RESEARCH ARTICLE

ALBUMIN-ADJUSTED CALCIUM: ARE PREVIOUSLY PUBLISHED REGRESSION EQUATIONS RELIABLE FOR YOUR LABORATORY? – A PILOT STUDY

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ABSTRACT

Background: Most of the laboratories use previously published regression equations for estimation of calcium which may not fit for their population. So deriving locally a regression equation for albumin-adjusted calcium (Ca_{Ad}) is a mainstay to avoid population-based differences.

Aims & Objective: To derive regression equation for albumin-adjusted calcium in our laboratory and validate it for the local population.

Material and Methods: Total 575 normal healthy individuals of 35-65 years were included in the present study and were estimated for serum total calcium (CaT), ionized calcium (Ca^{2+}), and albumin. The linear regression equation for the binding of calcium and albumin was derived in a cohort of 450 normal healthy individuals of 35-65 years, and the albumin-adjusted calcium equation was internally validated in a separate cohort of 125 subjects. The performance of this equation was compared with a previously published equation: Ca_{Ad} (mmol/L) = CaT (mmol/L) + 0.02 (40 - [albumin] (g/L).

Results: The local adjustment equation obtained from the derivation subset was expressed by the relationship; Ca_{Ad} (mmol/L) = CaT (mmol/L) + 0.03 (37.33 – [albumin] (g/L)). The equation was internally validated with an adjusted r2 shrinkage value of 0.0009 in a validation subset. Bland-Altman plot showed statistically significant difference (Mean = 0.13 mmol/L) when both formulae were compared for the population.

Conclusion: A locally derived and internally validated albumin-adjusted calcium equation differed significantly from previously published equations. Individual laboratories should determine their own linear albumin-adjusted regression equation for calcium rather than relying on published formulas.

KEY-WORDS: Serum Calcium; Total Protein; Corrected Calcium; Albumin; Albumin-adjusted Calcium

Introduction

Calcium is the fifth most common element, and the most prevalent cation, in the body.[1] Over 98% of total body calcium is present in bone, of which about 1% appears to be freely exchangeable with ECF through both physicochemical and cell-mediated mechanisms. Calcium circulates in the ECF in three distinct fractions: about 50% is the biologically important ionized fraction, 40% is protein-bound and is not filterable by the kidney, and 10% is complexed to small diffusible inorganic and organic anions such as bicarbonate, citrate, lactate, phosphate, and sulphate.[2] About 80% of protein-bound calcium is associated with albumin & the remaining 20% associated with globulins.[1]

Disorders that lower albumin also lower serum total calcium (CaT), but have a lesser effect on serum ionized calcium concentration (Ca^{2+}). In the seventies, numerous formulae were proposed to correct the serum calcium for the serum protein levels which aroused much controversy[3] and most of them studies suggested that adjusted serum total calcium (Ca_{Ad}) was a poor substitute for the direct measurement of serum Ca^{2+}. Several authors including Payne et al[4,5] used to calculate values of Ca^{2+} with inter-individual variations of Ca^{2+} corrected with a formula of individual correction and, while others preferred to measure serum Ca^{2+} directly.[3]

At present, Ca^{2+} level seems to be the best measure of serum calcium which is biologically active, and must be used whenever possible, but is based on a rigorous procedure which needs technician time. Numerous Indian laboratories do not have Ca^{2+} analysers, which are expensive. Even in foreign

countries like France most of the clinicians use the formula given by Payne et al.[4] Variation in serum albumin concentration therefore alters the concentration of serum total calcium, while the concentration of physiologically important ionized calcium remains constant.[1] Equations to adjust total calcium for albumin, such as the frequently cited Ca_{Ad} (mmol/L) = CaT (mmol/L) + 0.02 [40 – albumin (g/L)] are routinely used in clinical practice to give an estimate of calcium concentration in patients with hypoalbuminemia.[1-3]

To our knowledge, only a few studies in the nineties investigated the serum Ca^{2+} formulae correcting CaT and showed that the correlation between Ca^{2+} and CaT is weak, in both the general population and the elderly[6,7] and that the variability of serum Ca^{2+} increases with age.[8] These equations were derived in single laboratories over 20 years ago by determining the linear regression relationship of serum calcium on albumin concentration in patients deemed free of calcium disorders. Changes in laboratory analytic techniques, including a shift to the use of the bromocresol purple (BCP) albumin binding reagent, may influence the CaT to albumin relationship with other methods[4,5] and impact the assessment of calcium status when using these equations.

