of 30 subjects, 2 (6.7%) subjects had decreased level of magnesium while 28 (93.3%) subjects had still normal level of magnesium.

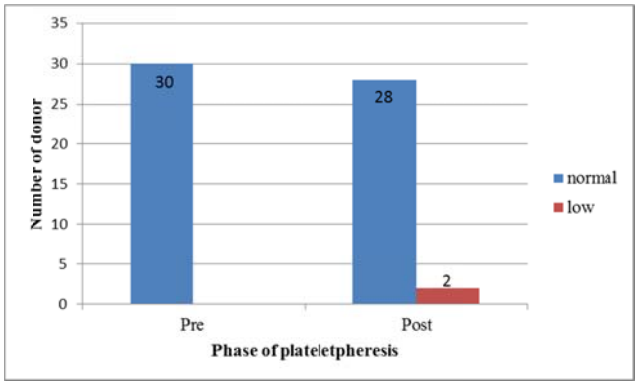

Fig. 8: Distribution of donor by magnesium status in pre and post-plateletpheresis donation.

4.9. Distribution of subjects by 25-OH vitamin D status in pre and post-plateletpheresis donation

Fig. 9 shows the distribution of subjects by 25-OH vitamin D status in pre and post-plateletpheresis donation. For 25-OH vitamin D level, result that is lower than 10 ng/mL is considered as deficient, between 10 to 29 ng/mL is considered as insufficient, between 30 to 100 ng/mL is considered as sufficient, and more than 100 ng/mL is considered as toxic. In pre-plateletpheresis donation, from a total of 30 subjects, only 3 (10%) subjects had sufficient level of 25-OH vitamin D while 27 (90%) had insufficient level of 25-OH vitamin D. In post-plateletpheresis donation, 2 (6.7%) subjects had sufficient level of 25-OH vitamin D while 28 (93.3%) had insufficient level of 25-OH vitamin D.

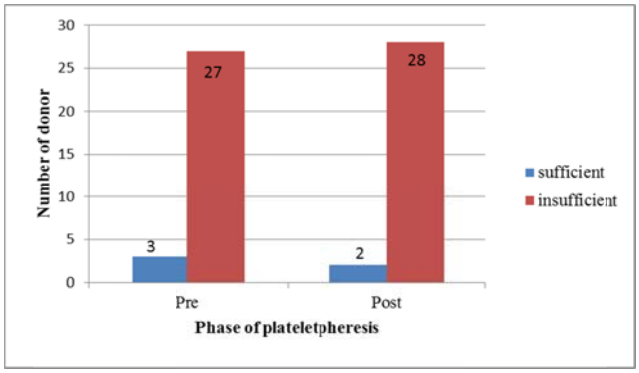

Fig. 9: Distribution of subjects by 25-OH vitamin D status in pre and post-plateletpheresis donation

4.10. Comparison of biochemical results between pre and post-plateletpheresis donation

Table 1 shows the biochemical results after being process using SPSS software. Paired t-test was chose to compare mean of two groups (before and after plateletpheresis donation) of four biochemical results on the same subjects (n=30). For parathyroid hormone, mean (SD) in pre-donation was 4.76 (2.88), while 13.47 (6.99) in post-donation. For calcium, mean (SD) in pre-donation was 2.36 (0.11), while 2.19 (0.11) in post-donation. For magnesium, mean (SD) in pre-donation was 0.88 (0.06), while 0.09 (0.11) in post-donation. For 25-OH vitamin D, mean (SD) in pre-donation was 24.29 (5.85), while

22.58 (5.55) in post-donation. The p-value for parathyroid hormone, calcium, and 25-OH vitamin D was <0.001 while 0.326 for magnesium.

Table 1: Results of paired t-test of biochemical results between pre and post-plateletpheresis donation

	Mean	Standard deviation	n	95% confidence interval		t-statistic (df)	p-value
				Lower	Upper		
Parathyroid hormone	-8.707	6.018	30	-10.95	-6.46	-7.924 (29)	<0.001
Calcium	0.170	0.102	30	0.13	0.21	9.109 (29)	<0.001
Magnesium	0.017	0.091	30	-0.02	0.05	1.000 (29)	0.326
25-OH vitamin D	1.713	2.082	30	0.94	2.49	4.507 (29)	<0.001

4.11. Spearman's correlation between frequency of previous donation and iPTH, iCa, iMg, 25-OH vitamin D respectively

Table 2 shows the correlation between frequency of previous donation and four parameters; iPTH, iCa, iMg, 25-OH vitamin D. Spearman correlation is used in this because the data are not normally distributed. Based on the results, frequency of donation is negatively correlated (-0.004) with iPTH followed by iMg (-0.212), and 25-OH vitamin D (-0.332). Frequency of donation is weakly correlated with iCa (0.163). All of the parameters are not significant.

