Annals of Clinical and Analytical Medicine
Original Research

Relationship between free calcium and adjusted calcium for albumin in patients with hypoalbuminemia

Calcium measurement methods in hypoalbuminemia

Esma Özdemir Anayurt, Yasemin Erdoğan Döventaş, İbrahim Yılmaz, Macit Koldaş
Department of Medical Biochemistry, Faculty of Medicine, Ministry of Health University, Haseki Training and Research Hospital, Istanbul, Turkey

Abstract

Aim: Measurement of total blood calcium is an important test that reflects bone and mineral metabolism. Total calcium is adjusted for albumin in many laboratories. Besides, it should be taken into consideration that adjusting the total calcium for albumin may cause an inaccurate evaluation of free calcium. This study aims to investigate the necessity of adjusted calcium used in biochemistry laboratories and whether ionized calcium measurement provides more benefit in determining the amount of active calcium in the body.

Material and Methods: We retrospectively selected 2092 samples with simultaneous measurements of free calcium, total calcium, albumin, total protein, and pH from the laboratory information system between January 2020 and June 2021. Samples with pH values outside the reference range of (7.35-7.45) and albumin values above the 4 g/dL were not included. In this study, the correlation between corrected total calcium calculated by Payne formula (usually expressed as albumin-adjusted calcium (mg/dL) =total calcium (mg/dL) + [4-albumin (g/dL)]. 0.8) and total calcium levels, total calcium and free calcium level were evaluated in hypoalbuminemia (<4 g/dL).

Results: Moderate relationship was found between free calcium and total calcium (r:0,48). The adjusted calcium values calculated with the Payne formula do not reflect free calcium (r= 0,47). A significant correlation was found between adjusted calcium and total calcium (r:0.72).

Discussion: Adjusted calcium application does not show a free calcium level. With the widespread use of free calcium measurement by most laboratories, the adjusted calcium application should be abandoned.

Keywords

Hypoalbuminemia, Total Calcium, Corrected Calcium, Lonized Calcium

DOI: 10.4328/ACAM.21266 Received: 2022-06-20 Accepted: 2022-09-28 Published Online: 2022-1011
Corresponding Author: Yasemin Erdoğan Döventaş, Department of Medical Biochemistry, Ministry of Health University, Haseki Training and Research Hospital, Istanbul, Turkey.
E-mail: yasemined@gmail.com P: +90 532 548 59 51
Corresponding Author ORCID ID: https://orcid.org/0000-0003-3166-841X

Introduction

Calcium is the fifth most abundant element in the human body. It plays a key role in skeletal mineralization, as well as a wide range of biological functions. Calcium is an essential element that comes into the body only through dietary sources. Calcium requirement is dependent on the state of calcium metabolism, which is regulated by three main mechanisms: intestinal absorption, renal reabsorption, and bone turnover. These in turn are regulated by a set of interacting hormones, including parathyroid hormone (PTH), 1,25-dihydroxy vitamin D [1,25(OH)2D], free calcium itself, and their corresponding receptors in the gut, kidney, and bone [1].

In serum, calcium exists in 3 forms: protein-bound, ionized (free), and complexed (chelated). Protein-bound calcium, which accounts for 40% of serum calcium, cannot be used by tissues. Albumin and globulin are the primary calcium-binding proteins in the serum whereas calmodulin is the primary calcium-binding protein in the cell. Chelated calcium, which accounts for 9% of the serum calcium, allows calcium to be absorbed by various tissues or carried between parts of the body. Serum calcium is often chelated into the ionic complexes of calcium phosphate, calcium carbonate, and calcium oxalate. Finally, free calcium, which makes up 51% of serum calcium, is utilized by the body to maintain physiologic functions [2].

Free calcium measurements in arterial blood collection are affected by many preanalytical factors such as sample collection, storage, transportation, pH changes, hemolysis, calcium-binding by the anticoagulant and sample dilution by the anticoagulant solution [3].

In addition, total calcium measurement is affected by albumin-bound calcium, pH, protein, and especially albumin levels. Due to such preanalytical and biological variables, total calcium levels are corrected for albumin. The most widely used correction formula was proposed by Payne in 1973 [4].

