ORIGINAL ARTICLE

Comparison between Serum Calcium Levels Measured Using Direct Ion-selective Electrodes and Photometric Method in Automated Analyzers

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Abstract

Introduction: Serum calcium is measured by photometric methods or ion-selective electrodes (ISEs). The ISEs measure free ionized calcium (FCa), which is not bound to proteins like albumin and is corrected using algorithms to calculate the total calcium, TCa (TCa_calc). The TCa obtained by photometry (TCa_meas) requires correction for albumin by several formulae to obtain the corrected Ca (TCa_corr).

Aims and objectives: In this study, we aim to find the agreement between TCa levels calculated from direct ISE results (TCa_calc) and TCa levels obtained by spectrophotometric methods after correction using formulae given in the literature (TCa_corr) at different levels of serum albumin.

Materials and methods: In this study, 332 serum samples were analyzed for TCa and albumin on Roche Modular P800 and FCa by direct ISE on XI-921 (Caretium) and converted to TCa_calc. The results of TCa_calc and TCa_corr were compared using paired *t* test.

Results: Significant difference was observed between TCa_calc (2.45 ± 0.34 mmol/L) and TCa_meas (2.07 ± 0.27 mmol/L). The TCa_meas was corrected for albumin using several commonly used formulae. However, significant differences still existed between TCa_calc and TCa_corr. The cases were further subdivided into three groups on the basis of serum albumin; however, significant differences were observed between TCa_calc and TCa_corr values in all subgroups.

Conclusion: Caution should be exercised while interchangeable usage and interpretation of serum calcium levels from direct ISE *vis-à-vis* photometric methods.

Clinical significance: With the infiltration of point-of-care devices in casualties and intensive care units, awareness needs to be created among clinicians regarding the potential misinterpretations of the tests involved. Regulatory guidelines to the same effect may also be considered.

Keywords: Albumin, Direct ISE, Free calcium, Ionic calcium, oCPC method, Total calcium.

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Introduction

In all, 98% of calcium in the body is present in bones in the form of hydroxyapatite. Rest of the 2% is distributed between the plasma and interstitial fluid. Calcium present in the plasma are of three forms, the free ionized calcium (FCa) which constitutes about 50% of the total calcium (TCa). Forty percent calcium is bound to proteins mainly albumin and 10% is complexed with low-molecular-weight ligands such as bicarbonate, phosphate, lactate, citrate, and others. The percentage of the calcium ions bound to albumin and other inorganic anions thus is subject to variation depending upon the changes in pH and alterations in the concentration of proteins, albumin, and other small anions. Although the TCa concentration in the serum can affect the neurological, cardiac, neuromuscular, renal, and gastrointestinal functions, FCa is the metabolically active form responsible for these physiological functions.

The methods used for quantifying calcium in serum measure either the FCa or the TCa. The FCa can be measured by direct ion-selective electrodes (ISEs) and is the best indicator of calcium status in the body because it is the biologically active form and its levels are tightly regulated by parathyroid hormone and vitamin D. Serum TCa in the laboratory is measured mostly by (i) photometric methods such as ortho-cresolphthalein complexone (oCPC) and arsenazo III. Often, FCa, which is measured by direct ISEs, is corrected using algorithms to calculate TCa (TCa_calc). On the contrary, TCa obtained by oCPC (TCa_meas) requires correction for albumin to give corrected Ca (TCa_corr). Various equations are used for this purpose. However, there is a lack of agreement between the TCa results obtained by both the methods and also by different mathematical formulae. Through this study we aim to compare the agreement in the values of serum TCa_calc (direct ISEs) with TCa_corr (from oCPC method). We also try to evaluate the results obtained in the light of serum albumin levels (<3 g/dL, 3–4.99 g/dL, and ≥5 g/dL) to find out whether these two methods give comparable results in any of the subgroups.

