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ORIGINAL ARTICLE

Albumin adjusted calcium: Study in a tertiary care hospital

Zaki Anuar MOHD ARIFFIN*, Farhi Ain JAMALUDDIN

Department of Pathology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur Malaysia

Abstract

Introduction: One commonly used equation which continues to be widely mentioned in text books and hence familiar to clinical people is total calcium + 0.02 (40 – albumin). This equation was derived using cresophthalein complexone and bromocresol green (BCG) methods for measuring serum total calcium and serum albumin respectively. However this equation maybe invalid when applied to calcium and albumin results generated by alternative assays. Hence we aim to derive an albumin-adjusted calcium equation specific to our laboratory's total calcium and albumin methodologies. *Materials and Methods*: A total of 3,175 adult University Malaya Medical Centre (UMMC) patients deemed free of any calcium metabolism disorders were selected and divided into two groups for derivation and validation. Simple linear regression associating total calcium and albumin was constructed from the data in the derivation group. The new albumin-adjusted calcium equation was validated in the validation group. Differences in calcium status classification following adjustments based on existing and new albumin-adjusted calcium equation was compared in a 469 hypoalbuminaemic patients. *Result*: The new albumin adjusted calcium equation was: *total calcium + 0.014 x (39-albumin)*. Of the 469 hypoalbuminemic patients, 78 were classified differently based on new equation. Based on the new equation, 55 normocalcemic patients were classified as hypocalcemic and 22 were classified as normocalcemic instead of hyperclacaemic. *Conclusion***:** Based on the newly derived albumin-adjusted calcium equation 17% of patients had different adjusted calcium classifications. This could potentially impact in the management. It is recommended that laboratories derive equations specific to their calcium/albumin methods and analytical platforms.

Keywords: Total calcium, serum albumin, corrected calcium, albumin-adjusted calcium equation

INTRODUCTION

A healthy adult has approximately 25 mol of calcium in the body. More than 99% of this total body calcium can be found in the bone and the remainder is in the extracellular fluid (ECF) compartment.[1] Three organs (the intestines, the kidneys and the bones) under the influence of parathyroid hormone and active Vitamin D are involved in the homeostasis of ECF calcium. About 20% (10 mmol) of dietary calcium intake is absorbed by the intestines and the rest is lost through faecal excretion. About 5 mmol of calcium per day is secreted in the intestine, hence the net calcium absorption is about 5 mmol per day. The kidneys excrete 5 mmol of calcium per day and generally, in a healthy individual, if the dietary intake and intestinal absorption of calcium is normal, the kidney excretion of calcium is equal to the net intestinal absorption of calcium.[1] Bone formation and remodelling are balanced processes resulting in no net change in ECF calcium proportion.[2]

ECF calcium is distributed in three forms. Firstly, the biologically active form of calcium is in its free ionised form which accounts for 50% of ECF calcium and maintaining a normal free ionised calcium level is critical for proper functioning of the organs as calcium involved in a variety of key cellular actions such as neuromuscular activity, hormone release and action, enzyme regulation, modulation of cell membrane permeability and it is also involved in the clotting process. Secondly, a major fraction (about 40%) of calcium is bound to proteins principally to albumin and the remaining is complexed to other anions such as phosphate or bicarbonate.[3] Many conditions can lead to the disorders of calcium metabolism and therefore hypocalcemia and hypercalcemia are common

**Address for correspondence:* Zaki Anuar Bin Mohd Ariffin, Department of Pathology, Faculty of Medicine, University of Malaya, 50603, Kuala Lumpur. Malaysia. Tel: +60379492025. Fax: +60379492818. Email: *zaki.anuar@ummc.edu.my*

especially in hospitalised patients.[4] Primary hyperparathyroidism and malignancy are the two most common causes of hypercalcaemia.[5] Other rare causes of hypercalcemia are hyperthyroidism, sarcoidosis and tuberculosis. Medications such as thiazide, lithium and vitamin D taken in excess can also cause hypercalcemia.[6] On the other hand, hypocalcemia is common in chronic kidney disease patients due to low albumin level as well as the inability of the kidney to produce adequate calcitriol for dietary calcium absorption in the intestine as well. Vitamin D deficiency and hypoparathyroidism due to congenital defect or inadvertent destruction of the parathyroid gland during neck surgery are also some of the causes of hypocalcemia.[7]

