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

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DOI: 10.5455/ijmsph.2013.2.209-214  Received Date: 02.12.2012  Accepted Date: 03.12.2012

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 r^2 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. 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. About 80% of protein-bound calcium is associated with albumin & the remaining 20% associated with globulins.

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 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 al14,51 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.

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.\textsuperscript{[4]} Variation in serum albumin concentration therefore alters the concentration of serum total calcium, while the concentration of physiologically important ionized calcium remains constant.\textsuperscript{[1]} Equations to adjust total calcium for albumin, such as the frequently cited Ca\textsubscript{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.\textsuperscript{[1-3]}

To our knowledge, only a few studies in the nineties investigated the serum Ca\textsuperscript{2+} formulae correcting CaT and showed that the correlation between Ca\textsuperscript{2+} and CaT is weak, in both the general population and the elderly\textsuperscript{[6,7]} and that the variability of serum Ca\textsuperscript{2+} increases with age.\textsuperscript{[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\textsuperscript{[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\textsuperscript{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 1\textsuperscript{st} January 2009 and 31\textsuperscript{st} 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\textsuperscript{[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.\textsuperscript{[13]} The selected normal healthy subjects were bled, taking 5 ml of whole blood in each case and the serum specimens obtained. 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\textsuperscript{2+} and albumin. O-cresolphthalein complexone method was used to determine CaT\textsuperscript{[14]}, ion selective electrode direct potentiometry for Ca\textsuperscript{2+}\textsuperscript{[15]}, and Bromocresol green (BCG) method for albumin\textsuperscript{[16]}.

**Study Procedures**

For the present study, biological measurements and analysis were standardized in order to determine Ca\textsuperscript{2+} in accordance with approved guidelines (NCCLS, 1995).\textsuperscript{[17]} An anaerobic overnight fasting venous blood sample was drawn between 7–9 am in supine position. Ca\textsuperscript{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\textsuperscript{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. 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}.

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). Level of significance, P < 0.05 was taken significant. A simple (ordinary least squares) linear regression equation for the association between CaT and serum albumin was determined 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 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.
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<tr>
<th>Table-1: Demographics of the Derivation Sample</th>
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<tr>
<td>Male</td>
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<tr>
<td>No. of Subjects</td>
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<td>Mean Age (yr)</td>
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<th>Table-2: Biological Characteristics of the Derivation Sample Subjects</th>
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<tr>
<td>Parameter</td>
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<tr>
<td>S. Total Calcium (mmol/l)</td>
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<td>S. Ionized Calcium (mmol/l)</td>
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<td>Serum Albumin (g/l)</td>
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<th>Table-3: Correlation between Total Calcium and S. Albumin</th>
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<tr>
<td>Measured Parameters</td>
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<tr>
<td>Total Calcium (mmol/L)</td>
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<th>Table-4: Demographics of the Validation Sample</th>
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<tr>
<td>Male</td>
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<td>No. of Subjects</td>
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<th>Table-5: Biological Characteristics of the Validation Sample Subjects</th>
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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.[27]

Since, in the developing countries like India, the facilities for the direct estimation are not available for Ca²⁺ in most of the diagnostic scenarios, the locally derived & validated regression equation in a laboratory for Caₐd 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**


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

\[
\text{Adjusted Ca} = \text{Total Ca} - (\text{slope} \times \text{[albumin]}) + \text{mean total Ca} - \text{intercept Ca} \\
= \text{CaT} - (0.03 \times \text{[albumin]}) + 2.33 - 1.21 \\
= \text{CaT} - (0.03 \times \text{[albumin]}) + 1.12 \\
= \text{CaT} + 1.12 - (0.03 \times \text{[albumin]}) \\
= \text{CaT} + 0.03 (1.12/0.03 - \text{[albumin]}) \\
= \text{CaT} (\text{mmol/L}) + 0.03 (37.33 - \text{[albumin] (g/L)})
\]

**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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Source of Support: Nil
Conflict of interest: None declared