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Materials and Methods

This cross-sectional study was conducted in the clinical biochemistry and emergency laboratory, at a tertiary care teaching hospital in north India. A total of 332 consecutive serum samples received in emergency laboratory over a period of 20 days were included in the study. Serum was separated by centrifugation at 2000 rpm for 10 minutes and analyzed for FCa and TCa_calc by direct ISEs on XI-921, Caretium. The FCa was directly measured by the ISEs, whereas TCa_calc is the TCa obtained by multiplying FCa with 1.95 per the algorithm provided by the manufacturer. Serum was then used to measure TCa_meas by o-CPC method and albumin by Bromocresol green (BCG) method on Roche modular P800 autoanalyzer in the routine biochemistry lab. Samples with less than 500 µL separated serum, with visible hemolysis and lipemia were excluded. Routine quality control was performed daily per the accepted guidelines on both the analyzers. The TCa_meas was then corrected for albumin using the following formulae taken from literature:

- TCa_corr_Orrell = TCa_meas + 0.0176 (34 − albumin)[1]
- TCa_corr_Payne = TCa_meas + 0.0246 (40.4 − albumin)[2]
- TCa_corr_Berry = TCa_meas + 0.0227(46 − albumin)[3]
- TCa_corr_Clase = TCa_meas + 0.018 (35 − albumin)[4]

All the data were compiled in Microsoft Excel and analyzed using STATA software. The results of TCa_calc were compared to TCa_meas and TCa_corr using paired *t* test. Further, all the subjects were divided into three subgroups on the basis of serum albumin levels. Serum albumin (i) <3 g/dL ($n = 115$), (ii) 3–4.99 g/dL ($n = 157$), and (iii) ≥5 g/dL ($n = 60$). Comparison between TCa_calc with TCa_meas and TCa_corr was done within the subgroups in a similar manner.

To find out the clinical significance and whether the results from both machines could be used interchangeably, we calculated the number of samples that gave difference between TCa_cal and TCa_meas or difference between TCa_cal and TCa_corr of more than ±5%. Since the percentage coefficient of variation for serum calcium measurement on our chemistry autoanalyzer was 5% (±2.5%), the percentage difference of ±5% was taken to increase the sensitivity of results. If the percentage difference was less than ±5%, the results were considered to be similar in both the machines. However, if the percentage difference was more than ±5%, the results of both machines could not be used interchangeably and it was considered to be an analytical error. Analysis was done in all patients as well as in all subgroups divided on the basis of serum albumin to find the formulae that gave the best results.

Results

The TCa_calc obtained from direct ISE results was compared with the TCa_meas by oCPC method as well as TCa_corr obtained after correction of TCa_meas with formulae by Payne, Orrell, Berry, and Clase using paired *t* test as mentioned above. Table 1 summarizes the results. Without subgrouping per albumin levels when all samples were analyzed together, significant difference ($p < 0.05$) was observed between TCa_calc (2.45 ± 0.28 mmol/L) and TCa_meas (2.12 ± 0.27 mmol/L). The TCa_meas was further corrected for albumin using the following formulae: (i) Orrell et al. 1971 (2.10 ± 0.19 mmol/L), (ii) Payne et al. 1973 (2.25 ± 0.21 mmol/L), (iii) Berry et al. 1973 (2.37 ± 0.20 mmol/L), and (iv) Clase et al. 2000 (2.12 ± 0.19 mmol/L). Significant difference ($p < 0.05$) was also observed between TCa_calc and TCa_corr.

When comparison was done between the subgroups on the basis of serum albumin, similar result was obtained with significant differences ($p < 0.05$) between the measured and corrected values (Tables 2A to 2C). To evaluate the clinical significance of these differences, the number of samples in each subgroup showing agreement (percentage difference between TCa_calc and TCa_meas or TCa_corr of ≤±5%) between both the methods was calculated. It was seen that, maximum agreement between TCa_calc and TCa_meas as well as TCa_corr was observed when

Table 1: Result of total calcium measured by direct ISEs (TCa_calc) and by oCPC (TCa_meas) and corrected by formulae (TCa_corr) in all samples ($n = 332$)

TCa_calc (mmol/L)	*Formula*	*TCa (mmol/L)*	*p value*	*%Difference* ≤5%; number of samples (%)*	*%Difference* >5%; number of samples (%)*	*Mean% difference**
2.45 ± 0.28	TCa_meas	2.12 ± 0.27	<0.05	58 17.5)	274 (82.5)	12.97
	TCa_corr_Orrell	2.10 ± 0.19	<0.05	56 (16.9)	276 (83.1)	13.42
	TCa_corr_ Payne	2.25 ± 0.21	<0.05	119 (35.8)	213 (64.2)	7.08
	TCa_corr_ Berry	2.37 ± 0.20	<0.05	182 (54.8)	150 (45.2)	2.27
	TCa_corr_ Clase	2.12 ± 0.19	<0.05	58 (17.5)	274 (82.5)	12.69

