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Stable Ionized Calcium Concentration in Uncapped Plasma Samples

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Abstract

Background: Blood ionized calcium (iCa) concentration has been shown to be pH dependent. In the clinical laboratory, plasma specimen for iCa measurement is routinely rejected when exposed to air prior to analysis. A preanalytical variable such as specimen exposure to air is believed to alter the pH and consequently the iCa concentration. Specimen exposure leads to the loss of carbon dioxide resulting in an increase pH and decrease iCa concentration. The purpose of this study is to investigate the rate at which these changes affect iCa concentration. We hypothesize that the changes are slow and insignificant to warrant the rejection of plasma specimen.

Methods: Appropriately collected whole blood specimen were centrifuged and analyzed for iCa concentration per laboratory procedure on AVL by Roche. The first, at time zero and subsequent measurements were made at various time intervals of sample exposure to air. The pH of plasma and whole blood samples exposed to air at various intervals were also measured to evaluate for changes.

Results: The iCa concentrations remained stable in plasma samples exposed to air for a considerable period of time. Exposed plasma pH measurements were equally stable (for up to ninety minutes), compared to that of whole blood specimen. The average changes seen in whole blood pH (~0.5 units) were ten times greater than that of plasma pH (~0.05 units) for the same length of time.

Conclusion: Plasma samples for iCa that are accidentally exposed to air should not be summarily rejected because both the pH and iCa level are stable for almost eighty minutes. The changes in whole blood pH are due to the respiring red cells. Red cells contain carbonic anhydrase (not found in plasma) that catalyzes the reversible interconversion of carbon dioxide and bicarbonate ion. Unnecessary specimen rejection/redraw request is costly. Besides, repeat injection is painful, exposes the patient to infections and hematoma at the injection site.

Keywords: Whole blood; Plasma; Ionized Ca; CO_2; pH; Avl analyzer

Abbreviations: iCa: ionized Calcium; CO_2: Carbon Dioxide; PTH: Parathyroid Hormone; 1, 25 (OH) 2D: 1, 25 di-hydroxyl vitamin D; TAT: Turn Around Time

Introduction

Although not generally included in the basic metabolic profile, calcium is a major electrolyte assayed from whole blood, plasma or serum specimen for the diagnosis and management of many disease conditions especially in transfusion, cancers, septic, neonates and critically ill patients [1-3]. In blood, calcium exists in two forms; bound and unbound. The bound form is physiologically inactive as the calcium is tethered to negatively charged amino-acid residues on proteins, mainly albumin (40%) and other anions (10%) such as phosphates, carbonates, citrate and lactate. The remaining unbound (~50%) is known as ionized (free) calcium. The ionized form is tightly regulated and interacts with receptors and ion channels to drive many biochemical processes within the body [2-4]. Because it is the most physiologically active form, iCa (unlike total calcium) level provides the best measure of a patient's calcium status especially in conditions of abnormal blood levels of albumin or immunoglobulin's [1,4,5]. In classifying calcium status, total calcium (tCa) frequently disagrees with iCa in parathyroid disease conditions [6].

The equilibrium that exist between the bound and iCa is influenced by many diseased conditions [5], including changes in the blood pH [7,8]. Consequently, any factor that influences blood pH should also impact the iCa concentration. Hyperventilation induced respiratory alkalosis, for example will decrease iCa whereas hypoventilation induced respiratory acidosis will increased ionize calcium concentration [9,10]. The protonation or deprotonation of amino and carboxyl groups of charged or uncharged protein molecules should alter the calcium equilibrium and the proportion of the iCa. In general, blood calcium is tightly regulated by the parathyroid hormone (PTH), and 1, 25 dihydroxylvitamin D, 1, 25 (OH) 2D between the intravascular and extracellular fluids [1,3,4,9] and calcitonin [11].

