Original

New formulas for iCa estimation over a wide range of serum albumin concentrations measured by a modified bromocresol purple method

†Takuya Ishigaki[*1], Akari Kameda*[*1], Hayato Ichinari*[*1], Shunsuke Yamada*[*2], Kiichiro Fujisaki*[*2], Toshiaki Nakano*[*2], Taeko Hotta*[*1], Dongchon Kang*[*1,3]*

†Corresponding author: MHSc, Department of Clinical Chemistry and Laboratory Medicine, Kyushu University Hospital, 3-1-1, Maidashi, Higashi-ku, 812-8582, Fukuoka, Japan. E-mail: ishigaki.takuya.472@m.kyushu-u.ac.jp
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*[1]Department of Clinical Chemistry and Laboratory Medicine, Kyushu University Hospital, 3-1-1, Maidashi, Higashi-ku, 812-8582, Fukuoka, Japan.
*[2]Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, 3-1-1, Maidashi, Higashi-ku, 812-8582, Fukuoka, Japan.
*[3]Department of Clinical Chemistry and Laboratory Medicine, Kyushu University, Graduate School of Medical Sciences, 3-1-1, Maidashi, Higashi-ku, 812-8582, Fukuoka, Japan.

ABSTRACT

Although the serum calcium concentration is affected by the serum albumin concentration and requires use of a formula, previous formulas were constructed using the bromocresol green (BCG) method, which has problems with accuracy. In this study, we constructed a new calcium formula using a modified bromocresol purple method, which overcame the limitations of the BCG method. The new formulas were constructed with the pH, ionized calcium (iCa), calcium, and albumin values of 706 patients. Sensitivity, specificity, and weighted kappa coefficient were evaluated with the values of 50 hemodialysis patients. We developed three formulas: to estimate iCa directly (Formula 1), to estimate iCa corrected to pH 7.4 (Formula 2), and to evaluate iCa corrected to pH 7.4 as an index (Formula 3). Using hemodialysis patients for validation, Pre-correction calcium and Formula 1 were tended to be classified as hypocalcemia than iCa or iCa corrected to pH 7.4, while Payne's formula and Kidney Disease Outcomes Quality Initiative's formula 2 were tended to be classified as hypercalcemia. Based on the weighted kappa coefficient, Formula 3 as corrected calcium was the best for assessment of calcium conditions. Since the serum calcium is widely used in daily practice, Formula 3 may be the most useful.

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Key Words
Bromocresol green method, Calcium formula, Ionized calcium, K/DOQI, Payne's formula, Modified bromocresol purple method

I. Introduction

Measurement of ionized calcium (iCa) is important for evaluating a patient's calcium level because of its direct role in physiological functions such as nerve and muscle excitation, blood coagulation, cell membrane permeability, and enzyme activation. However, because the measurement of iCa requires strict handling of the sample from blood collection to analysis [1], its determination in daily practice is limited. Instead of measurement iCa, corrected Ca (cCa), which is the total serum Ca (tCa) corrected by the albumin (Alb) concentration, is widely used [2)3)].

On the other hand, there are three problems with these formulas. First, these formulas use the Alb concentration measured by the bromocresol green (BCG) method,

which has been reported to react with other proteins [4)5)]. Second, these formulas do not take into account iCa as an index. Some studies comparing iCa and cCa have reported that cCa is a poor predictor of iCa abnormalities compared to uncorrected tCa [6)7)]. And finally, these formulas are only used for low Alb concentration.

The aim of this study was to construct new formulas to estimate or evaluate iCa for all Alb concentration based on the Alb measured by a modified bromocresol purple (mBCP) method which overcome the limitations of the BCG method [8)]. The mBCP method has been reported to show a strong correlation with immunonephelometry as the gold standard than with the BCG method [9)10)].

Because the iCa concentration fluctuates with pH, we first have constructed a formula to estimate iCa using pH, tCa, and Alb as variables. Then, we have also generated a formula to predict iCa corrected to pH 7.4 so that iCa can be easily estimated using only the Alb concentration. And finally, because tCa is widely used in daily practice, we have constructed a new formula for cCa that can be used to evaluate iCa more accurately than previous formulas [2)3)].

II. Materials and methods

1. Patients

To establish the new Ca formulas, we compiled the pH, iCa, tCa, and Alb values of 706 patients 20 years old and above whose arterial or venous blood gas analysis and biochemical tests were ordered and measured at the same time between January 2014 and October 2017 at Kyushu University Hospital and whose samples had pH values of 7.20–7.60.