No commercial vendors currently offer a largely automated instrument for the measurement of ionized free calcium [5], and blood gas analyzers are widely used for the measurement of ionized free calcium using the direct-selective electrode (ISE) method. Many authors claim that the measurement of free ionized calcium should be preferred to the albumin-adjusted total calcium based on formulas from the literature [6-,9]. However, if an albumin adjustment is to be made, each laboratory should establish its formula [5-9].

Material and Methods

Patient Population

Data were retrospectively collected from Haseki Training and Research Hospital laboratory information system from January 2020 to June 2021 (n=2092). Simultaneous venous blood collection was performed for serum total protein, albumin, and total calcium measurements, and arterial blood collection was performed for ionized free calcium and pH measurements. Patients with albumin above 4 g/dL and pH other than 7.35-7.45 were excluded. This study was approved by the Health Sciences University Istanbul Haseki Training and Research Hospital Ethics Committee with decision number 252.

Laboratory Measurement Data

The serum albumin, total protein, and total calcium were determined on BeckmanCoulterAU5800 (Beckman Coulter Inc., Brea CA, USA) from January 2020 to November 2020. Rochec701/Cobas 8000 (Roche Diagnostic, Mannheim, (Germany) was used for serum albumin, total protein, and total calcium from November 2020 to June 2021. The Bromocresol green method was used for albumin, the Bıuret Method was used for total protein, and serum total calcium was tested with the NM-BAPTA method on Rochec701/Cobas 8000® analyzer and tested with the Arsenazo method on Beckman Coulter AU5800. The concentration of free calcium was measured using an ion-selective electrode in an automated blood gas analyzer (Rapidlab 1265, Siemens, Germany). To apply Payne's formula, albumin values were converted to g/dL (g/Lx1/10=g/dL) and ionized calcium values to mg/dL (mmol/Lx4=mg/dL). Standard internal and external quality control procedures were followed for all analytical methods.

Statistical Analysis

Statistical analyzes were performed with the SPSS 22.0 program. The mean and standard deviation values of the data were calculated. Spearman's Correlation analysis was performed for the data that did not fit the normal distribution with the Shapiro-Wilk test. The statistical significance limit was accepted as $p<0.005$.

Results

Data of 2092 (female 42.3% and male 57.7%) patients whose total albumin level was below 4 g/dL were analyzed. The mean age was 49.9±12.5 years. The mean and standard deviation values of total calcium, ionized calcium, total protein, albumin, and adjusted calcium are given in Table 1. Of the 2092 patients with albumin values below 4 g/dL, the number of patients with ionized calcium values below 1 mmol/L was 686 (14.3%), and the number of patients with total calcium values below 8 mg/dL was 551 (11.5%). In 76.9% of patients with ionized calcium values <1 mmol/L, albumin values were below 3.5 g/dL. The number of patients with hypocalcemia according to both ionized calcium and total calcium values was observed as 260 (12.4%). As shown in figures 1 and 2, the correlation between total calcium and adjusted calcium is significant. The linear regression equation between the two methods was 'Adjusted calcium = 0.657 X Total calcium + 3.57'. The adjusted total calcium value is significantly higher than the measured total calcium value. A moderate relationship was found between free calcium and total calcium (r:0,48). The adjusted calcium values calculated with the Payne formula do not reflect free calcium (r:0,47).

Table 1. Total protein, total calcium, adjusted calcium, free calcium, m and albumin levels

	Mean±SD (n :2092)
Total protein (g/L)	60.5±9.2
Total calcium (mg/dL)	8.4±0.7
Adjusted calcium (mg/dL)	9.11±0.58
Free calcium (mmol/L)	1.02±0.11
Albumin (g/dL)	3.13±0.54

SD: standard deviation

Discussion

Measurement of total calcium is the most commonly used technique worldwide owing to its ready availability, low cost, and resistance to variable transport and storage conditions. It is thus very important to understand the need of estimating free ionized calcium for the management of critically ill patients with disorders of calcium metabolism, especially in settings of cardiac or renal ailments [10].

