Ionized vs serum calcium in the diagnosis and management of primary hyperparathyroidism: which is superior?

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Abstract

BACKGROUND: The diagnosis of primary hyperparathyroidism (PHPT) is based on the presence of an elevated serum calcium level. The study objective was to compare ionized calcium levels to serum calcium levels with respect to parathyroid hormone level (PTH) and several patient outcomes.

METHODS: The study population comprised a retrospective cohort of 268 patients with PHPT who underwent primary parathyroidectomy. Serum calcium levels were compared with ionized calcium levels regarding their association with PTH level, presence of multiglandular disease, adenoma size, and extent of neck exploration.

RESULTS: Serum calcium level was correlated with ionized calcium level ($R^2 = .68$, 95% confidence interval [CI], .56 to .79; $P < .0001$) and PTH was associated with both serum ($R^2 = .19$; 95% CI, .04 to .33; $P = .012$) and ionized ($R^2 = .23$; 95% CI, .07 to .38; $P = .004$) calcium levels. Ionized calcium level was a more sensitive indicator of PHPT because there was a greater incidence of ionized calcium being elevated without concordant serum calcium elevation than vice versa ($P < .0001$). Ionized calcium was also more linearly associated with adenoma size than serum calcium ($P = .0001$). There were no differences between serum and ionized calcium levels in predicting the presence of multiglandular disease or the extent of neck dissection.

CONCLUSIONS: Serum calcium level is an appropriate first-line biochemical test for the diagnosis of PHPT. However, ionized calcium measurements may provide additional benefit in certain cases of PHPT because it is correlated with PTH level and adenoma size, and it may be a more sensitive marker of disease severity than serum calcium.

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Primary hyperparathyroidism (PHPT) is the most common cause of hypercalcemia.¹ With an incidence of approximately 1% in the adult population and increasing to 2% after age 55 years, PHPT is a relatively common endocrine disorder characterized by elevated parathyroid hormone (PTH) and calcium levels.²⁻⁴ PHPT is most commonly detected incidentally through routine biochemical laboratory testing, although symptoms that include nephrolithiasis, musculoskeletal complaints, neuropsychiatric disorders, and abdominal symptoms may occur.⁴,⁵ PHPT may arise because of a solitary parathyroid adenoma (90%), multiple parathyroid adenomas (5%), 4-gland...
hyperplasia (5%), or parathyroid carcinoma (<1%) that produce inappropriately elevated levels of PTH and cause hypercalcemia. For each of these causes, surgical excision of the pathological glands is the cornerstone of treatment and is the only option that allows an enduring cure.

Calcium homeostasis is normally tightly regulated, but in PHPT it is deranged by inappropriate production of PTH. PTH acts on the kidneys (decreasing urinary calcium excretion), gastrointestinal tract (increasing calcium absorption), and bone (stimulating resorption and breakdown of calcium), leading to hypercalcemia. In the body, circulating calcium exists as either serum calcium or ionized calcium. Although both serum calcium and ionized calcium may be quantified through routinely available biochemical methods, only the ionized calcium reflects the biologically active form of calcium in the body.

Serum calcium measurements have the advantage of allowing delayed processing and sample decapping, whereas ionized calcium measurements require anaerobic collection and more rapid sample processing, making them less practical for outpatient evaluation. However, the measurement of serum calcium is affected by serum proteins and pH, both of which may fluctuate and introduce inaccuracies. Although serum calcium and ionized calcium levels are correlated, regression comparisons show substantial scatter when they are measured concomitantly in patients with PHPT.

Currently, the diagnosis of PHPT is based upon elevated serum calcium levels (serum calcium >1.0 mg/dL or .25 mmol/L) rather than elevated ionized calcium levels, in addition to other criteria. However, there may be diagnostic and predictive advantages to measuring ionized calcium, when compared with serum calcium, in the setting of PHPT. Therefore, we hypothesized that ionized calcium is a superior measurement compared with serum calcium with respect to biochemical markers of PHPT (PTH levels and diagnostic sensitivity), pathologic characteristics of PHPT (number and size of parathyroid adenomas), and extent of surgical exploration.