As the most active form physiologically, iCa measurement represents the best indicator of a patient's calcium status. Current laboratory protocol calls for specimen for iCa measurement to be collected anaerobically on ice in PST gel and lithium heparin tube. The collected specimen is immediately transported to the lab for processing; where it is centrifuged and analyzed for results as quickly as possible. However and occasionally after centrifugation uncapped plasma specimen are not analyzed immediately. These accidentally exposed specimens are generally rejected, compromising turn-around-

rime (TAT) and delaying patient's treatment. Braid [5] indicated that uncapped sample may undergo significant changes in the pH due to the loss of CO_2. The loss of CO_2 associated with exposure leads to an increased in pH and a decreased in the iCa concentration. These changes in the pH and iCa level are well documented in whole blood samples [7,8]. Unlike whole blood, changes in routinely assayed iCa level using heparinized plasma samples has not been documented in uncapped specimen accidentally exposed to air. Furthermore, the rate at which these changes (if any) occur in exposed samples to warrant specimen rejection needs to be investigated.

Specimen rejection is generally followed by redraw/recollect request. Besides the poor TAT and treatment delays, redraw or recollection request is risky and can be costly to the patient and the healthcare community. We investigated the kinetics or rate of change in iCa concentration when centrifuged specimen (plasma) is exposed to air to clearly define the time interval for rejection of samples or analyzed for results without compromising effective patient care. We hypothesize that iCa level in uncapped plasma specimen should remain stable because there isn't plenty of dissolved carbon dioxide [12] escaping (if any) from the sample to significantly change the pH and hence the iCa concentration.

Materials and Methods

Whole Blood specimen was collected by venous puncture into tubes containing lithium heparin. The tube was immediately placed in ice and transported to the laboratory for processing and analysis. Institutional Review Board (IRB) approval is filed with the office of Sponsored Programs at Winston Salem State University. Specimen for iCa measurements were hospital specimens destined for disposal after reporting patient result. All samples for pH measurements were obtained from healthy volunteers within our clinical laboratory science program. These specimens were centrifuged at 4000 rpm for 4 min to separate plasma from red cells. The centrifuged sample tubes were immediately uncapped to measure the iCa concentration and the pH. The first measurements were recorded as time 0. The first iCa measurement was the reported patient result per standard operating procedure (SOP). All subsequent measurements followed the SOP except that the specimen continuously left uncapped, exposed to air after the first measurement for both iCa level and pH at the stipulated time intervals.

A total of twelve specimens from different individuals were processed at various intervals for iCa on the AVL 9180 Electrolyte Analyzer by Roche following calibrations and quality control performance per manufacturer's instructions [13]. Whole blood and plasma pH measurements were performed on OAKION pH 2100 series following a three point calibration using buffer 4.01, 7.00 and 10.01 per manufacturer's package insert [14]. To prevent precipitation during pH measurement, a small stirring bar was used to keep the red cells uniformly suspended in the whole blood sample throughout the measurements.

Data analysis

The initial pH values for all specimens fell between 7.4 - 7.6 pH change was calculated by subtracting the initial from all subsequent pH data over time. A total of 10 (6 plasma and 4 whole blood) samples were processed to identify pH changes. The mean pH ± error was calculated using Microsoft excel program and plotted as a function of time for both plasma and whole blood specimen.

Results

Previous evidence indicate that under anaerobic conditions, iCa levels remain stabled for several hours at 15°C-30°C and for one week at 2°C-8°C (Piedmont Lab. protocol). Under aerobic conditions, our results were equally stable. We did not see any significant change in iCa concentration in uncapped samples. ICa is measured routinely to diagnose and assess treatment progress in many diseased states. Because samples are accidentally left uncapped and exposed to air for several minutes are summarily rejected, we wanted to document the impact of such exposure on plasma iCa levels. Post centrifugation specimens were uncapped and iCa measured at defined time intervals as described under Methods. Table 1a shows the iCa levels in twelve uncapped samples. Throughout the measurement interval there were very little variations in the measured values. We observed also no significant changes in plasma iCa levels between samples that were incubated for 10 min on ice, room temperature or at 37°C before centrifugation (data not included).

It is well documented that iCa concentration is pH dependent in whole blood [1,7-9]. The changes are pH is linked to the CO_2 levels in blood. The loss of CO_2 or increase in the pH leads to decreased iCa. Surprising, in uncapped centrifuged plasma samples we did not observe any significant decrease or change in iCa levels over several min of measurement.

Because changes in iCa concentration were not significant, we decided to assess pH changes in plasma and whole blood samples. In Figure 1, plasma average pH measurements were equally stable. Within the first 90 min of measurements, the average change in the pH was less than ~0.05 units in plasma. Unlike plasma, the average pH change in whole blood samples was ~0.5 units; approximately ten times greater than that of plasma over the span of measurement. These results support the observation that iCa is stable in plasma and this stability is maintained even when the sample is exposed to air. Significant changes in the pH of whole blood supports published findings that iCa is pH dependent.

