Original Article

Ionic Calcium Measurement in Blood : A Comparative Analysis of Direct Ion-Selective Electrode Method and Formula-based Predictions

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Abstract

Background : Free ionic calcium, the biologically active component of Total Calcium (TCa) in blood, reflects the actual calcium status in health and disease.

Materials and Methods: This study analyzed 200 heparinized arterial blood gas samples for free calcium using the direct Ion-selective Electrode (ISE) method and total calcium and plasma albumin using Arsenazo III and Bromocresol Green (BCG) methods, respectively. Our study categorized patients into three groups based on plasma pH: acidosis (pH<7), normal (pH=7), and alkalosis (pH>7). The results revealed that the calculated ionic calcium was lower than the measured ionic calcium, indicating the superior reliability of the direct Ion Selective Electrode (ISE) method for ionic calcium measurement.

Result : In the acidosis and normal pH groups, measured ionic calcium showed a significant positive correlation with both total calcium and calculated free calcium (acidosis: r=0.42, p=0.026 and r=0.49, p=0.008; normal: r=0.54, p<0.0001 and r=0.44, p<0.0001). However, no significant correlation was observed in the alkalosis group.

In the hypoalbuminemia group, there was a positive correlation between total calcium and both measured free calcium (r=0.481, p<0.0001) and calculated free calcium (r=0.800, p<0.0001). Additionally, calculated free calcium was positively correlated with measured free calcium (r=0.508, p<0.0001). The Bland-Altman method revealed a lack of agreement between measured free calcium and calculated free calcium across different pH levels and albumin concentrations.

Conclusion : The direct ISE method proves to be a more reliable approach compared to formula-based predictions.

Key words : Total calcium, calculated free calcium, pH, Measured Free calcium, ISE.

Calcium, the most abundant cation in the body, is often referred to as the “king of minerals” due to its critical role in numerous physiological processes such as blood clotting, neuromuscular conduction, maintaining normal skeletal and cardiac muscle tone and excitability, stimulating exocrine gland secretion, and maintaining cell membrane integrity and permeability[1]. In the bloodstream, calcium exists in three forms: anion-bound, protein-bound, and free or “ionized” calcium. The concentration of these fractions is influenced by hydrogen ions, anions, and plasma proteins[2]. While total calcium measurement accurately represents overall calcium homeostasis in healthy individuals, ionized calcium is the preferred test in patients with abnormal pH or aberrant protein or anion concentrations. Although approximately 50% of the calcium in the blood is in free form (ionized), 40% is bound to proteins and 10% is complexed with other anions (bicarbonate, lactate, and citrate), it is the ionized calcium that is biologically active[3]. Equations and nomograms have been developed to connect total calcium to ionized calcium, primarily to correct total calcium values for the impact of protein

Editor's Comment :

- Direct ion-selective Electrode (ISE) measurement of free ionic calcium provides a more reliable assessment of calcium status than formula-based calculations, across varying pH and albumin concentrations.
- Measured ionic calcium shows significant positive correlations with total calcium and calculated free calcium in acidosis and normal pH states, but not in alkalosis, highlighting pH-dependent variations.
- Bland-Altman analysis confirms poor agreement between measured and calculated free calcium, underscoring the importance of direct measurement for clinical decision-making.

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binding[4-6]. However, the accuracy of these corrections has been disputed, and direct measurement of ionized calcium was previously unfeasible due to methodological challenges[7]. With advancements in technology resolving these challenges over time, the measurement of ionized calcium is now anticipated to become a standard test.

Despite these advancements, resource-limited settings may still face challenges in implementing direct measurement of ionized calcium. This study aims to address this gap by comparing measured ionic calcium using the Ion Selective Electrode (ISE) method with derived values through total corrected calcium using formulas. This comparison could provide valuable insights for healthcare settings with limited resources and contribute to improving patient care.

Study Design and Methodology :

This study is a prospective analysis of 200 patient samples from the Maternal ICU, Department of Obstetrics and Gynecology at Ram Prakash Gupta Mother and Child State Referral Hospital. The samples were received in the Department of Biochemistry at Dr Ram Manohar Lohia Institute of Medical Sciences from December, 2021 to May, 2022.

Sample Collection and Analysis :

Blood samples were collected using a heparinized syringe and centrifuged at 3500 rpm for 15 minutes to separate the plasma. The plasma was then analyzed for ionic calcium and total calcium levels. Total calcium was measured using a commercially available kit (based on the Arsenazo II method) on an automated analyzer (Beckman Coulter AU480). Ionic calcium was measured using an ion-selective electrode method on a Combiline Eschweiler automated analyzer. Calibration and quality control samples were processed before each experiment according to the manufacturer's instructions.