Changes in albumin levels do not affect free calcium levels. Since calcium has less available albumin for binding, hypoalbuminemia should theoretically decrease the amount of bound calcium and lead to a decreased reported total calcium. Therefore, a patient's total calcium level may appear low even though free calcium is normal, which can lead to an incorrect diagnosis of hypocalcemia or an overestimation of the extent of existing hypocalcemia.This reason gave rise to formulae for adjusting total calcium for albumin concentration, and there are several formulas in the literature to adjust total calcium levels for albumin [4,11- 15].

In this study, we evaluated the Payne formula [4] for the correction of total calcium and the utility of ionized free calcium measurements in clinical laboratory practice. In our study, in low albumin concentration (albumin <4 g/dL) the correlation between ionized free calcium and total calcium, and adjusted total calcium were moderate agreement. On the contrary total calcium and adjusted calcium showed better agreement. Therefore we propose that this adjustment formula should not be applied.

While the calculation of corrected calcium should theoretically provide a more accurate estimate of physiologically active free calcium in patients with hypoalbuminemia the commonly used correction equations become less accurate as hypoalbuminemia worsens. For example, Payne's assumption results in an overestimation of the total serum calcium after correction as compared to the free calcium.

Some authors suggest that their local formulas have better performance than formulas from the literature to predict total calcium concentration and also suggest that albumin modification of total calcium determinations should be abandoned in laboratories still using this practice [8, 9,16].

With the widespread use of ionized free calcium measurement in blood gas analyzers with the direction-selective electrode method in clinical laboratories, free ionized calcium should be measured instead of corrected calcium measurement, especially in chronic hemodialysis patients, patients with hyperparathyroidism, critical patients in intensive care units and patients with paraproteinemia such as multiple myeloma who require close monitoring of calcium metabolism [6,17-20].

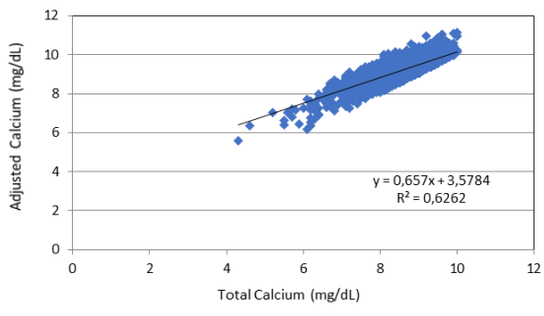


Figure 1. Linear Regression Chart Between Total Calcium and Adjusted Calcium

Limitations

One limitation of our study is that, first, there was a change in the biochemistry autoanalyzer used in the laboratory at the time of the study. Therefore, there were changes in reference values in serum albumin as well as calcium measurements and method differences for calcium. For calcium, the Arsenazo method was used in the Beckman Coulter autoanalyzer and the NM-BAPTA method was used in the Roche autoanalyzer. However, a recent study showed a good correlation between these methods [21].

Second, we could not collect creatinine, eGFR, parathyroid hormone, and electrolyte measurements because we evaluated the data retrospectively.

Since it is a retrospective study, the sources of preanalytical errors that may occur during the sampling of the samples have been ignored.

Conclusion

The commonly used correction formula Payne resulted in low levels of agreement with free ionized calcium and adjusted total calcium. Free ionized calcium measurements are frequently used in clinical biochemistry laboratories, despite the cost of direct ion-selective electrode measurement. We agreed that free ionized calcium measurements will be more accurate, especially in patients who require close monitoring of calcium, and correction formulas should be abandoned.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Funding: *None*

Conflict of interest

The authors declare no conflicts of interest.

References

1. Peacock M. Calcium metabolism in health and disease. Clin J Am Soc Nephrol. 2010;5 (Suppl 1):S23-30.

2. Yu E, Sharma S. Physiology, Calcium. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2021.

3. Baird G. Preanalytical considerations in blood gas analysis. Biochem Med (Zagreb). 2013;23(1):19-27.

4. Payne RB, Little AJ, Williams RB, Milner JR. Interpretation of serum calcium in patients with abnormal serum proteins. Br Med J. 1973;4(5893):643-6. DOI:10.1136/bmj.4.5893.643.

