Original Article

Comprehensive analysis of changes in clinically significant divalent serum cation levels during automated plateletpheresis in healthy donors in a tertiary care center in North India

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Abstract:

Background: Adverse effects due to apheresis are unusual. The most common apheresis-specific reaction is hypocalcemia due to citrate anticoagulation and induces ionized hypocalcemia and hypomagnesemia by chelating effect during the plateletpheresis; generally transient and self-limiting but has the potential of severely injuring donor. We have investigated total calcium (tCa^{++}) and magnesium (tMg^{++}) levels in sixty healthy plateletpheresis donors at different intervals during the procedure and 30 min post-procedure. **Materials and Methods:** A total of 60 procedures were performed on healthy donors. Blood samples were obtained from sterile diversion pouch placed on apheresis circuit. 5 ml sample in plain vials was obtained at different intervals during each procedure and 30 min after the end of the procedure. Samples were used for measurement of tCa^{++} and tMg^{++} levels. **Results:** There is continuous decrease in mean tCa^{++} from baseline levels (9.83 ± 0.64 mg/dl) till end of procedure (8.33 ± 0.78 mg/dl), but after 30 min, levels again reached near their respective baseline values (9.42 ± 0.54 mg/dl). Similarly, mean tMg^{++} fell from baseline levels (2.36 ± 0.3 mg/dl) till the end of procedure (1.39 ± 0.40 mg/dl). After 30 min, levels were again increased, their respective baseline values (2.25 ± 0.25 mg/dl). **Conclusion:** There is continuous, gradual, and significant fall ($P < 0.05$) in mean tCa^{++} and mean tMg^{++} from baseline levels to till the end of procedure but after 30 min of completion of procedure, levels again reached near their respective baseline values.

Key words:

Citrate anticoagulation, hypocalcemia, hypomagnesemia, plateletpheresis

Access this article online

Website: www.ajts.org

DOI: 10.4103/0973-6247.162688

Quick Response Code:

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Introduction

The collection of platelets by means of apheresis requires citrate infusion in order to prevent clotting of extracorporeal blood in apheresis circuit.[1] The most common apheresis-specific reaction is hypocalcemia due to citrate anticoagulation, which is usually mild, but also has the potential of severely injuring donor. Besides chelating free bioactive blood calcium needed by coagulation factors, with little alteration in total calcium (tCa^{++}), citrate also affects total magnesium (tMg^{++}).[2,3] Magnesium is not only the second most abundant divalent cation in blood; but also involved in many metabolic processes that are closely dependent on its presence.[4] Alterations in magnesium ion activity might also occur during the plateletpheresis, and its variations may have repercussions on both calcium metabolism and parathormone (PTH) response.[5]

The standard citrate infusion rates exist for plateletpheresis procedures, in which metabolism, redistribution, and short procedure duration prevent accumulation to toxic levels.[6-8] However, during longer and repeated procedures, citrate accumulation may outpace metabolism, resulting in markedly decreased ionized calcium levels and significant donor symptoms.[9] The reactions were found to be more frequent among platelet donors (12%) than either plasma (5.9%) or granulocyte donors (9.4%).[10] According to the literature, about 16-50% plateletpheresis donors develop citrate related reactions.[11] If hypocalcemia becomes more severe, symptoms can progress to frank tetany with spasm in other muscle groups, including life-threatening laryngospasm, Q-T prolongation, and fatal arrhythmias can also occur.[12-14]

Manifestations of hypocalcemia and hypomagnesemia are almost similar, and some symptoms may be falsely attributed to hypocalcemia. Therefore, calcium supplementation may be a failure in such cases.[1] Although, prophylactic calcium supplementation during apheresis is a routine practice in many of the transfusion centers, no information is available regarding magnesium supplementation in apheresis donors.[1,15]

Plateletpheresis is a routine procedure performed at our institute by using three automated cell separators named Fenwal Amicus separator, version 2.5 (Baxter Healthcare Corporation, Deerfield, IL, USA), Fresenius COM. TEC, version 4.00 XX (Fresenius Hemocare GmbH, Bad Homburg v.d.H, Germany) and Haemonetics MCS + separator (Haemonetics Corporation, Braintree, Massachusetts, USA). In the present study, tCa^{++} and tMg^{++} levels were analyzed in sixty healthy plateletpheresis donors at different intervals during the procedure and 30 min post-procedure.