Measurement of serum total calcium is the most common test requested for assessment of calcium status.[8] However, due to its albumin binding nature, calcium level interpretation has to be done cautiously as conditions that alter albumin concentration can affect the total serum calcium level, but not the ionized calcium level.[9,10] Where accurate calcium status in required, it is important to measure free ionised calcium.[9] But measurement of free ionised calcium by the ion-selective electrode method is cumbersome and its availability is mostly limited to the critical setting such as in the intensive care unit.[11] Alternatively, albumin-adjusted serum total calcium has been used as a surrogate for free ionised calcium and it is commonly used in clinical practice.[4] Commonly used adjusted calcium equation is: adjusted-calcium = total calcium + 0.02(40-albumin). This equation is used in our laboratory to report adjusted total calcium in patient with serum albumin level less than 35g/L.[12]

The method used for calcium measurement and the choice of reagents for albumin measurement are the indications to derive albumin-adjusted calcium equation that is specific to the methodologies and the resulting derived equation will be specific to the population served by the laboratory.[13] Davies *et al*. showed that even though changes in total calcium assays will affect the adjusted calcium equation, it may not necessarily of clinical significance but nevertheless the adjusted calcium equation should be reassessed following changes in the total calcium methodology.[14] On the contrary, the choice of albumin measurement reagent using the dye binding-technique of either bromocresol purple (BCP) or BCG has been shown to have a major impact on albumin-adjusted calcium level.[13] There is also difference in calcium status classification in hypoalbuminaemic patients based on locally derived adjusted calcium equation compared to established adjusted albumin equation as shown by James *et al*.[15]

Due to change in methodology for serum albumin measurement from BCG to BCP in our lab, it is necessary to derive adjusted calcium equation for this method. We expect that this locally derived adjusted calcium equation will be different from the existing adjustment equation being used leading to different calcium status classification in a number of our patients. Since we expect that the adjusted calcium calculated by the equation derived would be different, this might affect the calcium status classification of our patients.

MATERIALS AND METHODS

Results of serum calcium and albumin that were measured simultaneously during the period of 8th March to 7th October 2017 were extracted from the laboratory information system. Results of patients aged 18 – 65 years were included in this study. The results were from in-patients receiving treatment as well as out-patients attending the various outpatient clinics. We excluded results from patients with renal impairment when serum creatinine levels more than 200 μmol/L, hypomagnesaemia (with potassium less than 3.5 or more than 5.5 mmol/L used as surrogate marker), liver impairment with alanine aminotransferase (ALT) and alkaline phosphatase (ALP) more than the upper reference limit of 49 IU/L and 127 IU/L respectively and patients from oncology, endocrine and intensive care units.[8]

Biochemical analysis

Serum total calcium was measured by Arsenazo III dye binding method and serum albumin measurement was by BCP method. Both serum total calcium and serum albumin were measured on ADVIA® 2400 Clinical Chemistry System (Siemens Healthineers, Germany). Quality control (QC) for serum total calcium and albumin were observed using the Westgard multi-rules QC plan with two level controls measured in each run respectively.

Study Procedure

Study participants were divided randomly into two equal sizes as derivation and validation groups. An equation associating total calcium and albumin was obtained by linear regression analysis from the data of the derivation group.[8]

The regression equation obtained was then cross-validated in the validation group sample by calculating the amount of shrinkage in the predictive power of the equation.[15] This was done firstly by applying the regression equation generated in the derivation group samples to the validation group samples to obtain a predicted calcium value for each participant in this group. Measured calcium for the validation group was then regressed on predicted calcium of the same group to obtain an estimate of the variance accounted for (adjusted r^2) in the validation group. Adjusted r^2 from the validation sample group was subtracted from that of derivation group sample. The difference in the adjusted r^2 for both groups was the estimate of the amount of shrinkage which is 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.[15] For further assessment of internal validity of the equation, bootstrapping analysis was undertaken as mentioned by James *et al*.[15]