Table 2: Spearman's correlation between frequency of previous donation and four parameters respectively

	Frequency of donation	Intact Parathyroid hormone (iPTH)	Ionized calcium (iCa)	Ionized magnesium (iMg)	25-OH vitamin D
Frequency of donation	1.000				
Intact Parathyroid hormone (iPTH)	-0.004	1.000			
Ionized calcium (iCa)	0.163	-0.234	1.000		
Ionized magnesium (iMg)	-0.212	-0.002	-0.204	1.000	
25-OH vitamin D	-0.332	0.125	-0.117	0.147	1.000

5. Discussion

In this study, 30 plateletpheresis donors were selected. All donors were selected from Hospital Universiti Sains Malaysia. The general criteria for plateletpheresis donation as well as inclusion and exclusion criteria of the study were passed. Consent was taken from every subject. All demographic data of the subjects were collected and tabulated for analysis. These include gender, ethnic, weight, age and frequency of previous donation. The adverse reactions that happened during and after the donation process were observed and recorded. Out of 30 subjects, 25 (83.3%) subjects were Malay while 5 (16.7%) subjects were Chinese and no any other ethnic involved. This result may reflect the distribution of ethnic in the state of Kelantan. Based on the population statistic, in 2011 provided by Department of Statistics Malaysia, in Kelantan, Malays constitutes about 1.493 million (92.3 %), Chinese; 52,700 (3.3 %), Indian; 4,500 (0.3 %) and others; 67,700 (4.2 %).

Age is one of the general criteria for all types of blood donation. Range of age permitting blood donation is 18 to 65 years old (Yasmin et al., 2008). Out of 30 subjects, 2 (6.7%) of them were age in the range of 18 to 30 years old, 10 (33.3%) of them were in the range of 31 to 40 years old, and 18 (60%) of them were in the range of 41 to 60 years old. Majority (60%) of the subjects were categorized as elderly rather than adolescence. Besides they were considered as regular donor. Low number of plateletpheresis donor may be due to the level of awareness of individual. Elderly group is more aware of the important of donating platelet. Besides, it may also be due to inconvenient long period of plateletpheresis process. The donation process commonly takes approximately 90 minutes depending on donor's weight. However the total number of plateletpheresis donor (n=30) in this study is weak to represent the whole population.

In normal blood donation, donor who has low body weight (< 45 kg) is not fit for blood donation. However, in this study, weight issue does not arise because those who has been considered as regular donor especially plateletpheresis donor always have weight of more than 55 kg. Besides the interval between last donations should not be less than 8 weeks for normal blood donation and 2 weeks for plasma or platelet donation (Olson et al., 1977). In the study, the weight of all subjects falls within 62 to 93 kg. Majority (40%) of their weight falls in the range of 81-90 kg. This followed by range of 71-80 kg (33.3%), 61-70 kg (23.3%), and 91-100 kg (3.3%).

Frequency of previous donation was expected to have an impact on the alteration of four parameters investigated. The data on frequency of previous donation were taken in the same time as demographic data were collected. The highest number of previous donation is 19 times while the lowest number of previous donation is 2 times. To observe the correlation between these two variables, Spearman's correlation was used. Instead of Pearson's correlation, Spearman's correlation was used because we had determined that the relationship is not linear and not normally distributed.

The linearity was determined through the skewed distribution pattern in histogram. Table 2 shows that none of the parameters (iPTH, iCa, iMg, 25-OH vitamin D) investigated has p-value of < 0.05. The p-value for iPTH, iCa, iMg, and 25 OH-vitamin D is 0.984, 0.391, 0.260, and 0.073 respectively. All parameters show insignificant in correlation. Three parameters; iPTH, iMg and 25-OH vitamin D are negatively correlated. The iCa shows weak correlation with value of 0.163. This means that frequency of previous donation does not contribute to the alteration of those biochemical compounds. In other word it can be defined as no strong correlation between frequency of previous donation and alteration of four parameters investigated.