Next, to test the usefulness of the newly constructed Ca formulas, we obtained the values of 50 hemodialysis patients who had agreed with written informed consent at Kyushu University Hospital before hemodialysis (**Table 1**). This study was approved by the Kyushu University Institutional Review Board for Clinical Research (approval number: 30-394, 2022-7). Since the concentration of iCa changes depending on the volume of blood collected and the time from blood collection to measurement [1)11)], we collected the required volume of blood and measured the iCa concentration within 15 min of blood collection. For iCa and pH, blood was collected using a BD Preset Syringe with Heparin for Arterial Blood (Becton, Dickinson and Company, Franklin Lakes, New Jersey). For tCa and Alb, blood was collected using a Insepack II-D tube (Tokuyama Sekisui Co., Ltd., Yamaguchi, Japan), then it was coagulated and centrifuged, and the serum was used for the measurement.

We examined the sensitivity, specificity, and weighted kappa coefficient of each formula (**Figure 1**). Pre-correction Ca, Payne's formula [2)], Kidney Disease Outcomes Quality Initiative's (K/DOQI's) formula 1 and K/DOQI's formula 2 were used for comparison [3)].

2. New formula

A formula for direct estimation of iCa was constructed by multiple regression analysis with pH, tCa, and Alb as variables (Formula 1). Because iCa and pH show a linear relationship from pH 7.2 to pH 7.6 [12)], a formula to estimate iCa corrected to pH 7.4 was constructed by multiple regression analysis with tCa and Alb (Formula 2). On the other hand, because tCa is frequently used in daily practice instead of iCa, cCa formula to evaluate iCa corrected to pH 7.4 as an index was constructed with tCa and Alb. To minimize the effect of a correction factor at normal levels of Alb, the reference interval of Alb was included in the new formula, and the value of X that was most closely correlated with iCa corrected to pH 7.4 was

Table 1 Patient demographics and laboratory values

Variable	Data for construction of new calcium correction formulas	Hemodialysis patients
Total	706	50
Age (year; mean ± SD)	58.9 ± 18.1	62.2 ± 11.5
Male (%)	411 (58)	29 (58)
Serum chemistries		
Total albumin (g/dL; mean ± SD)	3.16 ± 0.81	3.19 ± 0.62
Total calcium (mg/dL; mean ± SD)	8.61 ± 0.96	8.55 ± 0.59
Blood gus tests		
pH (mean ± SD)	7.392 ± 0.062	7.360 ± 0.039
iCa corrected to pH 7.4 (mmol/L; mean ± SD)	1.117 ± 0.113	1.155 ± 0.055
iCa (mmol/L; mean ± SD)	1.113 ± 0.116	1.130 ± 0.057

Total albumin (g/L) = 10 × Total albumin (g/dL), Total calcium (mmol/L) = 0.2495 × Total calcium (mg/dL)

iCa corrected to pH 7.4 was calculated with pH show a linear relationshio
from pH 7.2 to pH 7.6. iCa corrected to pH 7.4=iCa[1+0.53(pH–7.4)]

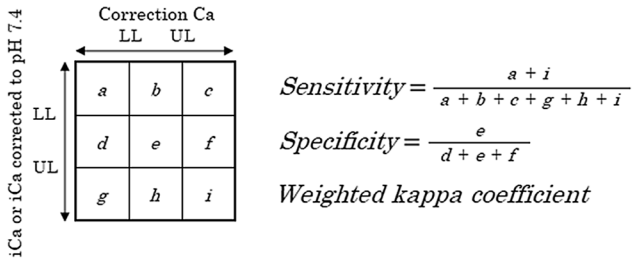

Figure 1 Method for evaluating the sensitivity, specificity, and weighted kappa coeffi-cient. LL: lower limit, UL: upper limit. Sensitivity: percentage of patients de-termined to be hypercalcemic or hypocalcemic using iCa or iCa corrected to pH 7.4. Specificity: percentage of patients determined to be within the refer-ence range using iCa or iCa corrected to pH 7.4. Weighted kappa coefficient: used to evaluate the agreement with iCa or iCa corrected to pH 7.4 on an or-dinal scale. Even if the determination is discrepant, the agreement score will be higher for *b*, *d*, *f* , and h than for *c* and *g*.

determined by Pearson's correlation coefficient (Formula 3).