Materials and Methods

When the donor was found eligible for platelet donation, consent form was filled, and plateletpheresis was performed on any of the three plateletpheresis machines based on either continuous or intermittent flow technology, available at our center. A total of 60 procedures were performed on healthy donors. No prophylactic calcium or magnesium supplementation was administered. Of these 60 procedures, 7 were performed on Fenwal Amicus separator, version 2.5, a continuous flow machine (Baxter Healthcare Corporation, Deerfield, IL, USA), 34 on Fresenius COM. TEC, version 4.00 XX, a continuous flow machine (Fresenius Hemocare GmbH, Bad Homburg v.d.H, Germany) and 19 on Haemonetics MCS + separator, an intermittent flow machine (Haemonetics Corporation, Braintree, Massachusetts, USA). All these procedures were carried out following the departmental standard operating procedures using closed system plateletpheresis kits and acid, citrate and dextrose-A (ACD-A) as anticoagulant in the proportion of 1:10-1:12 (blood flow rate: 60-80 ml/min).

Sample collection

Blood samples were obtained from sterile diversion pouch placed on apheresis circuit. 5 ml sample in plain vials were obtained at 0 min (baseline), 30 min, 60 min during each procedure and 30 min after the end of the procedure. Samples were centrifuged, supernatant removed and used for measurement of tCa^{++} and tMg^{++} levels. Centrifugation and separation took place within 2 h of collection and were stored at –20°C to –30°C for <4 weeks and then analyzed in one run.

Biochemical measurement of calcium and magnesium

There are various chemical methods available for measurement of serum calcium and magnesium viz. precipitation method, visual complexometric method, Cathode ray polarography, automated stopped-flow analyzing method, atomic absorption spectrophotometry, and spectrophotometry complexed with different dyes (colorimetric spectrophotometry).[16-22]

VITROS DT 60 II chemistry system (Ortho Clinical Diagnostics, Johnson and Johnson Company, New York) is a colorimetric spectrophotometer that quantitatively measure calcium and magnesium concentration in the donor serum.[22] VITROS DT 60 II module is a high throughput analyzer that gives 65 tests/hour and used for the magnesium measurement. While its VITROS DTSC II module gives 20 tests/hour and used for calcium determination.

A 10 µL sample was deposited on the slide and was evenly distributed by spreading a layer. The bound calcium was dissociated from binding proteins and formed complex with arsenazo III dye, causing a shift in absorption maximum. Reflection density of colored complex was measured spectrophotometrically at 680 nm wavelength. Amount of colored complex was proportional to calcium concentration in the sample. Similarly, magnesium from sample combined with formazan dye. The high magnesium affinity of the dye dissociated from magnesium from binding proteins and resulted in Mg^{++} - dye complex causing the shift in absorption maximum. Reflection density of colored complex was measured spectrophotometrically at 660 nm wavelength. Amount of colored complex was proportional to magnesium concentration in the sample.

Statistics

Statistical analysis was done using SPSS statistical computer software (version 13, USA). Serum calcium, magnesium levels were analyzed as mean ± standard deviation, comparison between successive samples was made by means of the paired *t*-test and the $P < 0.05$ was considered statistically significant.

Results

Sixty healthy male donors (mean age 28.2 ± 5.19 years) weighing 65.8 ± 12.7 kg underwent plateletpheresis. The mean blood volume processed in Haemonetics MCS + cell separator was 3.49 ± 0.45 L and 307.6 ± 36.3 ml of ACD was used. In Fresenius COM. TEC 2.92 ± 0.53 L of whole blood was processed using 296.3 ± 49.3 ml of ACD. While in Fenwal Amicus cell separator 2.68 ± 0.44 L of whole blood was processed, and 264.4 ± 45.6 ml of ACD was used.

The tCa^{++} and tMg^{++} levels were analyzed at different intervals during procedures and 30 min post-procedure. There is continuous and gradual decrease in mean tCa^{++} from baseline levels (9.83 ± 0.64 mg/dl) till end of the procedure (8.33 ± 0.78 mg/dl) but after 30 min of completion of procedure, levels again reached near their respective baseline values (9.42 ± 0.54 mg/dl). The change in total calcium level was statistically significant ($P < 0.05$) as seen in Table 1 and Figure 1. Similarly, mean tMg^{++} concentration fell from baseline levels (2.36 ± 0.3 mg/dl) till end of procedure (1.39 ± 0.40 mg/dl). After 30 min of completion of procedure, levels were again increased and reached near their respective baseline values (2.25 ± 0.25 mg/dl) and difference was significant ($P < 0.05$) as shown in Table 2 and Figure 2.