A new albumin-adjusted calcium equation was derived from the regression equation determined in the derivation group by adding to the y-intercept, the difference between the value of y-intercept and the mean of serum total calcium of the participants. Following the derivation of the new albumin-adjusted calcium equation, calcium status classification was compared in a group of hypoalbuminemic patients (indicated by the serum albumin of less than 32 g/L). In this group of patients, albumin-adjusted calcium was determined by both equations (existing equation and the newly derived equation) and the differences in albumin-adjusted calcium determined using both equations were assessed by using Bland-Altman plot. The agreement between both equations were further assessed by using weighted kappa statistic for the calcium status classification (hypocalcemia, normocalcemia and hypercalcemia).[15] Wilcoxon signed-rank test was used for the comparison of calcium status classification following application of newly derived albumin-adjusted calcium equation.[15] Statistical analyses were performed using MedCalc Statistical Software version 14.8.1 (Ostend, Belgium) and IBM SPSS Statistics for Windows Version 20.0 (Armonk, New York).

RESULTS

The present study consisted of 3,175 participants and they were divided into derivation group (1,587 participants) and validation group (1,588 participants). Table 1 below describe the biological characteristics of the participants in the derivation and validation groups. Both serum total calcium and serum albumin concentration of the subjects in the derivation group were normally distributed upon graphical assessment. The mean level for serum total calcium and serum albumin was 2.30 mmol/L and 39 g/L respectively. The relationship of total calcium and albumin in this group is expressed by total calcium = (0.01421 x albumin) + 1.7915 with a correlation coefficient of 0.32 (FIG. 1). The new adjusted calcium equation obtained is: adjusted calcium = total calcium + 0.014 x (39-albumin). Good internal validity of the equation is evidence by a small value of the amount of shrinkage of the adjusted r^2 which is 0.026. Bootstrapping analysis yielded similar values further confirming the validity of the equation. Adjusted calcium levels in a group of 469 patients with hypoalbuminemia were determined using both the newly derived and the currently used equation to assess the agreement between them. Bland-Altman analysis showed the mean difference of -0.11 mmol/L between both equations (95% limit of agreement between -0.04 to -0.17 mmol/L) (FIG. 2).

Calcium status classification determined by both equations is shown in Table 2. The results showed that 78 patients differed in their calcium status classification.55 patients who were deemed

TABLE 1: Biological characteristic of the subjects

	Derivation Group (n=1577)		Validation Group (n=1588)	
	Mean	**Range**	**Mean**	**Range**
Total serum Ca (mmol/L)	2.3	2.0-2.7	2.3	2.0-2.7
Serum albumin (g/L)	39	20-50	39	20-50

