



Extra-Analytical Quality Control of Serum Total Calcium (Collection of Blood in Supine State and Correction Using Serum Albumin Levels)

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Abstract

This study aimed to verify two protocols used to avoid pre-analytical errors of serum total calcium results: collecting the blood samples after supine status, and correct the result of serum total calcium if the albumin level is fall below 40g/l. The effect of supine for half an hour and correction of final result using serum albumin concentration in serum total calcium was determined by collecting 5 ml of blood 0 hr again after half an hour. Weight and height were measured to calculate the body max index. Blood samples were collected from 101 healthy normal individuals, 102 known diabetic patients, and 100 known hypertensive patients. Serum samples were analyzed for total calcium and albumin levels by automated chemical analyzer. Repeated measures ANOVA followed by Tukey's test was performed to compare between control and test values, and P value was considered significant if it was less than 0.05. the results shows that the serum calcium levels were significantly different after ½ hr supine and ½ hr supine with correction compared to 0 hr uncorrected and corrected calcium levels.

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The results were similar overall and in groups that consisted of normal population, diabetics or hypertensive. The results were also similar in different weight groups as per body mass index. As a conclusion we can say that the supine state of the patient for ½ hr alters the serum Ca⁺⁺ levels significantly compared to 0 hr values.

Keywords: extra-analytical; total calcium; supine; pre-analytical errors.

1. Introduction

Calcium is an important mineral required for bone formation, neurotransmission, contraction of myocardial and skeletal muscles, and blood clotting. Serum total calcium levels are used to diagnose osteoporosis, renal failure, rickets, parathyroid disorders, malignancy and to determine growth in children [1].

Extra-analytical quality control refers to steps taken for preventing errors during the pre-analytical and post-analytical phases of the total analytical process [2]. It is well known that most of the errors in biomedical analysis occur during the pre-analytical and post-analytical phases [3]. One of the steps in the pre-analytical phase is patient preparation. For determination of total serum calcium levels, the patient is kept in supine position for half an hour. The post-analytical quality control for serum total calcium level involves while verifying, validating, and correcting the results by simultaneously measuring serum albumin levels.

In the circulation, about 45% of calcium is protein bound (35% to albumin, 10% to globulins) and the remainder is in the filterable fraction, of which about 48% is ionized and the rest is bound to anions such as phosphate, citrate or carbonate. About 250 mM of calcium is filtered at the glomeruli each day and over 95% is reabsorbed, leaving less than 7.5 mMol for excretion in urine [4]. Calcium results need correction because of unstable serum albumin levels due to physiological and pathological factors [5].

The patient's posture is known to affect results of some biochemical parameters while many other laboratories are not affected significantly. The biochemical tests that are most affected by posture are proteins such as enzymes, albumin, and globulins and protein-bound substances such as triglycerides, cholesterol, calcium, and iron [3].

The present study was aimed to determine the influence of posture on serum total calcium levels after application of correction factor by simultaneous estimation of serum albumin levels.

2. Materials and Methods

A hospital based case control, cross sectional study was conducted in Khartoum state (Sudan). Samples were collected at different hospitals that included Jabir Abu Alizz diabetes center, Omdurman hospital, Alsarorab hospital, and Aljazeera Islang hospital. The study was conducted between June, 2011 and July, 2013.

Volunteers were randomly selected and informed consent was obtained from the all the volunteers. The study was approved by the ethical committee of Omdurman Islamic University. Medication and disease history was obtained from all the participants and individuals taking drug(s) that is known to influence serum calcium levels

or those suffering from disease known to affect serum total calcium levels and/or serum albumin levels were excluded from the study. Similarly individuals less than 15 years of age and those above 65 years of age were excluded from the study.

A total of 303 samples were collected, 104 samples from males and 199 samples from females. The study populations were aged between 17-63 years at the time of study. The subjects were divided into 3 groups: The first group consisted of 101 normal healthy volunteers; the second group included 102 known diabetic patients while the last group had 100 known hypertensive patients.