Methods

Study design

This was a retrospective cohort study of 268 sequential parathyroidectomies for PHPT carried out at a single tertiary care center for patients who had never undergone prior parathyroid surgery. The objective of the study was to compare ionized calcium with serum calcium for biochemical, pathologic, and clinical outcomes. The primary outcome of interest was correlation with PTH as a biochemical marker of disease severity and diagnostic sensitivity. Secondary outcomes included association with clinical indicators of disease complexity (such as multiglandular disease and adenoma size) and extent of neck surgery defined as bilateral neck exploration (BNE) compared with unilateral neck exploration (UNE).

Data collection

Parameters that included patient demographics, preoperative biochemical studies (including all calcium measurements), operative findings (including intraoperative PTH measurements), extent of operative exploration (BNE vs UNE), adenoma size, postoperative biochemical studies (including all calcium measurements), and pathologic diagnoses were reviewed. The mean patient follow-up, as defined by the date of the last serum calcium measurement, was 140 days. All patients included had a minimum planned 30-day biochemical follow-up.

Laboratory analysis

Total calcium determinations were performed on the Siemens Advia 1650 and the Siemens Advia 1800 clinical chemistry analyzer (Siemens Medical Solutions, Malvern, PA). Ionized calcium determinations were performed in the Radiometer ABL 700 and ABL 800 (Radiometer, Ltd, West Sussex, UK) series of blood gas analyzers. Intact PTH determinations were performed on the Siemens Immulite 2000 and 2500 instruments (Siemens Medical Solutions) by 2-site chemiluminescent sandwich assay, and intraoperative intact PTH determinations were performed on the Roche Cobas e411 (Roche Diagnostics, Indianapolis, IN) by 2-site electrochemiluminescent sandwich assay. All assays were performed in accordance with manufacturer protocols.

Data analysis

The primary outcome of interest, correlation with PTH level, was analyzed by linear regression models. Serum calcium was plotted against ionized calcium to confirm a linear relationship and correlation. This was carried out to corroborate previous studies demonstrating this relationship and to verify integrity and quality of the data. PTH was plotted against serum calcium and ionized calcium levels. Linear regression relationships between PTH and both serum and ionized calcium were performed, and 95% confidence intervals (CIs) of regression parameters were obtained using bootstrapping methods.

The diagnostic sensitivity of ionized calcium was compared with that of serum calcium by evaluating all patients with PHPT with discordant calcium measurements. We examined patients who had high serum calcium levels (defined as being higher than the 50th percentile of the cohort) and low serum calcium levels (defined as being lower than the 50th percentile of the cohort) and vice versa. We further examined discordant cases by creating 3 tiers of calcium level within the cohort: (1) less than the 25th percentile, (2) between the 25th and 75th percentiles, and
(3) higher than the 75th percentile. The proportion of discordant cases was evaluated using the Pearson chi-square test.

Secondary outcomes were analyzed by a univariate unpaired \( t \) test and multivariate logistic regression. Regression analysis was performed on serum and ionized calcium measurements against the following variables: solitary vs multiple adenomas, adenoma size at the 50th percentile or higher vs lower than the 50th percentile, and UNE vs BNE during parathyroidectomy. Regression analysis was performed using calcium measurements as both continuous variables and categorical variables divided into quintiles. Multivariate logistic regression was carried out controlling for patient age and sex. All data analyses were performed using Stata, version 11.2 (Stata Corp, College Station, TX) and JMP Pro, version 9.0.2 (SAS Institute, Cary, NC). This study was approved by our institutional research ethics review board.

Results

Study patient demographics are summarized in Table 1. Linear regression models evaluating the biochemical relationship between PTH and calcium levels are summarized in Table 2. Although PTH demonstrated a linear relationship with serum and ionized calcium levels, neither measurement appears to be superior to the other based on overlapping 95% CIs (Table 2). Fig. 1 illustrates the linear relationship between serum calcium and ionized calcium levels. Although both serum and ionized calcium levels are highly correlated, there is a substantial amount of scatter between the 2 variables, suggesting that this relationship may not be perfectly precise (Fig. 1).