The aim of the present study was to investigate the relationship of CaT and Ca^{2+} with serum albumin and also to check whether the correction formulae in the previous studies are suitable for the Indian population. The purpose of this study was to derive and internally validate an albumin-adjusted calcium equation by applying linear regression to our own laboratory data for total calcium and serum albumin measurements. We hypothesized that a locally derived equation would differ from the previously published equations.

Materials and Methods

Participants

According to the inclusion criteria, normal healthy individuals of 35-65 years were selected & enrolled between 1st January 2009 and 31st March 2010 in the hospital. After taking informed written consent, 575 normal healthy individuals underwent the protocol for clinical examination previously approved by the Institutional Ethical Committee.

Exclusion criteria were: serum creatinine > 200 mmol/L, albumin < 20 g/L or > 50 g/L, total calcium > 3.0 mmol/L, or elevations in serum parathyroid hormone, alkaline phosphatase, or alanine transaminase above the reference range & persons receiving hemodialysis & with acid-base imbalances. Only the first simultaneous CaT and albumin results per patient during the study period were employed. The distribution of serum calcium concentrations was assessed graphically and found to follow a normal distribution.

The study cohort was divided randomly into ~75% derivation sample and ~25% validation sample. A simple (ordinary least squares) linear regression equation for the association between CaT and albumin was determined from the derivation sample[9-12], and the regression equation was then cross-validated in the validation sample.

Biochemical Analysis

Consecutive serum CaT and albumin measurements were determined by using instruments and reagents from a single vendor (Transasia Erba Diagnostics Ltd). Laboratory quality control was satisfactory during the study period and assured by use of multi-rule quality control procedures at three levels.[13] The selected normal healthy subjects were bled, taking 5 ml of whole blood in each case and the serum specimens obtained. The serum specimens were then analyzed for CaT, Ca^{2+} and albumin. O-cresolphthalein complexone method was used to determine CaT[14], ion selective electrode direct potentiometry for Ca^{2+}[15], and Bromocresol green (BCG) method for albumin[16].

Study Procedures

For the present study, biological measurements and analysis were standardized in order to determine Ca^{2+} in accordance with approved guidelines (NCCLS, 1995).[17] An anaerobic overnight fasting venous blood sample was drawn between 7–9 am in supine position. Ca^{2+} was measured within 1/2 hr after collection of blood in heparinized tube. The sample was kept at 4–8°C. Determination of Ca^{2+} was measured by ion

selective electrode direct potentiometry based Medica Easylyte electrolyte analyzer (Detection Range: 0.6 – 6 mmol/L at 6.0 – 8.0 pH units; Resolution: 0.01 mmol/L) (Reference range: 1.13 to 1.32 mmol/L), and was adjusted for pH 7.4 and 37 ± 0.1°C temperature.[18] Simultaneously, CaT with O-CPC method (Reference range: 2.10 to 2.55 mmol/L), and serum albumin level with nephelometric BCG method (Reference range: 37 to 53 g/L) were analyzed on ERBA Excel-300 fully automated random access chemistry analyzer. All the parameters under study were kept under strict quality control so that ascertain whether differences obtained between variables or groups are outside the critical differences for each method.

Among numerous previously published formulae for correcting CaT, the formula of Payne et al (1973) was selected for comparison: Ca_{Ad} (mmol/L) = CaT (mmol/L) + 0.025 x [40 – albumin; (g/L)]. Ca^{2+} being theoretically equal to 50% of Ca_{Ad}.[2]

In the present study, SI units have been used for global uniformity in the measurements and used 'mmol' instead of 'mg' assuring that the relationship obtained will solely be interrelated to the quantity of analytes rather than their weights.
Formula for interchanging units of calcium: Calcium (mmol/L) = Calcium (mg/dL) x 0.25