Two blood samples were collected from each of the participant. Five ml of blood sample was obtained at the beginning and the participant was asked to rest in supine position for 30 min. Then, another five ml blood samples was collected. Blood samples were obtained via standard venipuncture techniques [6] in SST gel tubes. Following collection, samples were left to clot and were centrifuged for 5 min at 2000 RPM and immediately the serum was transported in labeled Eppendorf tube with cap. Samples refrigerated (<4 °C) until analysis. The serum total calcium levels were determined by Red Arsenazo III method while bromocresol method was used for estimation of serum albumin levels.

The weight and height of the participants were recorded to determine the body mass index. The participants were classified based on BMI as underweight, normal, overweight and obese (Table 1).

Table 1: Criteria for classification of participants based on BMI [7]

<i>Categories</i>	<i>BMI</i>
Underweight	Less than 18.5
Normal weight	18.5 - 24.9
Overweight	25 - 29.9
Obese	30 or higher

The serum total calcium levels were corrected according to serum albumin levels by using the following formula [8]:

$$\text{Corrected Calcium (mg/dl)} = \text{Calcium (mg/dl)}_{\text{measured}} + 0.8 (4 - \text{Serum albumin g/dl})$$

Statistical analysis: All values are mean±SD. The changes in serum calcium level were analyzed using repeated measures ANOVA followed by Tukey’s multiple comparison test while the difference in serum albumin level was analyzed using one-way ANOVA followed by Tukey’s multiple comparison test. The statistical analysis was performed using GraphpadInstat (vesion 3.01, 32 bit for Win 95/NT). P value was considered significant if it was less than 0.05.

3. Results

The changes in serum calcium level after correction, supine position for ½ hr and supine position for ½ hr with

correction is shown in Table 2. The overall comparison of calcium level in all the subjects irrespective of diseases status revealed that the correction of calcium level at 0 hr did not produce any significant change in the results as compared to uncorrected results. Supine status for ½ hr produced a significant change in the both corrected and uncorrected serum calcium levels as compared to 0 hr uncorrected calcium levels and 0 hr corrected calcium levels. However, similar to 0 hr values, the correction of calcium level after supine status for ½ hr did not alter the values significantly as there was no significant difference between supine status uncorrected and corrected values (Table 3). Since the study population was screened for presence of diabetes and hypertension, the changes in serum calcium levels due to supine status and correction were also compared in normal, diabetic patients and hypertensive patients. The results were similar to overall results in diabetic and hypertensive patients while in the normal population, the value of corrected calcium levels at 0 hr was significantly different from uncorrected 0 hr value and also the value of corrected calcium levels at 1/2 hr was significantly different from uncorrected 1/2 hr value. The other result in normal population was similar to overall results and those observed in diabetic patients and hypertensive patients (Table 2). The serum albumin level was significantly less in diabetic population compared to normal population in the present study.

Table 2: Comparison of serum calcium levels at 0 hr and ½ hr supine (corrected and uncorrected) and serum albumin levels in different disease states.

Group	n	Serum Albumin level (g/dl)	Ca ⁺⁺ 0 Hr- Uncorrected (mg/dl)	Ca ⁺⁺ 0 Hr- Corrected (mg/dl)	Ca ⁺⁺ ½ Hr- Uncorrected (mg/dl)	Ca ⁺⁺ ½ Hr- Corrected (mg/dl)
Normal	101	4.18 ± 0.284	10.83 ± 0.522	10.68 ± 0.428 ^{a2}	10.29 ± 0.580 ^{a3,b3}	10.14±0.502 ^{a3,b3,c2}
Diabetic	102	4.00 ± 0.465 ^{***}	10.51 ± 0.872	10.49 ± 0.734	10.13 ± 0.739 ^{a2,b2}	10.14 ± 0.771 ^{a2,b2}
Hypertensive	100	4.11 ± 0.253	10.66 ± 0.468	10.58 ± 0.404	10.05 ± 0.591 ^{a3,b3}	9.97 ± 0.558 ^{a3,b3}
Overall	303	--	10.66 ± 0.657	10.58 ± 0.548	10.16 ± 0.647 ^{a3,b3}	10.08 ± 0.624 ^{a3,b3}

All values are mean±SD, ***P<0.001 compared to normal population group, ^{a2}P<0.01, ^{a3}P<0.001 compared to Ca⁺⁺ 0 hr-uncorrected values, ^{b2}P<0.01, ^{b3}P<0.01 compared to Ca⁺⁺ 0 hr-corrected values, ^{c3}P<0.01 compared to Ca⁺⁺½hr-uncorrected values.