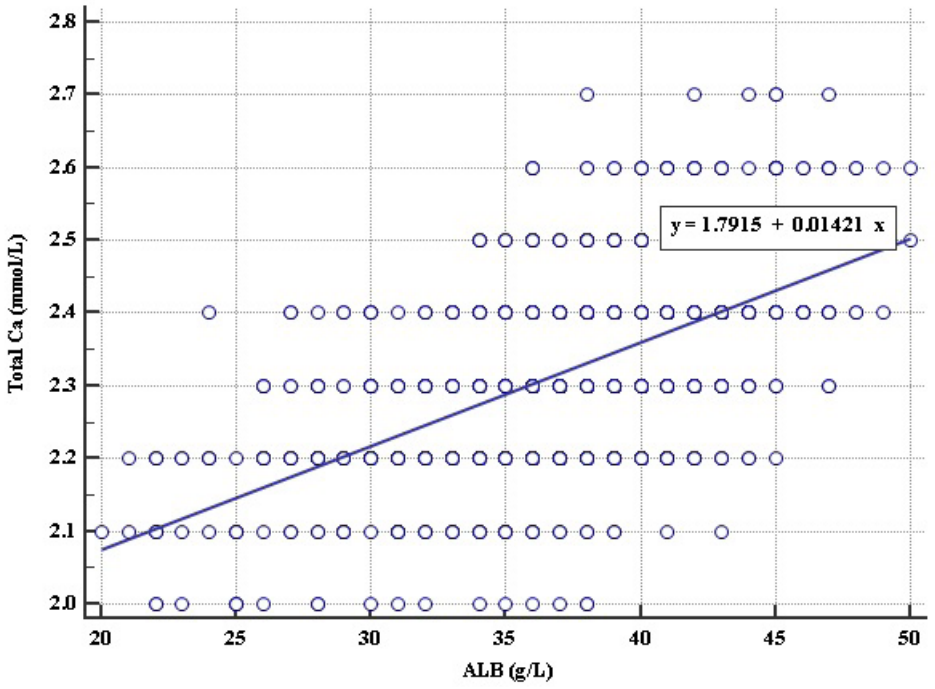

FIG. 1: Simple linear regression of total calcium against albumin.

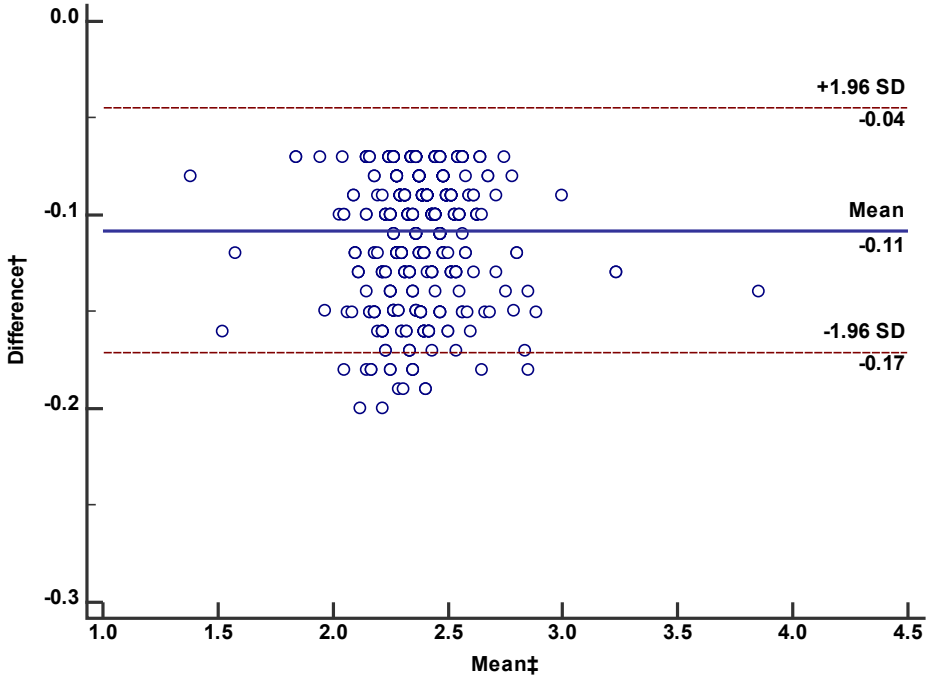

FIG. 2: Bland-Altman plot showing the difference plot of albumin-adjusted calcium concentration between the existing equation and the new equations
†Difference of albumin-adjusted calcium value between existing and new albumin-adjusted calcium equation. ‡Mean of albumin-adjusted calcium value between existing and new albumin-adjusted calcium equation.

TABLE 2: Comparison of calcium status classification in 469 hypoalbuminemic patients using the existing and new equations

Existing albumin-adjusted ca equation*[1]	New albumin-adjusted Ca equation*[2]		
	Hypocalcemia *(Ca < 2.2 mmol/L)*	**Normocalcemia** *(Ca 2.2-2.6 mmol/L)*	**Hypercalcemia** *(Ca > 2.6mmol/L)*
Hypocalcemia (n=27) *(Ca < 2.2 mmol/L)*	**27**	**0**	**0**
Normocalcemia (n=397) *(Ca 2.2-2.6 mmol/L)*	**55**	**342**	**0**
Hypercalcemia (n=45) *(Ca > 2.6mmol/L)*	**0**	**23**	**22**

