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ORIGINAL RESEARCH

Comparison between serum ionised, total and corrected calcium at different albumin concentrations

[1]Dr. B.Shameem, [2]Dr. D.Kalpana, [3]Dr. Niveditha Devi Rajendran, [4]Dr. V.G.Karpaghavalli

[1]Associate Professor, [2]Assistant Professor, [4]Professor, Department of Biochemistry, Government Medical College, Kallakurichi, India
[3]Assistant Professor, Department of Biochemistry, National Centre for Ageing, Madras Medical College, Chennai, India

Corresponding Author
Dr. B.Shameem
Associate Professor, Department of Biochemistry, Government Medical College, Kallakurichi, India

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ABSTRACT

Introduction: Calcium is the most abundant mineral of human body with highest content around 98% lying stably in bones in the form of hydroxyapatite. There are three types of calcium found in plasma or serum: free or ionized calcium, calcium attached to proteins, and complexed or chelated calcium, which is attached to a number of anions. All three types of calcium are regularly measured in laboratories to determine the overall calcium concentrations.This is because total calcium is influenced by albumin concentrations and other bound anions, but not the levels of free ionized calcium.In light of this, the current study was designed to compare serum ionized, total, and adjusted calcium at various albumin concentrations. **Methodology:** This study was done as an observational study. Serum samples submitted to clinical biochemistry laboratory over a period of 3 months were included in the study. Based on albumin levels they were divided into three groups.For calculating corrected calcium following formula was used: Corrected Serum Calcium (mg/dl)= Total serum calcium (mg/dl) + 0.8 [4- serum albumin (g/dl)]. **Results:** The mean age range of patients was 54.65±15.4 years. In our study group among 250 samples, 141 cases had normal albumin levels, 85 cases had hypoalbuminemia and rest 24 cases had hyperalbuminemia.In our study samples, total calcium showed a positive correlation with ionized calcium (r=0.416, p value= < 0.001) and corrected calcium(r=0.685, p value <0.001). **Conclusion:** Measuring free ionized calcium is crucial when precise calcium status is needed. However, using the ion-selective electrode approach to assess free ionized calcium is difficult and primarily restricted to critical settings like the intensive care unit. As an alternative, albumin-adjusted serum total calcium is frequently utilized in clinical practice and has been used as a stand-in for free ionized calcium.

Keywords: calcium, total, corrected, ionised, albumin

INTRODUCTION

Calcium is the most abundant mineral of human body with highest content around 98% lying stably in bones in the form of hydroxyapatite. The extracellular compartment, which is found in serum and interstitial fluid, contains the majority of the soluble fraction. The three types of this soluble fraction are as follows: Calcium ions attached to albumin, calcium in combination with organic anions like phosphate, bicarbonate, and citrate, and free calcium—the physiologically active form—are all involved in a wide range of metabolic and physiological processes. Serious effects on neurological, renal, cardiac, and gastrointestinal processes could result from any disturbance in total calcium levels.[1]

Calcium has a wide range of physiological significance. Its intracellular and extracellular functions are crucial, unique, and interconnected. Many physiological processes, such as muscle contraction, hormone production, glycogen metabolism, and cell division, are critically regulated by intracellular calcium. In addition to providing a consistent supply of calcium for intracellular usage, extracellular calcium is crucial for membrane integrity and coagulation.[2]

There are three types of calcium found in plasma or serum: 1) free or ionized calcium, 2) calcium attached to proteins (mostly albumin), and 3) complexed or chelated calcium, which is attached to a number of anions (phosphate, bicarbonate, sulfate, citrate, and lactate).[2,3]. The diffusible portion of calcium is made up of both complexed and ionized calcium. Because this component can cross biologic membranes, it may also be referred to as ultrafilterable calcium. Albumin

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binds around 90% of the protein-bound calcium, while other globulins bind the remaining 10%. The interpretation of total calcium is dependent on serum albumin and total protein**3** levels since around half of calcium is protein bound.[4]

Ionized calcium is the physiologically active form of serum calcium and is implicated in calcium homeostasis in both healthy individuals and patients with parathyroid abnormalities, according to experiments by Moore[5] and McLean and Hastings[6,7] Numerous formulas have been put forth to calculate the concentrations of ionized calcium [8,9]. In essence, these are empirical equations that are based on a correlation between an observed concentration of albumin or another substance [10] and the ionized calcium concentration as determined by the ion selective electrode method.

All three types of calcium are regularly measured in laboratories to determine the overall calcium concentrations. Assuming that the albumin concentration was normal, the corrected calcium concentration calculates the total concentration. These formulas are used in clinical labs to determine adjusted calcium levels. Some utilize total protein**1**, while others use the albumin level. The advent of ion-selective electrodes3 revolutionized the precision of ionized calcium measurement. Several investigations have found that when patients get transfusions of citrated blood, the direct measurement of ionized calcium.

There has always been discussion about which is a better method and more appropriate for clinical settings: the estimation of total calcium, calcium adjusted for albumin, or free ionized calcium measured by direct ISE. This is because calcium ions bound to albumin and organic anions can have varying values.