Calculation of Free Calcium :

Total protein, albumin, and globulin were estimated to derive calculated free calcium. The following formulas were used[8] :

(1) Percentage (%) Protein-bound = 0.8 × Albumin (g/L) + 0.2 × Globulin (g/L) + 3

(2) Calculated Free Calcium (mg/dL) = Total calcium – Protein-bound calcium

(3) Calculated free calcium (mmol/L) = Calculated free calcium (mg/dL) × 0.25

Adjusted total Calcium was calculated by using Payne's formula as follows[9] :

(1) Adjusted total calcium (mg/dl) = total calcium(mg/dl) + 0.8(4-albumin(gm/dl)

(2) Adjusted total calcium (mmol/L) = adjusted total calcium (mg/dL) × 0.25

Data Analysis :

Data analysis was performed using SPSS Statistics for Windows, version 21.0 (IBM, USA). Median and quartile ranges were used for normal data distribution. Pearson correlation coefficient was used to calculate the correlations between variables, and Bland-Altman Plot analysis was used to assess the agreement between variables. A $p<0.05$ was considered statistically significant.

Result

Patients were categorized into three groups based on plasma pH: acidosis (pH<7), normal (pH=7) and alkalosis (pH>7). Tables 1 and 2 reveals that the calculated ionic calcium was lower than the measured ionic calcium, indicating the superior reliability of the direct Ion Selective Electrode (ISE) method for ionic calcium measurement. In the hypoalbuminemia group, there was a significant decrease in total calcium and calculated free calcium compared to the normal albuminemia group ($p<0.0001$). However, no significant differences were observed across different pH groups.

Correlation among the Calcium Values in Acidosis (pH<7), Normal (pH=7) and Alkalosis (pH>7) Groups

As shown in Table 3, measured ionic calcium in the acidosis group showed a positive correlation with total calcium and calculated free calcium ($r=0.42$, $p=0.026$ and $r=0.49$, $p=0.008$, respectively). Similarly, in the normal pH group, measured ionic calcium was positively correlated with total calcium and calculated free calcium ($r=0.54$, $p<0.0001$ and $r=0.44$, $p<0.0001$ respectively). However, no significant correlation was observed in the alkalosis group.

Correlation Analysis of Calcium Variables Based on Albumin Levels

In the hypoalbuminemia group, there was a positive correlation between total calcium and both measured free calcium ($r=0.481$, $p<0.0001$) and calculated free calcium ($r=0.800$, $p<0.0001$). Additionally, calculated free calcium was positively correlated with measured free calcium ($r=0.508$, $p<0.0001$). In the normal

Table 1 — *Comparative description of ionic and calculated calcium along with total calcium among different categories based on plasma pH*

Variables	Acidosis; n=31 Median (Q1-Q3)	Normal; n=93 Median (Q1-Q3)	Alkalosis; n=76 Median (Q1-Q3)	P1	P2	P3
FCM (mmol/L)	0.83 (0.71-1.00)	0.77 (0.60-0.90)	0.82 (0.64-0.96)	0.217	0.526	0.060
FCC (mmol/L)	0.84 (0.75-0.91)	0.81 (0.74-0.85)	0.82 (0.77-0.89)	0.238	0.959	0.411
TC (mg/dL)	7.90 (7.50-8.40)	8.20 (7.60-8.90)	8.30 (7.80-8.70)	0.108	0.019*	0.424

Acidosis: pH<7.35, Normal: pH- 7.35 to 7.45, Alkalosis: pH>7.45, FCC: Free calcium (calculated), FCM: Free calcium (measured), TC: Total calcium The Mann-Whitney test was used to calculate the p-value. *p-value <0.05 was considered as statistically significant. P1: p-value between acidosis and normal, P2: p value between acidosis and alkalosis, P3: p-value between normal and alkalosis.