Formula 1: Direct estimation for iCa with pH, tCa, and Alb as variables

Formula 2: Direct estimation for iCa corrected to pH 7.4 with tCa and Alb as variables

Formula 3: cCa=tCa+X([the median value of the reference interval of Alb]–Alb)

R version 4.2.0 was used for weighted kappa coefficient and multiple regression analysis.

3. Laboratory Tests

iCa and pH were measured using a Rapidpoint 500 system (Siemens Healthineers AG, Erlangen, Bayern). An enzymatic method, Accuras Auto Ca (Shino-Test Corporation, Tokyo, Japan), was used for the tCa measurement, and the Aqua-auto Kainos ALB test Kit (KAINOS Laboratories, Inc. Tokyo, Japan), an mBCP method, was used for the Alb measurement. The LABOSPECT 008 (Hitachi High-Tech Corporation, Tokyo, Japan) was used for the biochemical analysis. The reference intervals for Alb and tCa were 4.1 g/dL–5.1 g/dL and 8.6 mg/dL–10.1 mg/dL, respectively [13]. The iCa reference interval ranged from 1.13 mmol/L–1.33 mmol/L and was preset by the blood gas analyzer. The reference interval for iCa corrected to pH 7.4, 1.12 mmol/L–1.24 mmol/L, was obtained from 80 healthy volunteers.

III. Results

To estimate iCa directly, a multiple regression analysis was performed using pH, tCa, and Alb, and the new formula estimated iCa (eiCa)=0.113tCa–0.043Alb–0.338 pH+2.781(Formula 1) was obtained. The p-value for all variables was $p<0.01$ and the adjusted R-squared value was 0.71. To estimate iCa corrected to pH 7.4, multiple regression analysis was performed using tCa and Alb, and the formula eiCa(pH7.4)=0.116tCa–0.045Alb+0.260(-Formula 2) was generated. The p-value for all variables was $p<0.01$, and the adjusted R-squared value was 0.71. For Formula 3, the median value of the reference interval of Alb was 4.6 g/dL since the reference interval for Alb is 4.1–5.1 g/dL [13]. To determine the value of X that was most closely correlated with iCa corrected to pH 7.4, the Pearson's correlation coefficient was checked at intervals of 0.1 from 0.0 to 1.0, and the value of X was determined to be 0.4 ($r=0.843$). Thus, Formula 3 was cCa=tCa+0.4(4.6–Alb).

We evaluated the agreement between each formula and the reference interval for iCa or iCa corrected to pH 7.4 using the data from 50 dialysis patients (**Figure 2**). Pre-correction Ca and Formula 1 were tended to be classified as hypocalcemia than iCa or iCa corrected to pH 7.4, while Payne's formula and K/DOQI's formula 2 were tended to be classified as hypercalcemia. Formula 1 had the highest sensitivity at 100%, followed by Formula 2 (90.5%), and Pre-correction Ca (76.2%). Formula 3 had the highest specificity at 93.1%, followed by K/DOQI's formula 2 (89.7%), and Payne's formula (79.3%). Using the weighted kappa coefficient, Formula 3 (0.68) performed the best, followed by Formula 2 (0.64) and Formula 1 (0.54) (**Table 2**).

IV. Discussion

To evaluate blood Ca levels in accordance with a patient's pathological state, it is necessary to measure iCa, which indicates the calcium bioactivity. However, the measurement of iCa requires strict preanalytical conditions and specialized equipment [1], and thus tCa is usually measured instead. Since the concentration of tCa is affected by the Alb concentration, Ca formulas have been reported by Payne et al. [2], as well as others [3][6][7] that take into account the Alb concentration. On the other hand, the BCG method has been reported to react with other