The analysis of serum calcium and magnesium in relation to the type of the cell separator used for the procedure with time is shown in Figures 3 and 4. In Fresenius COM. TEC, mean tCa^{++} fell from 9.81 ± 0.79-8.66 ± 0.75 mg/L at the end of procedure (60 min), difference was statistically significant($P < 0.001$). Similarly, on Fenwal Amicus cell separator, mean tCa^{++} fell from 9.98 ± 0..68-8.87 ± 0.68 mg/L at the end of procedure (60 min) and mean tCa^{++} fell from 9.72 ± 0.54-7.37 ± 0.38 mg/L on Haemonetics MCS + cell separator, difference was statistically

Table 1: Comparison of change in serum calcium levels with time

Time interval	Mean ± SD		Significance of change
	Serum calcium level (mg/dl)	Difference from baseline	*P*
Baseline	9.83±0.64	—	—
30 min	9.05±0.50	0.78±0.46	<0.001
60 min	8.33±0.78	1.50±0.84	<0.001
Post-30 min	9.42±0.54	0.41±0.67	<0.001

SD: Standard deviation

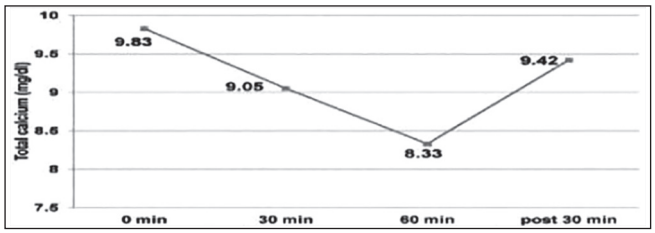

Figure 1: Comparison of the time course of total calcium in serum at different time intervals during plateletpheresis

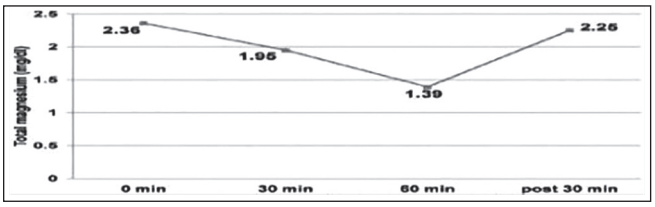

Figure 2: Comparison of the time course of total magnesium in serum at different time intervals during the plateletpheresis

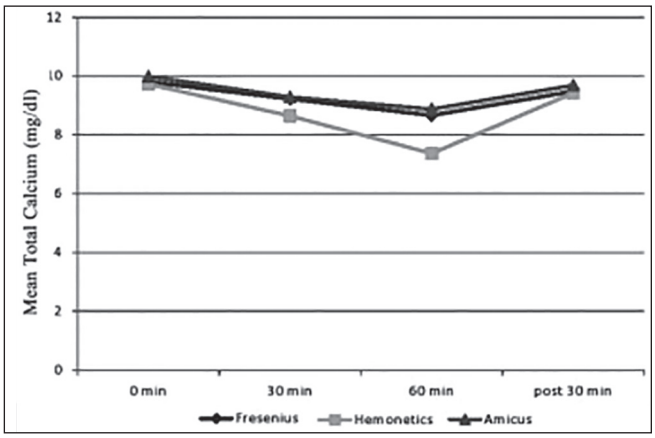

Figure 3: Comparison of total calcium levels on different apheresis machines with time

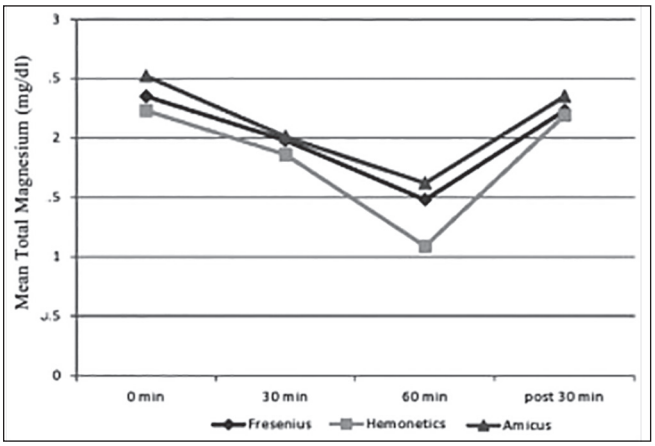

Figure 4: Comparison of total magnesium levels on different apheresis machines with time

Table 2: Comparison of change in serum magnesium levels with time

Time interval	Mean ± SD		Significance of change
	Serum magnesium level (mg/dl)	Difference from baseline	*P*
Baseline	2.36±0.30	—	—
30 min	1.95±0.32	0.41±0.19	<0.001
60 min	1.39±0.40	0.97±0.24	<0.001
Post-30 min	2.25±0.25	0.11±0.31	<0.001

SD: Standard deviation

significant($P < 0.001$). On all three cell separators, recovery of tCa^{++} levels were noted after 30 min of the procedure and levels reached almost their baseline values [Figure 3].