Due to their increased throughput, the majority of contemporary clinical laboratories employ the ortho-cresolphthaleincomplexone (oCPC) or Arsenazo III techniques, which are assessed by autoanalyzers, to report total calcium levels. Nonetheless, there is enough evidence in the literature to support the development of algorithms or formulae for adjusting calcium for varying levels of albumin, pH, mass action, etc.[11-15] with the free calcium to be calculated as 50% of these values. This is because total calcium is influenced by albumin concentrations and other bound anions, but not the levels of free ionized calcium.[16] Following that, equations for directly determining free calcium were also developed using Total Calcium, protein levels, albumin, pH, and other factors.[17,18]In light of this the current study was designed to compare serum ionized, total, and adjusted calcium at various albumin concentrations.

METHODOLOGY

This study was done as an observational study. Serum samples submitted to clinical biochemistry laboratory over a period of 3 months were included in the study. Only those samples were included that had pH in reference range and special care was taken to analyze the serum sample on autoanalyzer and ISE analyzer without significant delay. Based on albumin levels they were divided into three groups, i.e., a group with albumin less than reference range (hypoalbuminemia group), albumin in a reference range of 35–52 g/L (normoalbuminemia group) and albumin above reference range (hyperalbuminemia group).

Following analyses of the serum samples for albumin using the bromocresol green dye binding method and total calcium using the oCPC method on a XL 640autoanalyzer, the same samples were subjected to ionized free calcium potentiometry on a combiline direct ISE analyzer. To make them easier to utilize in formulas, the autoanalyzer's Total Calcium units were transformed from mg/dL to mmol/L using a conversion factor of 0.25 and albumin from g/dL to g/L using a conversion factor of 10. According to the direct ISE analyzer, the free ionized calcium was already expressed in mmol/L. For calculating corrected calcium following formula was used: Corrected Serum Calcium(mg/dl)= Total serum calcium (mg/dl) + 0.8 [4- serum albumin (g/dl)]

RESULTS

This study was done as an observational study. Serum samples submitted to clinical biochemistry laboratory over a period of 3 months were included in the study. The mean age range of patients was 54.65±15.4 years. In our study group among 250 samples, 141 cases had normal albumin levels, 85 cases had hypoalbuminemia and rest 24 cases had hyperalbuminemia. It has been noted that computed free calcium derived from formulas differs significantly from measured free calcium levels when all samples are taken into account.

A similar pattern emerged in patients with hypoalbuminemia when various subgroups are taken into account according to their albumin concentrations. However, there was no discernible difference between the estimated free calcium and the free calcium measured by direct ISE in patients whose albumin levels were within the normal range. The calculated and actual levels of free calcium in the hyperalbuminemia group differed significantly.

Table 1: Results of measured and corrected parameters in different groups

	Total Calcium measured (mmol/L)	Albumin (g/L)	Total Calcium corrected (mmol/L)	Free ionized Calcium (mmol/L)	P value
All patients	2.15±0.25	30.19±10.5	2.21±0.25	1.09±0.15	0.016*
Hypoalbuminemia	1.87±0.23	22.57±6.8	2.08±0.21	1.08±0.13	0.016*

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Normoalbuminemia	2.20±0.17	40.25±5.35	2.09±0.11	1.11±0.12	0.930
Hyperalbuminemia	2.35±0.15	49.63±1.89	2.04±0.15	1.02±0.06	0.001*

In our study samples, total calcium showed a positive correlation with ionized calcium (r=0.416, p value= < 0.001) and corrected calcium(r=.685, p value <0.001).

DISCUSSION

According to clinical research, in conditions including acute acid-base disturbances, dysproteinemia, hyperparathyroidism, renal stones, and renal insufficiency, free calcium is a more accurate indication of calcium metabolic abnormalities than total calcium[14].

The most widely used method for measuring total calcium globally is spectrophotometry because it is readily available, inexpensive, and resistant to changing storage and transportation conditions. It is now known, nevertheless, that the important and physiologically active portion of total calcium is free calcium. For the treatment of critically ill patients with problems of calcium metabolism, it is crucial to comprehend the necessity of estimating free ionized calcium, particularly in the context of cardiac or renal diseases. Formulae for determining free calcium from measured total calcium after adjusting for significant variables such albumin content were developed in response to this necessity.

Our findings demonstrate a considerable difference between measured and predicted free calcium, both in the hypoalbuminemia group and when all samples are taken into account. Although there have been prior instances of estimated free calcium values that do not match measured free calcium and albumin-adjusted calcium, they are still in use today[19,20]. For the treatment of critically ill patients with problems of calcium metabolism, it is crucial to comprehend the necessity of estimating free calcium, particularly in the context of cardiac or renal diseases.

Furthermore, there are some inherent problems with these formulae, such as the fact that they do not account for all the variables that influence the complex calcium equilibria, that variations in the analytical parameters used in the formulae have an impact on the calculations, and that different reference ranges for the same parameters also have an impact on the results. In one such investigation, even though the identical formula was used, the results of the BCG or bromocresol purple methods for determining albumin were noticeably different.[21]

CONCLUSION

The most often requested test for determining calcium status is the measurement of serum total calcium. However, because it binds to albumin, it is important to interpret calcium levels carefully because circumstances that change albumin concentration can affect total serum calcium levels but not ionized calcium levels. Measuring free ionized calcium is crucial when precise calcium status is needed. However, using the ion-selective electrode approach to assess free ionized calcium is difficult and primarily restricted to critical settings like the intensive care unit. As an alternative, albumin-adjusted serum total calcium is frequently utilized in clinical practice and has been used as a stand-in for free ionized calcium.Direct measurement of free calcium by direct ISE seems to be the better alternative despite its cost, low throughput and the necessity to maintain stringent anaerobic conditions while measurement.

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