The adverse reaction that appear during and immediate after plateletpheresis process were recorded. Out of 30 subjects, only 4 of them had adverse reaction. Among 4 (13.3%) subjects, 3 (10%) of them had paraesthesia while the remaining 1(3.3%) subject had twitching movement. Twenty six (86.7%) subjects had no symptoms. These adverse reactions as explained by Winters J.L were caused by decreased of calcium level. The number of those expressed adverse reaction in this study does not correlate with the findings of Bolan et al. in 2001 where by an average fall in ionized calcium of 33% from baseline contributed to signs and symptoms of citrate toxicity including paraesthesia, and twitching movement. In contrast, the reduction of ionized calcium level in this study which causes 4 subjects out of 30 to express discomfort feeling was only about 0.08% from pre-donation value. Nevertheless, the response of body toward citrate toxicity varies as this study focuses on two ethics only; Malays and Chinese (Newman, 2002; Newman et al., 2003; Newman, 2003; Trouern-Trend et al., 1999).

In the study, blood was drawn twice; pre and post-donation. Both collections were sent for analysis. Four parameters measured include intact PTH (iPTH), ionized calcium (iCa), ionized magnesium (iMg), and 25-OH vitamin D. Pre-plateletpheresis result shows that all subjects had normal level of ionized calcium. However, the plateletpheresis process which use citrate as anticoagulant did affect two of the subjects in which the level decreases below normal range at the end of donation. Despite the level of ionized calcium of 28 subjects out of 30 in post-donation phase falls within normal range, the difference between pre-donation group and post-donation group is significantly proved with p-value < 0.001. It is assumed that the level of ionized calcium was in the process of reversion to normal with two of the subjects remain low still. The significant decreased of ionized calcium between pre and post-donation clearly shows the action of citrate which bound to ionized calcium (Kaplan, 2012). Calcium in serum exists in three forms; ionized, protein-bound states, and complexes with citrate and phosphorus ion (Favus et al., 2006). Ionized calcium is the physiologically active form of calcium as this may explain it tendency of binding with citrate (Toffaletti, 1983). Citrate which binds to ionized calcium, as explained in the first chapter derived from apheresis machine. It is induced in the donor's body during the donation process. It is one of the compound used in the anticoagulant known as Acid Citrate Dextrose Formula–A (ACD-A). The chemical nature of the soluble calcium-citrate complex and its dissociation constants made it suitable for the development of ACD-A (Hester et al., 1983).

On the other hand, the result of intact PTH in pre-donation shows that 27 (90%) subjects were normal while 3 (10%) of them had high level of iPTH beforehand. In contrast to that, in post-donation phase, 26 (86.7%) subjects had high level of PTH and 4 (13.3%) remain normal. PTH is synthesized by parathyroid cell. It is a potent stimulator used to increase calcium levels in blood, whenever ionized calcium levels are lowered. In normal condition, the level of iPTH is not supposed to be high when level of ionized calcium lies within normal range. However in this study, the level of iPTH was high while the level of iCa was still falls within normal range. This situation has brought us to the assumption that the significant difference (< 0.001) of iCa level between pre and post-donation has made the iPTH level exceeded normal range. By group comparison, the p-value for iPTH is < 0.001 with mean difference of -8.707 and standard deviation of 6.018. It is significant that there is a difference in level of iPTH in pre-plateletpheresis and post-plateletpheresis process. The alteration of this hormone is expected to have an impact on its interrelated biochemical compound as PTH capable of controlling mineral fluxes across intestine, bone, and kidney (Favus et al., 2006). The theory made us to add another two parameters to be tested in the first place. Calcium, magnesium, and phosphate are regulated by 1.25-dihydroxyvitamin D_3 and PTH (Favus et al., 2006). Since it is interrelated, the decreased level of ionized calcium and increased level of iPTH may result in the alteration of magnesium and vitamin D level.

Magnesium is the most abundant divalent cation n the intracellular compartment. It exists 55% in the ionic state, 30% as protein-bound, 15% in complexes. Like calcium, ionized state is the active form of magnesium. Magnesium balance is regulated through the efflux and influx process across intestine, bone, and kidney. Unlike calcium, magnesium is less potent to be regulated by PTH (Favus et al., 2006). Nevertheless, Mercan D. et al. in 1997 stated that the infusion of citrate may also affect the level of ionized magnesium as this could subsequently influence the production of PTH. Back to our findings, the mean difference of ionized magnesium is 0.017 with standard deviation of 0.091 and p-value of 0.326. This means that there is no significant difference of level of iMg between pre and post-plateletpheresis donation. However out of 30 subjects, 2 of them were identified to have reduced and low iMg level after plateletpheresis process. The insignificant result of paired t-test may contrast the results of Mercan D et al. in 1997 where by the ionized magnesium fell by 30 ± 4 percent during

infusion of citrate. Furthermore, the percentage of iMg reduction was steeper compared to iCa. Therefore we assume that the normal level of ionized calcium in post-donation may explain the insignificant result of ionized magnesium.