5. Baird GS. Ionized calcium. Clin Chim Acta. 2011;412(9-10):696-701.

6. Jain A, Bhayana S, Vlasschaert M, House A. A formula to predict corrected calcium in haemodialysis patients. Nephrol Dial Transplant. 2008;23(9):2884-88.

7. Lian IA, Åsberg A. Should total calcium be adjusted for albumin? A retrospective observational study of laboratory data from central Norway. BMJ Open. 2018;8(4):e017703.

8. Pekar JD, Grzych G, Durand G, Haas J, Lionet A, Brousseau T, et al. Calcium state estimation by total calcium: the evidence to end the never-ending story. Clin Chem Lab Med. 2020;58(2):222-31.

9. Ridefelt P, Helmersson-Karlqvist J. Albumin adjustment of total calcium does not improve the estimation of calcium status. Scand J Clin Lab Invest. 2017;77(6):442-7.

10. Mir AA, Goyal B, Datta SK, Ikkurthi S, Pal A. Comparison Between Measured and Calculated Free Calcium Values at Different Serum Albumin Concentrations. J Lab Physicians. 2016;8(2):71-6.

11. Berry EM, Gupta MM, Turner SJ, Burns RR. Variation in plasma calcium with induced changes in plasma specific gravity, total protein, and albumin. Br Med J. 1973;4(5893):640-3.
12. Clase CM, Norman GL, Beecroft ML, Churchill DN. Albumin-corrected calcium and ionized calcium in stable haemodialysis patients. Nephrol Dial Transplant. 2000;15(11):1841-46.
13. Gardner MD, Dryburgh FJ, Fyffe JA, Jenkins AS. Predictive value of derived calcium figures based on the measurement of ionised calcium. Ann Clin Biochem. 1981;18 (Pt 2):106-9.
14. Marshall RW, Hodgkinson A. Calculation of plasma ionised calcium from total calcium, proteins and pH: comparison with measured values. Clin Chim Acta. 1983;127(2):305-10.
15. Orrell DH. Albumin as an aid to the interpretation of serum calcium. Clin Chim Acta. 1971;35(2):483-9.
16. Jain A, Bhayana S, Vlasschaert M, House A. A formula to predict corrected calcium in haemodialysis patients. Nephrol Dial Transplant. 2008;23(9):2884-88.
17. Buege MJ, Do B, Lee HC, Weber DM, Horowitz SB, Feng L, et al.. Corrected calcium versus ionized calcium measurements for identifying hypercalcemia in patients with multiple myeloma. Cancer Treat Res Commun. 2019;21:100159.
18. Shirakabe A, Kiuchi K, Kobayashi N, Okazaki H, Matsushita M, Shibata Y, et al. Importance of the Corrected Calcium Level in Patients With Acute Heart Failure Requiring Intensive Care. Circ Rep. 2020;3(1):44-54.
19. Yu Y, Yu J, Yao R, Wang P, Zhang Y, Xiao J, et al. Admission Serum Ionized and Total Calcium as New Predictors of Mortality in Patients with Cardiogenic Shock. Biomed Res Int. 2021;2021:6612276.
20. Byrnes MC, Huynh K, Helmer SD, Stevens C, Dort JM, Smith RS. A comparison of corrected serum calcium levels to ionized calcium levels among critically ill surgical patients. Am J Surg. 2005;189(3):310-14.
21. Bourguignon C, Dupuy AM, Coste T, Michel F, Cristol JP. Evaluation of NM-BAPTA method for plasma total calcium measurement on Cobas 8000®. Clin Biochem. 2014;47(7-8):636-9.

How to cite this article:
Esma Özdemir Anayurt, Yasemin Erdoğan Döventaş, İbrahim Yılmaz, Macit Koldaş. Relationship between free calcium and adjusted calcium for albumin in patients with hypoalbuminemia. *Ann Clin Anal Med 2022; DOI: 10.4328/ACAM.21266*