In Figure 4, comparison of tMg^{++} levels on different apheresis machines with time is shown. Mean tMg^{++} fell from initial value of 2.35 ± 0.38 mg/L-1.48 ± 0.21 mg/L at the end of procedure (60 min) on Fresenius COM. TEC, on Fenwal Amicus cell separator, mean tMg^{++} fell from 2.52 ± 0.32–1.62 ± 0.16 mg/L and 2.23 ± 0.34-1.09 ± 0.08 mg/L on Haemonetics MCS + cell separator. The difference was statistically significant ($P < 0.001$) on all three cell separators. On all three cell separators, recovery of tMg^{++} levels was noted after 30 min of the procedure and levels reached almost their baseline values.

Discussion

Citrate is used as a primary anticoagulant in plateletpheresis procedures in order to prevent clotting of extracorporeal blood in apheresis circuit. It is well-known that citrate induces ionized hypocalcemia and hypomagnesemia by chelating calcium ions and magnesium ions to produce a soluble complex causing decrease in ionized calcium (active form) that increases excitability of nerve membranes and lead to spontaneous depolarization causing perioral and acral paresthesias. Some donors may experience shivering, nausea, vomiting, abdominal pain, chills and fever, lightheadedness, tremors, and muscle cramps.[11] If hypocalcemia becomes more severe, symptoms may progress to frank tetany, including life-threatening laryngospasm. These signs and symptoms may be accentuated by alkalosis due to hyperventilation.[23] We, therefore, evaluated biochemical changes in healthy donors during plateletpheresis procedure.

Previous studies on plateletpheresis have reported a modest reduction in tMg^{++} and tCa^{++}, a significant drop in ionized calcium and magnesium, and a quick rise in PTH with a continuous decrease thereafter.[24,25]

In present study, we have measured total (free as well as bound) calcium and magnesium levels at different intervals by a spectrophotometer. There is continuous, gradual, and significant fall (P value < 0.05) in mean tCa^{++} from baseline levels (9.83 ± 0.64 mg/dl) till the end of the procedure (8.33 ± 0.78 mg/dl), but after 30 min of completion of procedure, levels again reached near their respective baseline values (9.42 ± 0.54 mg/dl). Similarly, tMg^{++} concentration significantly fell (P value <0.05) from baseline levels (2.36 ± 0.3 mg/dl) till the end of the procedure (1.39 ± 0.40 mg/dl). After 30 min of completion of the procedure, levels again increased and reached near their respective baseline values (2.25 ± 0.25 mg/dl). The reason could be because of mobilization of these ions from skeletal stores as well as increased absorption by the kidney, increased

calcium levels are also due to increased parathyroid hormones and mobilization of calcium bound to serum albumin.[15,26] Das *et al.* noticed that mean tCa^{++} fell from 2.62 ± 0.12–2.36 ± 0.12 mmol/L and mean tMg^{++} from 0.89 ± 0.01-0.79 ± 0.01 mmol/L, however the difference was not significant. Moreover, drop in mean iCa from 1.33 ± 0.1–0.84 ± 0.1 mmol/L and mean iMg from 0.53 ± 0.01–0.35 ± 0.1 mmol/L was statistically significant ($P < 0.001$).[27] Other studies also reported that although, the fall in tCa^{++} and tMg^{++} were modest and not significant, the drop in iCa and iMg was statistically significant ($P < 0.001$).[1,25,28]

The levels of iCa decreased up to 35% in procedures performed without prophylactic Ca, and more than 50% of such procedures were associated with citrate-related complaints, many of which were clinically significant. In contrast, only 20% of procedures performed with prophylactic Ca were associated with symptoms, most of which were mild.[8] Because it is now clear that acute hypocalcemia and hypomagnesemia occur during the plateletpheresis, both these cations are candidates for monitoring during citrate anticoagulated plateletpheresis. In recent years, progress in ionophore research and electrode design has made possible the development of magnesium selective electrodes. Such analytical devices allow concomitant measurement of the ionic activity of both magnesium and calcium in the blood.[29,30]

In short, plateletpheresis procedures are very safe for donors; in fact severe adverse reactions occur in only a very small percentage (0.89%) of donors.[31] Thus, efforts to understand the risk factors and etiologies of adverse donor reactions are important in protecting donor safety and retaining donors.

Conclusion

Oral or IV calcium administration in association with citrate infusions may provide an enhanced level of donor comfort, and permit increased component yields. Bolan *et al.* recommend administration of 2 g of oral Ca carbonate approximately 30 min before donation to mitigate the significant citrate-related effects associated with plateletpheresis.[15]

Magnesium supplementation deserves further investigation. Additional studies to ascertain the cumulative effects of these changes may be of benefit, particularly for frequent platelet donors.

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Cite this article as: Solanki A, Agarwal P. Comprehensive analysis of changes in clinically significant divalent serum cation levels during automated plateletpheresis in healthy donors in a tertiary care center in North India. Asian J Transfus Sci 2015;9:124-8.

Source of Support: Nil , **Conflicting Interest:** None declared.