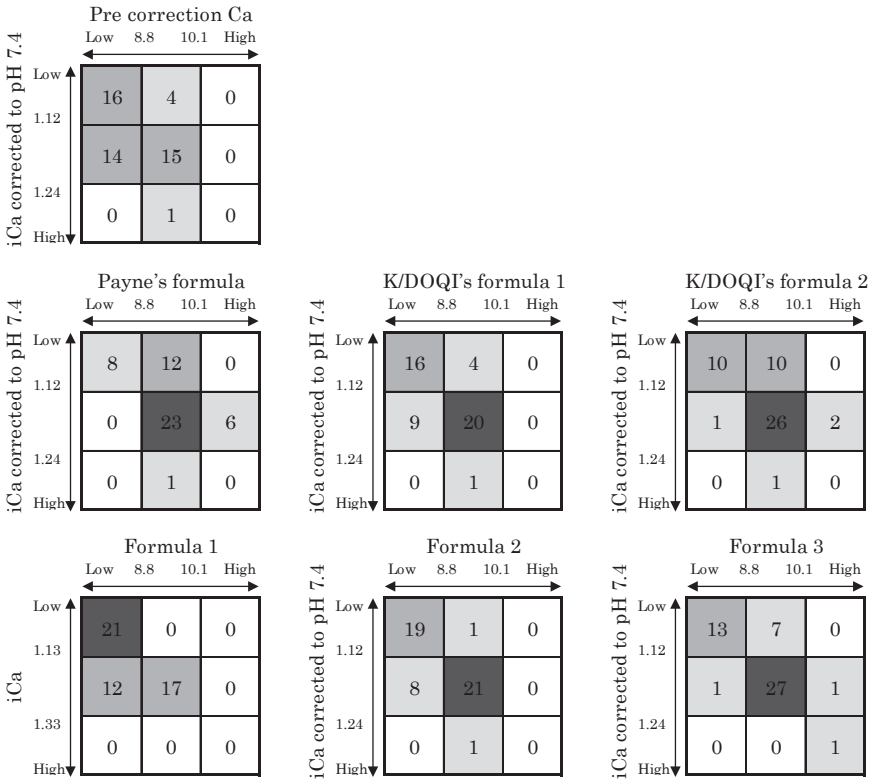

Figure 2 Classification of hemodialysis patients according to iCa or iCa corrected to pH 7.4. Based on iCa, 21 patients were classified as hypocalcemic (<1.13 mmol/L) and 29 patients were classified as normocalcemic. In contrast, based on iCa corrected to pH 7.4, 20 patients were classified as hypocalcemic (<1.12 mmol/L), 1 patient was classified as hypercalcemic and 29 patients were clas-sified as normocalcemic.

Table 2 Evaluation of the correction formulas for the determination of iCa and iCa corrected to pH 7.4

Ca formula		Sensitivity (%)	Specificity (%)	Agreement [1)]
Pre correction Ca	cCa (mg/dL) = tCa (mg/dL)	76.2	51.7	0.33
Payne's formula	cCa (mg/dL) = tCa (mg/dL) + 4 – Alb (g/dL)	38.1	79.3	0.43
K/DOQI's formula 1	cCa (mg/dL) = tCa (mg/dL) + 0.704(3.4 – Alb (g/dL))	76.2	69.0	0.48
K/DOQI's formula 2	cCa (mg/dL) = tCa (mg/dL) + 0.8(4 – Alb (g/dL))	47.6	89.7	0.49
Formula 1	ciCa (mmol/L) = 0.113tCa (mg/dL) – 0.338pH – 0.043Alb (g/dL) + 2.781	100.0	58.6	0.54
Formula 2	ciCa (mmol/L) = 0.116tCa (mg/dL) − 0.045Alb (g/dL) + 0.260	90.5	72.4	0.64
Formula 3	cCa (mg/dL) = tCa (mg/dL) + 0.4(4.6 – Alb (g/dL))	66.7	93.1	0.68

cCa: corrected Ca, tCa: total serum Ca, ciCa: corrected ionized calcium
Alb (g/L) = 10 × Alb (g/dL), Ca (mmol/L) = 0.2495 × Ca (mg/dL)
1) Weighted kappa coefficient, a value of 1.00 denotes perfect agreement

Sensitivity, specificity, and weighted kappa coefficient were evaluated using the data from 50 hemodialysis patients.

proteins [4)5)]. Although the BCP method has a high specificity to albumin, has been reported to underestimate the Alb concentration in patients with renal failure or dialysis [14)15)], and to react differently depending on the form of albumin, such as human mercaptalbumin (HMA) or human non-mercaptalbumin (HNA) [8)]. Therefore, the BCG and BCP methods may not be accurate enough to be used to precisely estimate iCa or cCa. In contrast, the mBCP resolves the difference in reactivity by adding sodium dodecyl sulfate and 5,5-dithiobis (2-nitrobenzoic acid), and achieves high specificity and stability to Alb [8)]. The Japanese Society for Dialysis Therapy guidelines recommends Payne's formula for patients with Alb concentration of less than 4.0 g/dL [16)], and the K/DOQI guideline also recommends that tCa concentration should be corrected when Alb concentration are low [3)]. In con-

trast, the reference interval of Alb determined by a large-scale study in Japan was 4.1 g/dL–5.1 g/dL, which is higher than these guidelines [13]. Collectively, this brings into question whether corrected Ca for a specific range of Alb concentration is appropriate for estimating or determining the iCa. In this study, we developed a new formula that can be corrected for all Alb concentration with iCa as the indicator using a mBCP method.