Statistical Analysis

All statistical analyses were accomplished with IBM SPSS Statistics version 20.0 (Released Aug 16, 2011) (IBM Corporation, Somers, New York).[19] Level of significance, $P < 0.05$ was taken significant. A simple (ordinary least squares) linear regression equation for the association between CaT and albumin was determined from the derivation sample[9-12], and the regression equation was then cross-validated in the validation sample in the following manner. First, the validity of the regression equation was examined by calculating the amount of shrinkage in the predictive power of the equation by McNemar Formula.[20] This was carried out by applying the regression equation to the validation sample to obtain a predicted calcium value for each subject. Measured calcium was then regressed on predicted calcium to obtain an estimate of the variance accounted for, in the validation sample. The adjusted r2 for the validation sample was subtracted from that of the derivation sample to arrive at an estimate of the amount of shrinkage, an indication of how much the predictive ability decreases when the equation is applied to other samples. If the shrinkage is small the regression is considered internally valid.[20] Moreover, in a sensitivity analysis a bootstrapping procedure was undertaken as an additional assessment of the internal validity. The Bootstrapping procedure conceptually involves copying samples of a data set on top of themselves infinitely creating a mega data file.[21] In Bootstrapping, A total of 1,000 re-samples were randomly drawn with replacement from the full sample. Analyses were then conducted on each new sample with regression parameters estimated for each sample including the original sample. Bootstrapping can provide regression coefficients, standard errors, and confidence intervals.[21] The mean agreement with 95% limits of agreement was assessed using a Bland-Altman plot. This technique plots the difference in calcium concentration between the two equations against the mean of the two values for each subject.[22]

Results

The cohort consisted of 575 subjects including 450 subjects in Derivation sample & 125 subjects in Validation sample. Table 1 & 2 describes the demographics of the derivation sample & its biological characteristics respectively. Statistically significant correlation was observed between CaT and serum albumin (Table 3; Figure 1), obtained from the derivation subset and was expressed by the regression equation: CaT (mmol/L) = 0.03 [albumin (g/L)] + 1.21.

The working form of the equation for Ca_{Ad} was derived as outlined in Appendix 1 and was expressed as: Ca_{Ad} (mmol/L) = CaT (mmol/L) + 0.03 (37.33 – [Albumin] (g/L)). Evidence of good internal validity was confirmed by an adjusted r2 shrinkage value of 0.0009 when the equation was applied to the validation subset. The Bootstrapping procedure also confirmed the validity of the derived equation.

The demographics of the derivation sample & its biological characteristics are designated by table 4

Table-1: Demographics of the Derivation Sample

	Male	Female	Total
No. of Subjects	288 (64%)	162 (36%)	450
Mean Age (yr)	44.23 ± 7.32	44.45 ± 7.67	44.31 ± 7.44

Table-2: Biological Characteristics of the Derivation Sample Subjects

Parameter	Mean ± SD	Range
S. Total Calcium (mmol/l)	2.70 ± 0.25	2.11 – 3.35
S. Ionized Calcium (mmol/l)	1.27 ± 0.13	1.11 – 1.80
Serum Albumin (g/l)	49.40 ± 8.07	31.40 – 69.90

Table-3: Correlation between Total Calcium and S. Albumin

Measured Parameters	Sr. Albumin (g/L)
Total Calcium (mmol/L)	r = 0.972, P < 0.001 (r = correlation coefficient)

Table-4: Demographics of the Validation Sample

	Male	Female	Total
No. of Subjects	75 (60%)	50 (40%)	125
Mean Age (yr)	43.76 ± 7.60	44.78 ± 7.37	44.17 ± 7.49

Table-5: Biological Characteristics of the Validation Sample Subjects

Parameter	Mean ± SD	Range
S. Total Calcium (mmol/l)	2.67 ± 0.26	2.12 – 3.25
S. Ionized Calcium (mmol/l)	1.23 ± 0.07	1.10 – 1.40
Serum Albumin (g/l)	49.19 ± 8.40	31.40 – 69.90