Time (min)	A	B	C	D	E	F	G	H	I	J	K	L
0	1.15	1.17	1.02	1.21	1.26	1.21	1.21	1.21	1.1	1.2	1.09	1.2
30	1.18	1.16	1.02	1.2	1.24	1.2	1.21	1.18	1.11	1.22	1.09	1.19
60	1.18	1.16	1.02	1.18	1.23	1.19	1.21	1.18	1.11	1.21	1.05	1.19
90	1.18	1.13	1	1.2	1.24	1.16	1.18	1.18	1.1	1.2	1.05	1.19
120	1.16	1.15	1	1.16	1.26	1.17	1.19	1.174	1.09	1.18	1.05	1.17

150	1.16	1.14	1.01	1.18	1.25	1.17	1.16	1.16	1.08	1.21	1.05	1.18
180	1.14	1.13	0.98	1.19	1.25	1.14	1.17	1.15	1.1	1.18	1.03	1.12
210	1.16	1.16	1	1.17	1.22	1.18	1.17	1.14	1.07	1.17	1.06	1.13

Table 1a: Measurements were recorded at various time intervals in uncapped adult specimen (A-L). The measurement at time zero was the reported patient result for each sample. Subsequent measurements every 30 min for 210 min total. Total calcium measurement for all 12 samples was within the normal reference range of 8.5 mg/dL-10.2 mg/dL; (b) The average iCa value for each specimen compared to the initial measurement at time zero was within the calculated error and not significantly different.

	A	B	C	D	E	F	G	H	I	J	K	L
Average	1.164	1.15	1.006	1.186	1.243	1.178	1.181	1.171	1.095	1.196	1.059	1.171
	± 0.015	± 0.015	± 0.014	± 0.017	± 0.014	± 0.023	± 0.029	± 0.022	± 0.014	± 0.018	± 0.021	± 0.029

Table 1b: The average iCa value for each specimen compared to the initial measurement at time zero was within the calculated error and not significantly different.

Discussion

The stability of plasma iCa concentration is significant to specimen handling as pre-analytical variables account for most errors in the clinical laboratory. Our results demonstrate that rejection of exposed plasma samples for iCa measurement should be reconsidered. Because of the stability in the iCa levels, accidentally exposed specimen should be processed to prevent unnecessary delay in patient treatment.

The prevailing rational for rejection of specimen is based on changes in the pH when samples are accidentally exposed to air. Exposure lead to the loss of carbon dioxide and pH change which significantly alters the iCa concentration. As a consequently, processing and reporting the result of such specimen can compromise patient care. The above rational is true for whole blood specimen [7-9]. Our results with centrifuged plasma sample demonstrates that iCa concentration remained basically unchanged from the first measurement at time zero and several minutes after in uncapped, exposed samples (Table 1b). ICa levels in centrifuged specimens were previously found to be stable for hours at 4°C, 22°C and 37°C; and for days at 4°C, in sealed tubes where pH change are unlikely. However, and surprisingly, we also found the same stability in iCa concentration when specimen were uncapped. It was not evident that the pH in aerobic tubes changed enough to alter the iCa concentration. There was no significant change in iCa levels when plasma specimen were exposed at room temperature (~22°C) or 37°C water bath for 10 min prior to processing.

In whole blood specimen, pH changes were significant, ten times greater than plasma pH (Figure 1). Unlike plasma, red cells in whole blood contain carbonic anhydrase, an enzyme that catalyzes the reversible inter conversion of dissolved carbon dioxide and the dissociated ions of carbonic acid (bicarbonate and protons). In exposed whole blood sample, the carbon dioxide produced is lost to the atmosphere-shifting the equilibrium towards the direction of more carbon dioxide loss (Figure 2) and pH increase. Such increase in pH has been shown to decrease iCa concentration in whole blood.