The active form of vitamin D is 1.25-dihydroxyvitamin D. It is produced enzymatically from 25-OH vitamin D in the kidney. The concentration of active form of vitamin D is about 1/1000 that of 25-OH vitamin D. The half-life of 1.25-dihydroxyvitamin D is 4 to 6 hours while the half-life of 25-OH vitamin D is 2 to 3 weeks (Miller & Gallo, 2010). This may explains why 25-OH vitamin D is more suitable for estimating total level of vitamin D in the body rather than 1.25-dihydroxyvitamin D. Besides the cost for measurement of 1.25-dihydroxyvitamin D is higher compared to 25-OH vitamin D (Miller & Gallo, 2010). A quite serious pattern has been observed in this study where by about 90% of total subjects had insufficient level of 25-OH vitamin D beforehand. Out of 30 subjects, only 3 of them had sufficient level (30-100ng/mL) of 25-OH vitamin D. This may be due to an elderly group which comprises about sixty percent of total number of subjects. Elderly subjects may have slightly insufficient level of 25-OH vitamin D compared to younger subjects. This situation becomes more serious when the p-value of comparing two groups of 25-OH vitamin D level between pre and post-plateletpheresis is <0.001 with mean difference of 1.713 and standard deviation of 2.082. This means that the at hand insufficient level of 25-OH vitamin D in pre-plateletpheresis would significantly reduce further to a more risky level when plateletpheresis process is done. As explained by Cantorna M T et al. in 2004, vitamin D is used to regulate calcium homeostasis. The balance is achieved through the bone formation and resorption process. Vitamin D is synthesized through photolysis process from sunlight exposure. It can also be derived from diet. The dietary intake of vitamin D is problematic. This is because foods which rich in vitamin D are too few. Low level of vitamin D may cause disease state. In conjunction to that, the insufficiency of level of vitamin D was attributed to cause inflammatory bowel disease (Cantorna et al., 2004). Based on the relationship of iCa, iPTH and 25-OH vitamin D, we expected that the persistent high of iPTH may worsens the state of vitamin D level insufficiency.

6. Conclusion

In conclusion, the cause of altered calcium, PTH, and 25-OH vitamin D level is citrate. The presence of citrate through infusion, binds with ionized calcium in donor's blood result in decreased level of ionized calcium. A state of hypocalcaemia leads to a markedly increased in the production of iPTH. Since PTH is a potent regulator of many chemical compounds, the above normal range level of iPTH may cause 25-OH vitamin D to reduce further. Based on the results of vitamin D level in pre-donation phase, in a state of insufficiency, we expect donor may not be able to withstand further reduction of vitamin D particularly 25-OH vitamin D in a long period as this may lead to disease state. On the other hand, the result of iPTH which shows significant high in level of iPTH without significant reason (normal ionized calcium level) is assumed to be the root cause of further reduction of vitamin D level.

The changes of iPTH and 1.25-dihydroxyvitamin D levels may altered bone metabolism (Winters, J.L., 2006). The problem of this situation may not appear in a short period. Dettke et al. in 2006 had investigated this effect. In their study, bone mineral density of the femoral necks and lumbar spines of apheresis donor with more than 100 donations (n=45) and those less than 50 donations (n=40) were compared. The result shows that thirty five percent of the donors with more than 100 donations exhibited osteopenia while there was no similar changes were seen in those who had donated less than 50 times. Although the frequency of donation of a donor in HUSM does not as great as 100 times, this finding has raised our concern because no similar study has been conducted on Malays and Chinese ethnics (Dettke et al., 2003).

The objectives in this study have been achieved. All of the parameters suggested including intact parathyroid hormone, ionized calcium, ionized magnesium, and 25-OH vitamin D have been measured. Besides the comparison between pre and post groups for all four parameters have been done using paired t-test.

Based on our findings, we would like to recommend that further study should be done to investigate long term effect of citrate toxicity emphasizing on the level of iPTH as well as 25-OH vitamin D. This is of concern because persistent derangement of these biochemical compounds could lead to problem of bone metabolism. Further investigation on the density of bone for donor with high frequency of apheresis donation should also be a prospect. Studies as suggested should focus on the local population as different ethnic may yield different result.

The limitation that has restricted our study includes time and budget. We recommend larger number of donors being recruited for such investigation as this may enhances the significance of results especially when it comes to evaluation of adverse reaction. Longer time scale for measurement of iPTH, iCa, iMg, and 25-OH vitamin D which emphasizes on the long term effect of citrate toxicity should also be considered.

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