*[1](albumin-adjusted calcium=total calcium+(0.02 × [40- albumin])
*[2](albumin-adjusted calcium=total calcium+(0.014 × [39-albumin])

to be normocalacemic by the current equation used in the lab were classified as hypocalcaemic by the new equation. The remaining 23 patients were noted to be normocalcaemic by the new equation, but were classified as hypercalcaemic by the existing equation. Wilcoxon signed-rank test showed that differences in calcium status classification by both equations is statistically significant ($p<0.05$).

DISCUSSION

Free ionised calcium measurement is crucial for true classification of calcium status especially in conditions such as cancer, renal transplant, dialysis and critical illness.[16] Its measurement by ion-selective electrode utilising potentiometry methodology is technically challenging and not widely available.[5] Lack of standardisation program for free ionised calcium method pose difficulty for a laboratory to monitor its performance.[5,17] Strict sampling procedures as well as the need for rapid analysis of the sample are some of the pre analytical factors that need to be considered before requesting for free ionised calcium.[18] Overall, free ionised calcium measurement is complicated pre-analytically as well as analytically and will incur a higher operational cost compared to the total serum calcium measurement.[16,17]

Measurement of total serum calcium and serum albumin are convenient from the laboratory perspective mainly because these assays are included in automated chemistry analyser.[19] Albumin-adjusted calcium as an alternative for free ionised calcium measurement will remain popular among clinicians in assessing patients' calcium status and classification. This is mainly due to the convenience in the measurements of both total serum calcium and serum albumin.[8] Our newly derived albumin-adjusted calcium concentration equation differs from the existing equation which is being used by our laboratory. This is in line with the suggestion from The Association for Clinical Biochemistry and Laboratory Medicine (ACB) which recommends the use of locally derived albumin-adjusted calcium equation specific for own population and methodology used for the measurement of total calcium and albumin.[8]

Significant difference in the calcium status classification based on the values produced by both equations was found where a percentage of those classified as normal became hypocalcaemic by the new equation. Errors in calcium status classification would potentially result in delayed or inappropriate treatment to patients. The use of our new equation generally resulted in lower adjusted calcium level and hence some patients were actually hypocalcaemic and normocalcaemic rather than normocalcaemic and hypercalcaemic.

A change in albumin measurement method from BCG to BCP contribute to the differences in both equations as evidenced by an increased number of patients being classified as hypercalcemia in the study by Labriola *et al*.[13] In contrast, our new albumin-adjusted calcium equation yielded a lower calcium level than the existing equation despite utilising BCP methodology for serum albumin.

However, the limitation of this study is that free ionised calcium was not measured due to the lack of a suitable analyser. Therefore, the comparison of the difference in calcium status classification by both adjustment equations in the

comparison group against true ionised calcium was not possible. Further studies are warranted to evaluate the performance of our new equation as the predictor of free ionised calcium in non-acute hospital settings.

The calcium and albumin levels of the participants were not categorised as from the inpatient or outpatient. Hence, the influence of different groups of patients towards the final albumin-adjusted calcium equation is unknown. We also excluded patients who had very low levels of serum albumin (< 20 g/L) as it is known that the relationship of total serum calcium and serum albumin deviates from linearity at low albumin level.[20] The newly derived equation is valid only for adjusting total serum calcium in patients whose serum albumin concentration range from 20 g/L up to 50 g/L.[21] It should be noted that the implementation of this newly derived albumin-adjusted calcium equation beyond our hospital setting may not be appropriate if the of total serum calcium as well as serum albumin differ from ours.

CONCLUSION

The result of this study demonstrated that the calcium status classifications were different in a number of our patients using the new adjustment equation. We recommend that where possible laboratories should derive adjustment equations from their own data.

Authors' contribution: Z.A carried out data collection and statistical analysis. Z.A and F.A discussed the findings and contributed equally in the manuscript preparation.

Conflict of interest: The authors declare no conflict of interest.

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