*Difference between TCa_calc and TCa_meas or TCa_calc and TCa_corr
All values are given as mean ± SD of the measured values. Value of $p < 0.05$ as predicted by paired *t* test is considered to be significant

Table 2A: Result of total calcium measured by direct ISEs (TCa_calc) and by oCPC (TCa_meas) and corrected by formulae (TCa_corr) in samples with serum albumin <3 g/dL ($n = 115$)

TCa_calc (mmol/L)	*Formula*	*TCa (mmol/L)*	*p value (paired t test)*	*%Difference* ≤5%; number of samples (%)*	*%Difference* >5%; number of samples (%)*	*Mean% difference**
2.33 ± 0.29	TCa_meas	1.91 ± 0.22	<0.05	9 (7.8)	106 (92.2)	17.52
	TCa_corr_Orrell	2.09 ± 0.20	<0.05	36 (31.3)	79 (68.7)	9.16
	TCa_corr_ Payne	2.32 ± 0.20	<0.05	73 (63.5)	42 (36.5)	−1.05
	TCa_corr_ Berry	2.42 ± 0.20	<0.05	94 (81.7)	21 (18.3)	−5.18
	TCa_corr_ Clase	2.12 ± 0.20	<0.05	37 (32.2)	78 (67.8)	8.18

*Difference between TCa_calc and TCa_meas or TCa_calc and TCa_corr
All values are given as mean ± SD of the measured values. Value of $p < 0.05$ as predicted by paired *t* test is considered to be significant

Table 2B: Result of total calcium measured by direct ISEs (TCa_calc) and by oCPC (TCa_meas), and corrected by formulae (TCa_corr) in samples with serum albumin 3–4.99 g/dL ($n = 157$)

TCa_calc (mmol/L)	*Formula*	*TCa (mmol/L)*	*p value (paired t test)*	*%Difference* ≤5%; number of samples (%)*	*%Difference* >5%; number of samples (%)*	*Mean% difference**
2.49 ± 0.26	TCa_meas	2.19 ± 0.22	<0.05	26 (16.6)	131 (83.4)	11.71
	TCa_corr_Orrell	2.13 ± 0.19	<0.05	17 (10.8)	140 (89.2)	14.09
	TCa_corr_ Payne	2.26 ± 0.19	<0.05	41 (26.1)	116 (73.9)	8.64
	TCa_corr_ Berry	2.38 ± 0.19	<0.05	75 (47.8)	82 (52.2)	3.72
	TCa_corr_ Clase	2.14 ± 0.19	<0.05	17 (10.8)	140 (89.2)	13.41

*Difference between TCa_calc and TCa_meas or TCa_calc and TCa_corr
All values are given as mean ± SD of the measured values. Value of $p < 0.05$ as predicted by paired *t* test is considered to be significant

Table 2C: Result of total calcium measured by direct ISE (TCa_calc) and by oCPC (TCa_meas) and corrected by formulae (TCa_corr) in samples with serum albumin ≥5 g/dL ($n = 60$)

TCa_calc (mmol/L)	*Formula*	*TCa (mmol/L)*	*p value (paired t test)*	*%Difference* ≤5%; number of samples (%)*	*%Difference* >5%; number of samples (%)*	*Mean% difference**
2.57 ± 0.23	TCa_meas	2.36 ± 0.16	<0.05	23 (38.3)	37 (61.7)	7.56
	TCa_corr_Orrell	2.05 ± 0.17	<0.05	3 (5)	57 (95.0)	19.87
	TCa_corr_Payne	2.08 ± 0.17	<0.05	5 (8.3)	55 (91.7)	18.61
	TCa_corr_ erry	2.23 ± 0.17	<0.05	13 (21.7)	47 (78.3)	12.79
	TCa_corr_ lase	2.06 ± 0.17	<0.05	4 (6.7)	56 (93.3)	19.44

*Difference between TCa_calc and TCa_meas or TCa_calc and TCa_corr
All values are given as mean ± SD of the measured values. Value of $p < 0.05$ as predicted by paired *t* test is considered to be significant

using the formula given by Berry et al. The percentage difference between both these values was less than ±5%. In the subgroup with albumin <3 g/dL, formula by Payne et al. also gave good agreement. In the subgroup with albumin ≥5 g/dL, none of the formulae gave comparable results. Of all the formulae, correction of TCa_meas based on the formula given by Berry et al. showed the best agreement in all the groups.