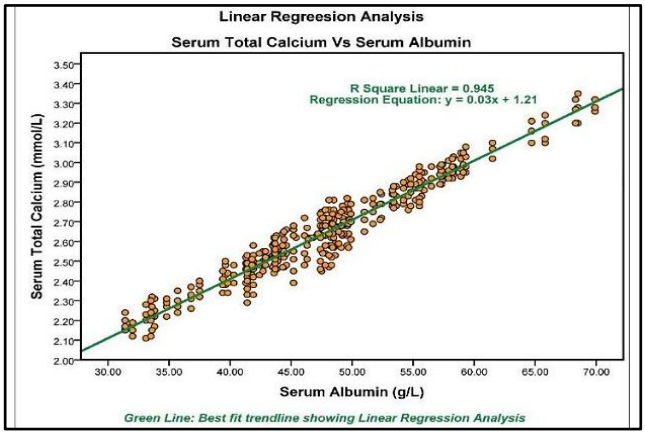

Figure-1: Locally derived Regression Equation for Serum Total Calcium

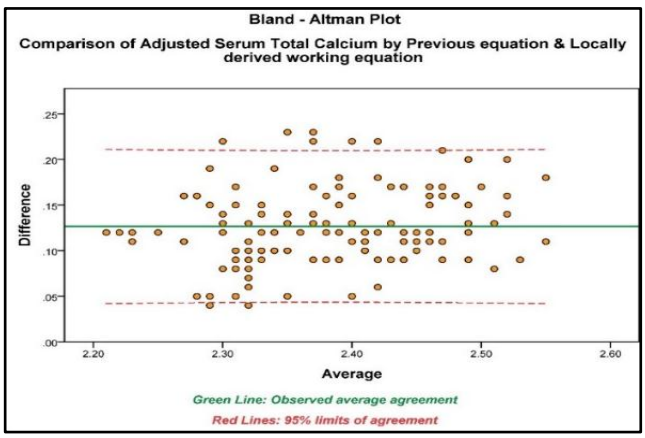

Figure-2: Comparison of Adj. Serum Total Calcium by Previous Equation & Locally derived Working Equation

& 5 respectively. To assess agreement between the derived and the previously published equation, we compared Ca_{Ad} results using the derived equation and the published equation. The Bland-Altman plot (see Figure 2) produced mean difference of 0.13 mmol/L in Ca_{Ad} concentration between the two formulae, 95% limits of agreement being 0.044 to 0.21.

Discussion

Hasting and McLean by their colossal experimental and clinical studies established unequivocally in the decade of 1930s that Ca^{2+} rather CaT is of primary physiological and clinical importance.[23-25] After these studies, numerous investigators have repeatedly confirmed and extended this crucial role of Ca^{2+} till date.[26-29] Despite of the well-established clinical significance of Ca^{2+}, even today the estimation of CaT is being used as a common initial test in the assessment of calcium status. Even if the measurement of Ca^{2+} would be ideal, it is more susceptible to measurement error due to the required precision in sampling techniques and so making it impractical to use as a screening test.[30] Thus in many outpatient scenarios, Ca_{Ad} prove necessary.

In the present study, we derived Ca_{Ad} equation using local laboratory data that differs from previously published equations, clearly indicating that the variations in protein-binding of calcium (mainly albumin) leads to deviations in the levels of serum total calcium.[31] While we did not pursue the reasons for the difference between the equations, a prospective explanation may be the racial differences between the populations and their dietary habits. Ashby et al noted a change in the regression coefficient for binding of calcium on albumin within a single laboratory following a change of analytical methodology.[32] Similarly, Barth et al observed differences in this relationship between laboratories using similar assays.[33] Changes in the formulation of the albumin binding BCG reagent have been supposed to be most accountable, arbitrated by differences in the degree to which the BCG reagent reacts with serum globulins.[33] In our study the BCG reagent was used for albumin measurement, alike all previous reported adjustment equations derived from work using the BCG reagent.[32-36]

Internal validation of the derived regression equation showed its suitability for the validation set of population. Bootstrapping procedure with the same results authenticated its applicability for the entire local population. This implies that the previously published equations available are not suitable for the population in different regions and unquestionably if dependent upon different method of albumin estimation.