In order to determine the influence of body weight on corrected and uncorrected calcium levels at 0 hr and ½ hr supine status, comparison were made between underweight, normal, overweight and obese patients using the criteria for classification mentioned above. The results were similar to that observed with overall comparison of the patients indicating that body weight does not have any significant influence on correction or supine status of the calcium levels (Table 3).

4. Discussion

In this study, Ca⁺⁺ level was measured in serum samples collected directly after participant arrived to hospital (0 hr samples), and after 30 min after the participants rested in supine state (½ hr supine). The results were corrected with albumin levels.

Table 3: Comparison of serum calcium levels at 0 hr and ½ hr supine (corrected and uncorrected) and serum albumin levels in different weight groups.

Group	n	Serum Albumin level (g/dl)	Ca ⁺⁺ 0 Hr- Uncorrected (mg/dl)	Ca ⁺⁺ 0 Hr- Corrected (mg/dl)	Ca ⁺⁺ ½ Hr- Uncorrected (mg/dl)	Ca ⁺⁺ ½ Hr- Corrected (mg/dl)
Underweight	33	4.18 ± 0.268	10.70 ± 0.468	10.57 ± 0.318	10.19 ± 0.555 ^{a3,b3}	10.04 ± 0.429 ^{a3,b3}
Normal	129	4.10 ± 0.414	10.66 ± 0.624	10.58 ± 0.454	10.12 ± 0.657 ^{a2,b3}	10.04 ± 0.652 ^{a3,b3}
Overweight	92	4.11 ± 0.331	10.69 ± 0.812	10.58 ± 0.751	10.17 ± 0.733 ^{a3,b3}	10.08 ± 0.726 ^{a2,b3}
Obese	49	4.00 ± 0.264	10.59 ± 0.539	10.58 ± 0.457	10.20 ± 0.500 ^{a3,b3}	10.19 ± 0.423 ^{a3,b3}

The aim of this study was to measure the role of ½ hr supine and correction with albumin on the Ca⁺⁺ levels. All values are mean±SD, ^{a2}P<0.01, ^{a3}P<0.001 compared to Ca⁺⁺ 0 hr-uncorrected values, ^{b2}P<0.01, ^{b3}P<0.01 compared to Ca⁺⁺ 0 hr-corrected values.

The Ca⁺⁺ levels after ½ hr supine and ½ supine with correction were significantly different than those observed when the sample were collected immediately after the patients arrived at the hospital. However, the corrections did not produce any significant change in the Ca⁺⁺ levels either at 0 hr or after ½ hr supine in the present study, indicating that corrections may not be required for Ca⁺⁺ levels. The findings of the present study are unique and contradicts the popular belief that calcium correction is essential [9].

The effect of common chronic disease such as diabetes and hypertension were also studied. However, the findings were similar to those observed in normal population or overall in all the tested population irrespective of disease states [10]. Similarly, the body mass index (BMI) did not influence the Ca⁺⁺ level measurements significantly.

So, this study is highly recommended whenever calcium investigation is requested to involve the albumin test; furthermore, for laboratory professionals, physicians and phlebotomist, it is recommended that to let the patient in supine rest for 30 minutes before blood collection.

5. Conclusion

The Ca⁺⁺ levels after supine status for ½ hr is significantly different compared to that observed at 0 hr while the correction of Ca⁺⁺ levels using serum albumin levels does not alter the Ca⁺⁺ significantly.

Conflict Of Interest

We declare that we have no conflict of interest.

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