Table 2 — *Comparative description of ionic and calculated calcium along with total calcium among different categories based on plasma albumin*

Variables	Hypoalbuminemia (n=120) Median (Q1-Q3)	Normal albuminemia (n=80) Median (Q1-Q3)	p-value
FCM (mmol/L)	0.78 (0.69-0.92)	0.80 (0.66-0.99)	0.873
FCC (mmol/L)	0.75 (0.71-0.80)	0.85 (0.80-0.92)	<0.0001*
Adjusted TCC (mmol/L)	2.10 (1.66-2.30)	2.23 (2.12-2.38)	<0.0001*
TC (mg/dL)	8.00 (7.60-8.40)	8.50 (7.80-9.10)	<0.0001*

FCC: Free calcium (calculated), FCM: Free calcium (measured), TC: Total calcium, TCC: Total calcium calculated. The Mann-Whitney test was used to calculate the p-value. *p-value <0.05 was considered as statistically significant.

Table 3 — *Correlation of Free calcium and Ionic calcium based on pH*

Variables	pH<7.35 Acidosis (n=31)		pH=7.35-7.45 Normal (n=93)		pH>7.45 Alkalosis (n=76)	
	FCC	FCM	FCC	FCM	FCC	FCM
FCC	1	r=0.49 p=0.008*	1	r=0.44 p<0.0001*	1	r= -0.06 p=0.588
TC	r=0.54 p=0.003*	r=0.42 p=0.026*	r=0.55 p<0.0001*	r=0.54 p<0.0001*	r=0.33 p=0.004*	r=0.04 p=0.739

FCC: Free calcium (calculated), FCM: Free calcium (measured), TC: Total calcium, r=Pearson correlation. The Pearson correlation coefficient was used to analyze the correlation between the biochemical variables *p-value <0.05, which was considered statistically significant.

Table 4 — *Correlation of Ionic calcium with total and free calcium on the bases of albumin*

Variables	Hypoalbuminemia(n=120)		Normal albuminemia(n=80)	
	FCC	FCM	FCC	FCM
FCC	1	r=0.508 p<0.0001*	1	r=-0.008 p=0.947
TC	r=0.800 p<0.0001*	r=0.481 p<0.0001*	r=0.417 p<0.0001*	r=0.011 p=0.924
Adjusted TCC	r=0.926 p<0.0001*	r=0.476 p<0.0001*	r=0.692 p<0.0001*	r=0.016 p=890

FCC: Free calcium (calculated), FCM: Free calcium (measured), TC: Total calcium, TCC: Total calcium calculated, r=Pearson correlation. The Pearson correlation coefficient was used to analyze the correlation between the biochemical variables*p-value <0.05, which was considered statistically significant.

albuminemia group, total calcium showed a positive correlation with calculated free calcium (r=0.417, p<0.0001), but no significant correlation was found with measured free calcium (Table 4).

Agreement analysis (Bland-Altman plot) for Calculated and Measured Ca++ ion

The Bland-Altman method (extensively used to evaluate agreement among two different instruments or measurement techniques) was used to calculate the mean difference (bias) between measured free calcium and calculated free calcium. The analysis revealed a lack of agreement between the two variables across different pH levels and albumin concentrations. However, no significant difference was observed between measured and calculated free calcium in all the groups.

Concordance (Measured and Calculated) in Ionic Calcium Level

A concordance study was conducted to categorize patients based on the reference range of ionic calcium levels using Cohen's Kappa statistic, a quantitative measure used to evaluate the level of agreement between two raters or judges who each classify items into mutually exclusive categories. Ionic calcium levels below 1.10 mmol/L were classified as decreased, while levels above 1.10 mmol/L were considered normal. The study found substantial agreement between both methods, with a Cohen's kappa (κ) value of 0.353, indicating statistical significance (p<0.0001) as shown in Table 5.

Discussion

Calcium, the most abundant mineral in the human body, is found in three forms in the bloodstream: free calcium, calcium ions bound to albumin, and calcium associated with organic anions such as phosphate, bicarbonate, and citrate. Free calcium is the physiologically active form and plays a crucial role in

Table 5 — *Concordance of Ionic calcium between measured and calculated*

FCC		FCM	
		<1.10 mmol/L	>1.10 mmol/L
	<1.10 mmol/L	187	08
	>1.10 mmol/L	02	03
Cohen's kappa (κ)		0.353	
p-value		<0.0001*	

FCC: Free calcium (calculated), FCM: Free calcium (measured). Cohen's Kappa values range between 0 and 1. A value of 0 indicates no agreement between the two raters, while a value of 1 indicates perfect agreement. The interpretation of different values for Cohen's Kappa can vary, but generally, a higher value indicates a higher level of agreement between the two raters.

various metabolic and physiological processes[10,11].