Discussion

Methods used commonly to measure TCa levels include colorimetric analysis with metallochromic indicators, indirect potentiometry, and atomic absorption spectrometry (AAS). The TCa is most commonly measured using spectrophotometric determination of the colored complex when various metallochromic indicators or dyes bind to calcium. The o-CPC and arsenazo III are the two most widely used indicators. Atomic absorption spectrophotometry is the reference method for determining calcium levels in serum.

Free calcium is the metabolically active fraction of TCa. It is thus very important to understand the need of estimating FCa for managing critically ill patients with disorders of calcium metabolism[5] especially in settings of cardiac or renal disorder.[6,7] Since factors such as pH, albumin, and plasma proteins affect the distribution of calcium, a number of formulae are commonly used to correct serum calcium concentration. In this study, we report the agreement between TCa levels calculated from direct ISE results and TCa levels obtained by spectrophotometric methods after correction using formulae given in the literature. Our results showed significant differences between TCa obtained from both these methods. We further divided our samples into three groups based on the levels of albumin and saw that differences were still seen between the TCa from direct ISEs and oCPC method in all the groups. On using the various correction formulae, we found that formulae given by Berry et al. and Payne et al. gave the best results. Upon correction with formulae by Berry et al., the maximum number of samples showed agreement using the two methods. However, none of the formulae were able to give good agreement in the albumin >5 g/dL group. Overall it was seen that in around 70% cases, TCa and TCa_meas or TCa_corr could not be used interchangeably. In these cases, the percentage difference between serum values of TCa and TCa_meas or TCa_corr was ≥ ± 5%.

In previous studies, the lack of agreement between calcium measured and that corrected for albumin level, using various formulae was reported.[8,9] In a study by Ladenson et al., of the 13 published algorithms none produced agreement between corrected TCa and free calcium.[10] Another study suggests abandoning the use of these formulae to correct calcium in clinical practice.[11] A number of studies using different analytical methods for measuring serum albumin and serum calcium have been carried out in different groups of patients both adults and children. There are studies of neonates,[12] patients on hemodialysis,[13,14] hyperparathyroidism,[15] and other critically ill subjects.[16] Earlier studies have reported discordant results in ionized and TCa in these patients.[10] The reason for disagreement is the delicate equilibrium of free and bound calcium bound to albumin/protein that is affected by a number of factors. Therefore, it is important to use ionized calcium in the patients since it is the physiologically active form. Besides, interchangeable use of reports with calcium obtained from different methods while monitoring a patient should be avoided since the results do not agree. Although more accurate, precise, and automatic, determination of free (ionized) calcium is much more expensive in resource poor settings. Furthermore, coating of proteins on the electrodes often lead to higher maintenance cost, leave alone the erratic results. The measurement of ionic calcium is affected by pH changes. Ideally, sample for measurement of ionized calcium should be collected by filling the tube up to the brim and ensuring that the sample remains sealed until just before analysis.

This sample should be analyzed without any delay. If the venous samples are kept in the open, the pH in the sample elevates due to the loss of CO_2 into the environment.[17] These changes significantly alter the value of ionized calcium. Some instruments report the results normalized to pH value of 7.4. Another interference is the sodium ion that significantly affects the plasma ionized calcium levels by altering the binding of calcium ions to plasma proteins. Therefore, in conditions of hypo- or hypernatremia clinically significant alteration is seen in the levels of ionized calcium.[18]

On the contrary, higher throughput of automated analyzers can give calcium values for a number of samples in less time and at a low cost. The testing by oCPC is more resistant to variable transport and storage conditions to some extent. However, it does not give FCa and is affected by the interference of magnesium ions. Calcium bound to citrate in massive blood transfusion or to monoclonal proteins or immunoglobulins in myeloma patients causes discordance in total and ionized calcium levels by increasing the total serum calcium.

However, in spite of the low reliability of formulae for correction of serum calcium, these are being used frequently in clinical practice. Wherever required much caution should be exercised and variables affecting calcium equilibrium in the serum should be kept in mind.

Conclusion

Summarily, caution should be exercised while interpreting the laboratory reports of serum calcium levels from direct ISEs and by photometric methods. For monitoring of a patient, interchangeable use of both of these is not advisable.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent

This study compared measurements of parameters sent for routine investigations using two different technologies to rule out analytical discrepancies, which implies consent.

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