There are some prophesied shortcomings to the present study like, first, the equation we report is applicable only over the albumin range studied (35–55 g/L), although this would include the majority of hypo-albuminemic population. Second, our equation cannot be generalized beyond our health region, given the inter-laboratory variation previously described. Furthermore, since the equation was derived in outpatient setting, its generality is delimited and cannot be made applicable to inpatient settings, reinforced by previous literature demonstrating poor reliability of this approach in the critically ill.[37]

Since, in the developing countries like India, the facilities for the direct estimation are not available for Ca^{2+} in most of the diagnostic scenarios, the locally derived & validated regression equation in a laboratory for Ca_{Ad} can prove to be an alternative for the same.

Conclusion

In conclusion, a locally derived and internally validated albumin-adjusted calcium equation differed from previously published equations. Our results illustrate that clinicians should be cautious applying a previously published albumin-adjusted calcium equation in modern settings with different analytical techniques. Using the methods described in this report, institutions must derive their own regression equations for adjusted calcium rather than relying upon existing published formulas.

References

1. Endres DB, Rude RK. Mineral and bone metabolism. In: Burtis CA, Ashwood ER, Bruns DE, editors. Tietz textbook of clinical chemistry and molecular diagnostics. 4th ed. New Delhi: Saunders Elsevier; 2006. p. 1892-905.
2. Bushinsky DA, Monk RD. Calcium. Lancet 1998;352:306-11.
3. Conceiaco SC, Weightman D, Smith PA, Luno J, Ward MK, Ker DNS. Serum ionised calcium concentration: measurement versus calculation. Br Med J 1978;1:1103-5.
4. Payne RB, Little AJ, Williams RB, Milner JR. Interpretation of serum calcium in patients with abnormal plasma proteins. Br Med J 1973;4:643-6.
5. Payne RB, Rowland KM, Phillips PJ, Duncan B, M. Lc L. Current ‘‘corrected’’ calcium concept challenged. Br Med J 1975;4:617-9.
6. Sorva A, Elfving S, Pohja P, Tilvis RS. Assessment of calcaemic status in geriatric hospital patients: serum ionized calcium versus albumin-adjusted total calcium. Scand J Clin Lab Invest 1988;48:489-94.
7. Sorva A, Elfving S, Sievers G, Tilvis RS. Calcemic status of geriatric patients: a longitudinal study. Gerontology 1992;38:87-91.
8. Watson JB, Lee K, Klein R, Klein BEK, Koch DD. Epidemiological evidence for the disruption of ionized calcium homeostasis in the elderly. J Clin Epidemiol 1997;50:845-9.
9. Payne R, Carver M, Morgan D. Interpretation of serum total calcium: effects of adjustment for albumin concentration on frequency of abnormal values and on detection of change in the individual. J of Clin Path 1979;32:56-60.
10. Ashby J, Wright D, Rinsler M. The adjusted serum calcium concept – a reappraisal. Ann Clin Biochem 1986;23:533-7.
11. Twomey P, Kroll M. How to use linear regression and correlation in quantitative method comparison studies. Int J Clin Pract 2008;62:529-38.
12. Barth J, Fiddy J, Payne R. Adjustment of serum total calcium for albumin concentration: effects of non-linearity and of regression differences between laboratories. Ann Clin Biochem 1996;33(Pt 1):55-8.
13. Westgard J, Barry P, Hunt M, Groth T. A multi-rule Shewhart chart for quality control in clinical chemistry. Clin Chem 1981;27:493-501.
14. Gitelman HJ. Determination of calcium using O-cresolphthalein complexone. Annal Biochem 1967;18:521.
15. D’Orazino P, Meyerhoff ME. Electrochemistry and chemical sensors. In: Tietz textbook of clinical chemistry and molecular diagnostics. 4th ed. Burtis CA, Ashwood ER, Bruns DE. New Delhi: Saunders Elsevier; 2006. p. 93-8.
16. Webster D, Bignell AH, Attwood EC. An assessment of the suitability of bromocresol green for the determination of serum albumin. Clin Chem Acta 1974;53:488-91.
17. NCCLS (National Committee for Clinical Laboratory Standards). 1995. Ionized calcium determinations: precollection variables, specimen choice, collection, and handling; approved guideline. Nccls 1995;15:1-19.
18. Ladenson JH. Clinical utility of ionized calcium. In: Moran RF, Galveston TX, editors. The ionized calcium: Its determination and clinical usefulness. Proceedings of an international symposium.