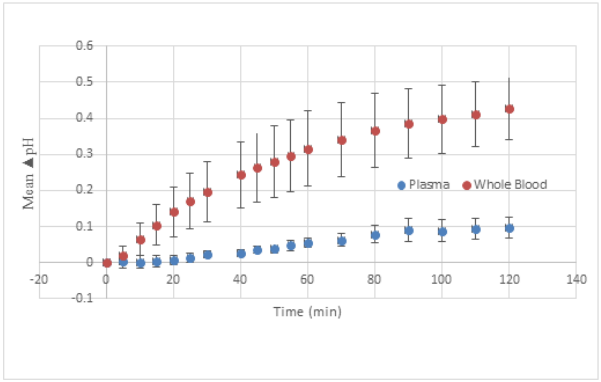

Figure 1: pH in plasma and whole blood specimens. The average pH change as function of time (min) of six plasma and four whole blood samples. As described under methods, data were normalized by subtracting first measured pH (measurement at time zero) from subsequent measurements. X-axis- time in minutes. Y-axis-mean pH change. The mean ± error is plotted as a function of time.

Oberleithner et al. [9] reported a drop in iCa concentration by 0.04 mmol/L per unit increase in pH in whole blood 7. In our study, the pH change for the first 80 min of measurements never exceeded 0.05 (twenty-fold decreased) and 0.1 (ten-fold decreased) throughout to affect any meaningful changes in the plasma iCa level. The iCa concentration were relatively constant and within the reference range of 4.4 mg/dL-5.4 mg/dL (1.06 mmol/L-1.35 mmol/L). The expected loss of dissolved CO_2 in plasma on exposure to air appears to be insignificant to alter any change in the pH and hence the iCa concentrations. On the other hand, pH increased on whole blood exposure specimen to air. In whole blood samples, carbonic anhydrase in respiring red cells CO_2 that is lost on sample exposure to air. The pH change observed in whole blood specimen (Figure 1) supports previous results that iCa concentration is pH dependent [1,8]. In reporting iCa concentration in such samples, it is important to include the pH to verify that the specimen was collected and handled properly.

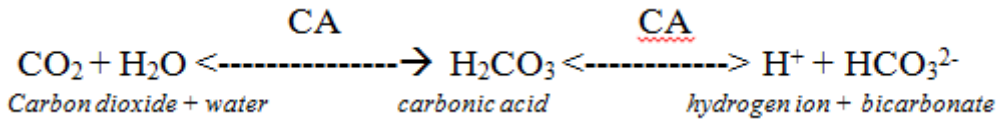

Figure 2: CO_2/HCO_3^{2-} equilibrium. The enzyme carbonic anhydrase (CA) in red cells catalyses the reversible interconversions of carbon dioxide and the ions of carbonic acid in red cells. In exposed whole blood samples, CO_2 loss shifts equilibrium toward the left-decreasing proton concentration and increasing specimen pH.

We have shown that iCa concentration does not change significantly for almost an hour and half when specimen are exposed to air. Similar findings had been reported for sealed samples stored under anaerobic conditions for several days. The stable iCa concentration in exposed samples corroborate with the minimal changes in the pH measurement of specimens over the same time. Previously reported results show no significant effect on total CO_2 concentration when serum specimen were exposure to room air for up to 2 h [10,15].

Because changes in iCa concentration is pH dependent in whole blood, our findings suggest that the rate of loss of carbon dioxide on centrifuged specimen (plasma) exposed to air is not significant to cause a significant change in the pH and hence the iCa concentration. Blood pH is influenced by the amount of dissolved CO_2. On exposure, whole blood pH increases as the CO_2 produced by the respiring cells, and catalyzed by carbonic anhydrase is loss to the environment.

In plasma, there was a minimal alteration in pH and the iCa concentration. Jafri et al. [10] reported minimal pH changes in serum and plasma samples exposed to air. Plasma plays very little role in buffering blood pH [12] because of the small fraction, less than 5% of dissolved CO_2 and the complete absence of carbonic anhydrase in plasma. Therefore, once the whole blood sample is centrifuged, iCa concentration is stable even under aerobic conditions.

While it is imperative to maintain the standard operating protocol for specimen processing for iCa, it is equally important to know that premature rejection of centrifuged sample that is accidently exposed to air would delay treatment and increase cost. The demonstrated stability in plasma iCa level in exposed samples for up to eighty minutes should minimized rejection. Besides treatment delay and increased cost, unnecessary rejection exposes patients to more pain from redraws, risks of infection and/or hematoma at the injection site.

Acknowledgement

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