Our study revealed a positive relationship between calculated free calcium and measured free calcium in acidic and normal pH conditions. This relationship was not observed in alkalotic conditions, indicating that pH plays a significant role in calcium homeostasis. This is consistent with previous literature that has reported changes in ionized calcium with impairments of calcium homeostasis due to variations in pH[11-13].

The mean of ionic calcium was found to be reduced to the reference range (0.84±0.59), indicating a discrepancy between measured and calculated values. This discrepancy becomes more pronounced in patients with hypoalbuminemia due to fluctuations in ionized calcium[14].

The Bland-Altman method revealed poor agreement between measured free calcium and calculated free calcium at different pH levels. This finding underscores the need for more accurate methods for measuring ionized calcium, especially in resource-limited settings.

The study findings highlight the complexities involved in accurately measuring ionic calcium levels, particularly in the context of varying pH and albumin concentrations. Patients with aberrant protein or anion concentrations are likely to have erroneous corrections due to fluctuations in ionized calcium. This is particularly evident in patients undergoing hemodialysis, where changes in pH, albumin, phosphate, and other anions due to therapeutic interventions can lead to incorrect pH of ionized calcium.

Our study aligns with the findings of Mir, *et al*[15] who found significant differences between predicted free calcium generated by formulas and measured free calcium, both in the hypoalbuminemia group and when all samples were considered combined. The Bland-Altman plots also failed to demonstrate agreement between the measured and calculated free calcium levels within the permissible range for any groups. When comparing calculated free calcium from Orrell's and Berry, *et al*'s formulas to measured free calcium in normal albuminemia and hyper-albuminemia, respectively, no significant differences were found between the two sets of conditions. However, none of the formulas were found to be universally applicable to all albumin values[16,17].

In our study, the Bland-Altman method revealed a poor agreement between measured free calcium and calculated free calcium at different pH levels. This finding underscores the need for more accurate methods for measuring ionized calcium, especially in resource-limited settings.

Accurately estimating free calcium is crucial, particularly in managing critically ill patients with calcium metabolism disorders, specifically in the context of cardiac or renal conditions. Our study revealed substantial agreement between measured and calculated ionic calcium, with patients classified into two categories according to reference range: Ionic calcium <1.10 mmol/L was considered as decreased and Ionic calcium >1.10 mmol/L as normal.

However, it's important to note that the formulas used to calculate ionic calcium have certain inherent flaws. They fail to account for all variables that affect the complex calcium equilibria, and there is a variance in the analytical parameters used in each formula. Additionally, varying reference ranges for the same parameters can affect the results. Studies have observed that these formulas do not correlate with directly measured free calcium in patients who are critically ill, have chronic kidney disease hyperparathyroidism, or receiving transfusions, or undergo hemodialysis[18-20].

The importance of estimating free calcium in managing critically ill patients, particularly those with cardiac or renal conditions, cannot be overstated. Abnormalities in calcium metabolism in these patients can have significant implications for their prognosis and treatment outcomes. However, the current formulas used for estimating free calcium have several limitations that need to be addressed[21].

Firstly, these formulas do not account for all the factors influencing the complex calcium equilibria. Calcium homeostasis is a multifaceted process involving various physiological mechanisms and pathways. By not considering all these factors, the formulas may not accurately estimate free calcium levels[22].

Secondly, variations in the analytical parameters involved in these formulas can affect the calculations. This introduces a degree of uncertainty and potential error in the estimation process. It is crucial to standardize these parameters to ensure consistent and reliable results.

Lastly, different reference ranges for the same parameters can also impact the results. This can lead to discrepancies in the estimated free calcium levels, making comparing results across different settings or populations challenging.

In light of these issues, there is a pressing need for more accurate and comprehensive methods for estimating free calcium. Future research should address these limitations and develop improved formulas that consider all relevant factors influencing calcium equilibria. This will enhance our ability to effectively manage patients with abnormalities in calcium metabolism, ultimately improving patient outcomes.

Conclusion

Despite the challenges associated with ion-selective electrode measurement of free calcium, such as cost, low throughput, and the need for strict anaerobic conditions, it remains a quick, easy, and reliable method for assessing calcium levels. If samples are carefully collected under anaerobic conditions, free calcium may reflect more accurately serum calcium status than total calcium.

However, in emergencies, formulas for estimating free calcium can be considered provided their limitations and the influence of albumin concentrations are considered. This balanced approach effectively manages patients' calcium levels while acknowledging the inherent complexities of calcium measurement. Future research should continue to explore more accurate and efficient methods for estimating free calcium to improve patient care.