Electrolyte blood gas division of the American association for clinical chemistry 1986. p. 5-11.
19. IBM SPSS Statistics 20 [computer program]. Version 20.0.0. Somers (NY): IBM Corporation; 2012.
20. Pedhazur E. Multiple Regression in Behavioural Research. Harcourt Brace Jovanovich 1982.
21. Thompson B. The use of statistical significance tests in research: bootstrap and other alternatives. J Experimental Education 1993;61:361-77.
22. Altman DG, Bland JM. Measurement in Medicine. The Analysis of Method Comparison Studies. Statistician 1983;32:307-17.
23. McLean FC, Hastings AB. A biological method for the estimation of calcium ion concentration. J Biol Chem 1934;107:337-50.
24. McLean FC, Hastings AB. The state of calcium in the fluids of the body. The conditions affecting the ionization of calcium. J Biol Chem 1935;108: 285-322.
25. McLean FC, Hastings AB. Clinical estimation and significance of Ca++ ion concentration in the blood. Am J Med Sci 1935;189:601-13.
26. Robertson WC, Marshakk RW. Ionized calcium in body fluids. Crit Rev Clin Lab Sci 1981;15:85-125.
27. Ladenson JH, Lewis JW, McDonald JM, Slatopoisky E, Boyd JC. Relationship of free and total calcium in hyperglycemic conditions. J Clin Endocrinol Metab 1979;48:393-7.
28. Ladenson JH, Bowers JRGN. Free calcium in serum II. Rigor homeostatic control, correlations with total serum calcium and review of data on patients with distributed calcium metabolism. Clin Chem 1973;19:575-82.
29. Toftaletti J. Ionized calcium through its first decade: A review of the analytical aspects and clinical importance of ionized calcium measurements. NOVA Biomedical 1986 (Available from NOVA Biomedical Inc., 200 propects St., Walthan, M.A. 02254-9141).
30. Payne R, Carver M, Morgan D: Interpretation of serum total calcium: effects of adjustment for albumin concentration on frequency of abnormal values and on detection of change in the individual. J of Clin Path 1979,32:56-60.
31. Sdd Rothman KJ, Greenland S: Modern Epidemiology. 2nd edition. Philadelphia: Lippincott-Raven Publishers; 1998.
32. Ashby J, Wright D, Rinsler M. The adjusted serum calcium concept – a reappraisal. Ann Clin Biochem 1986;23:533-7.
33. Barth J, Fiddy J, Payne R. Adjustment of serum total calcium for albumin concentration: effects of non-linearity and of regression differences between laboratories. Ann Clin Biochem 1996;33(Pt 1):55-8.
34. Orell D. Albumin as an aid to the interpretation of serum calcium. Clin Chim Acta 1971;35:483-9.
35. Berry E, Gupta M, Turner S, Burns R. Variations in plasma calcium with induced changes in plasma specific gravity, total protein and albumin. Br Med J 1973, 4:640-643.
36. Payne R, Carver M, Morgan D. Interpretation of serum total calcium: effects of adjustment for albumin concentration on frequency of abnormal values and on detection of change in the individual. J of Clin Path 1979, 32:56-60.
37. Slomp J, Voort P van der, Gerritsen R, Berk J, Bakker A. Albumin adjusted calcium is not suitable for diagnosis of hyper- and hypocalcemia in the critically ill. Crit Care Med 2003;31:1389-93.

Appendix 1: Derivation of Working Form of Albumin-adjusted Calcium Equation

Adjusted Ca	= Total Ca − (slope × [albumin]) + mean total Ca − intercept Ca
	= CaT − (0.03 × [albumin]) + 2.33 − 1.21
	= CaT − (0.03 × [albumin]) + 1.12
	= CaT + 1.12 − (0.03 × [albumin])
	= CaT + 0.03 (1.12/0.03 − [albumin])
Ca_{Ad} (mmol/L)	= CaT (mmol/L) + 0.03 − [37.33 − albumin (g/L)]

Adjusted r2 (Shrinkage): McNemar Formula:
Shrunken/Adjusted $R^2 = 1 - [(1 - R^2)*(N - 1)/(N - k - 1)]$
where N = Sample size, k = No. of independent variables or predictors

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