Three formulas were constructed using iCa or iCa corrected to pH 7.4 as an indicator and compared with the previous formulas using data from dialysis patients. Using Payne's formula, 20.7% of patients were judged to be hypercalcemic even though iCa corrected to pH 7.4 was at normal levels, and 60.0% of patients were failed to detect hypocalcemia, indicating that the correction using the mBCP method could not be used to evaluate Ca correctly with Payne's formula. In contrast, use of K/DOQI's formula 2 provided higher sensitivity, specificity, and agreement than Payne's formula, suggesting that it provides a result that is more reflective of the iCa condition than that obtained from Payne's formula. Formula 1, which directly estimates iCa, showed lower specificity and agreement than Formula 2 and Formula 3, albeit with higher sensitivity. Many formulas for estimating iCa concentration have been previously reported [17)-19)], but these formulas could not be used for comparison in this study because the reference interval differed depending on whether iCa was measured in serum [17)19)] or whole blood [18)]. Historically, it has been very difficult to calculate the iCa concentration accurately using only one formula because Ca homeostasis in the blood fluctuates depending on various factors such as pH, protein, parathyroid hormone, and calcitonin content [20)].

By comparison with Formula 1, Formula 2, which directly estimates iCa corrected to pH 7.4, and Formula 3, which was constructed from the relationship between the Ca reference interval and iCa corrected to pH 7.4, showed good specificity and agreement. In particular, Formula 3 provided superior sensitivity, specificity, and agreement with the results from Payne's formula and K/DOQI's formula 2, and showed the same level of agreement as Formula 2. This suggests that Formula 3 has the same performance as Formula 2, which can be used to directly estimate iCa. Furthermore, Formula 3 differs from formulas 1 and 2, which estimate iCa, in that it can be evaluated in terms of Ca concentration so it can be used in the same way as tCa is used in daily practice. Some formulas using the mBCP method have been previously reported [21)22)], none of them showed a better agreement than Formula 3 (Ohba's formula:0.49, Tanaka's formula:0.28).

Formula 3 uses iCa corrected to pH 7.4 instead of iCa as an index, and thus the iCa incorporated in the formula may be different from the actual in vivo iCa found in chronic kidney disease (CKD) patients with metabolic acidosis. The problem in CKD patients is that they suffer from hypocalcemia [3)]. The iCa concentration in patients with acidosis is higher than the iCa at pH 7.4 calculated by the pH adjustment formula. Therefore, unless hypocalcemia is determined using Formula 3, it is unlikely that the patient is actually hypocalcemic. Among the samples from the 50 hemodialysis patients analyzed in this study, there were seven patients whose iCa corrected to pH 7.4 was hypocalcemic but the Ca determined using Formula 3 was within the reference interval. Of these seven patients, three patients had acidosis and their iCa values were higher than the iCa corrected to pH 7.4, all within the reference interval of iCa. It is difficult to conclude which indicator is better to use to evaluate Ca in patients: iCa measured or iCa corrected to pH 7.4. However, it has been reported that iCa corrected to pH 7.4 is as useful as iCa measured in the evaluation of patients with chronic disorders of calcium metabolism [23)]. Therefo re, we consider that Formula 3 using iCa corrected to pH 7.4 as an indicator for correction is the most clinically practical, economic, and convenient method for Ca evaluation.

V. Conclusion

We developed Formulas 1, 2, and 3, which could be used across all Alb concentration, for more accurate estimation of iCa, iCa corrected to pH 7.4, and cCa, respectively. The formulas employed the mBCP method, which is the most accurate method for measuring the Alb concentration. On the basis that tCa is most commonly determined in the clinic, Formula 3 should be useful in daily practice even though its performance was essentially the same as that of Formula 2. We believe that the three new formulas will enable more accurate assessment of Ca conditions in the clinic.

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Author contributions

All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests

Authors state no conflict of interest.

Informed consent

Informed consent was obtained from all hemodialysis patients in this study. Owing to the retrospective nature of the study, informed consent was not required for the other patients.

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