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Conflict of Interest : None

References

1 Sava L, Pillai S, More U, Sontakke A — Serum calcium measurement: total versus free (ionized) calcium. *Indian Journal of Clinical Biochemistry* 2005; **20:** 158-61.

2 Rosol TJ, Capen CC — Calcium-regulating hormones and diseases of abnormal mineral (calcium, phosphorus, magnesium) metabolism. In Clinical Biochemistry of Domestic Animals 1997; **1:** 619-702.

3 Slomp J, van der Voort PH, Gerritsen RT, Berk JA, Bakker AJ — Albumin-adjusted calcium is not suitable for diagnosis of hyper-and hypocalcemia in the critically ill. *Critical Care Medicine* 2003; **31(5):** 1389-93.

4 Moore EW — Ionized calcium in normal serum, ultrafiltrates, and whole blood was determined by ion-exchange electrodes. *The Journal of Clinical Investigation* 1970; **49(2):** 318-34.

5 Hamroun A, Pekar JD, Lionet A, Ghulam A, Maboudou P, Mercier A, *et al* — Ionized calcium: analytical challenges and clinical relevance. *J Lab Precis Med* 2020; **5:** 22.

6 Baird GS — Ionized calcium. *Clinica Chimica Acta* 2011; **412(9-10):** 696-701.

7 Johnson PC, Ware JA, Cliveden PB, Smith M, Dvorak AM, Salzman EW — Measurement of ionized calcium in blood platelets with the photoprotein aequorin. Comparison with Quin 2. *Journal of Biological Chemistry* 1985; **260(4):** 2069-76.

8 Isselbacher, Isselbacher, Braunwad, Wilson, Martin, Fanci and Kasper — Harrison's principles of internal medicine, 1994. 13 th Ed. vol. 2, 2139.

9 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.

10 Berchtold MW, Brinkmeier H, Muntener M — Calcium ion in skeletal muscle: its crucial role for muscle function, plasticity, and disease. *Physiological Reviews* 2000; **80(3):** 1215-65.

11 Fogh-Andersen N, Christiansen TF, Komarmy L, Siggaard-Andersen O — Measurement of free calcium ion in capillary blood and serum. *Clin Chem* 1978; **24, 9:** 1545-52.

12 Fogh-Andersen N — Ionized calcium analyzer with a built-in pH correction. *Clin Chem* 1981; **27, 7:** 1264-67.

13 Thode J, Holmegaard SN, Transbøl I, Fogh-Andersen N, Siggaard-Andersen O — Adjusted ionized calcium (at pH 7.4) and actual ionized calcium (at actual pH) in capillary blood compared for clinical evaluation of patients with disorders of calcium metabolism. *Clin Chem* 1990; **36, 3:** 541-44.

14 Schwartz HD — New techniques for ion-selective measurements of Ca24 in serum after pH adjustment of aerobically sera. *Clin Chem* 1977; **23:** 611.

15 Mir AA, Goyal B, Datta SK, Ikkurthi S, Pal A — Comparison between measured and calculated free calcium values at different serum albumin concentrations. *Journal of Laboratory Physicians* 2016; **8(02):** 071-6.

16 Orrell DH — Albumin as an aid to the interpretation of serum calcium. *Clin Chim Acta* 1971; **35:** 483 9.

17 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:** 640 3.

18 Clase CM, Norman GL, Beecroft ML, Churchill DN — Albumin corrected calcium and ionized calcium in stable haemodialysis patients. *Nephrology Dialysis Transplantation* 2000; **15(11):** 1841-6.

19 Moore EW — Ionized calcium in normal serum, ultrafiltrates, and whole blood determined by ion-exchange electrodes. *The Journal of Clinical Investigation* 1970; **49(2):** 318-34.

20 Kihara Y, Grossman W, Morgan JP — Direct measurement of changes in intracellular calcium transients during hypoxia, ischemia, and reperfusion of the intact mammalian heart. *Circulation Research* 1989; **65(4):** 1029-44.

21 Ong GS, Walsh JP, Stuckey BG, Brown SJ, Rossi E, Ng JL, *et al* — The importance of measuring ionized calcium in characterizing calcium status and diagnosing primary hyperparathyroidism. *The Journal of Clinical Endocrinology & Metabolism* 2012; **97(9):** 3138-45.

22 Smaili S, Hirata H, Ureshino R, Monteforte PT, Morales AP, Muler ML, *et al* — Calcium and cell death signaling in neurodegeneration and aging. *Anais da Academia Brasileira de Ciências* 2009; **81:** 467-75.