Figure 2 illustrates discordance between high ionized and low serum calcium levels and vice versa based on the following cutoffs: higher than the 50th percentile or lower than the 50th percentile and higher than the 75th percentile vs lower than the 25th percentile. When dichotomizing calcium measurements by the 50th percentile, there were 33 cases for which the ionized calcium level was elevated relative to the serum calcium level, compared with 22 cases in which the serum calcium level was elevated relative to the ionized calcium level (\( P < .0001 \)). The mean PTH level in the case of high ionized calcium levels and low serum calcium levels was 18.3 ± 3.8 pmol/L compared with 13.9 ± 1.1 pmol/L for the opposite scenario. When more precisely stratifying calcium levels, there were 2 cases for which the ionized calcium level was greater than the 75th percentile and the serum calcium level was lower than the 25th percentile, and there were no cases with the opposite scenario (\( P < .0001 \)). In this more precisely stratified analysis, we were unable to compare mean PTH levels because there were no cases of relatively high serum calcium levels and low ionized calcium levels. Notably, we identified 41 cases (15.3%) in which the serum calcium level was found to be normal or only marginally elevated, whereas the ionized calcium level was found to be significantly elevated, and surgical and pathologic evaluation of the resected parathyroid glands confirmed PHPT.

Table 3 summarizes the results of secondary outcome analyses comparing serum vs ionized calcium with respect to \( t \) test for adenoma type, \( t \) test for adenoma size, and logistic regression analysis predicting extent of surgery (BNE vs UNE). There does not appear to be a significant difference between ionized and serum calcium levels in predicting complex adenoma (multiglandular

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD or N (%)</th>
<th>Range</th>
</tr>
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<tbody>
<tr>
<td>Age (y)</td>
<td>58.8 ± 13.4</td>
<td>19–89</td>
</tr>
<tr>
<td>Female sex</td>
<td>202 (75.4%)</td>
<td>NA</td>
</tr>
<tr>
<td>Preoperative calcium (mmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2.75 ± .19</td>
<td>2.28–3.58</td>
</tr>
<tr>
<td>Ionized</td>
<td>1.52 ± .13</td>
<td>1.12–2.18</td>
</tr>
<tr>
<td>Postoperative calcium (mmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2.33 ± .18</td>
<td>1.31–3.28</td>
</tr>
<tr>
<td>Ionized</td>
<td>1.26 ± .09</td>
<td>.97–1.63</td>
</tr>
<tr>
<td>PTH (pmol/L)</td>
<td>16.7 ± 14.1</td>
<td>4.5–94.5</td>
</tr>
<tr>
<td>Adenoma type (solitary)</td>
<td>235 (87.7%)</td>
<td>NA</td>
</tr>
<tr>
<td>Adenoma size (cm)</td>
<td>1.66 ± .76</td>
<td>.5–4.5</td>
</tr>
<tr>
<td>Extent of surgery (BNE)</td>
<td>91 (34.0%)</td>
<td>NA</td>
</tr>
</tbody>
</table>

BNE = bilateral neck exploration; NA = not applicable; PTH = parathyroid hormone.
Serum calcium vs ionized calcium levels were further compared in prediction of adenoma size by univariate and multivariate (controlling for patient age and sex) logistic regression models. The results suggested that higher serum and ionized calcium levels may be predictive of increasing adenoma size for both models ($P < .0001$ for both the univariate models). When categorizing calcium data into quintiles, there appeared to be a direct linear relationship with a higher ionized calcium level and larger adenoma size. This was not observed for serum calcium levels. Although the result was not significant, it may suggest that the ionized calcium level is more predictive of adenoma size than is the serum calcium level.

Logistic regression models evaluating the extent of neck exploration were also extended to include a multivariate analysis controlling for patient age and sex. The multivariate analysis was consistent with the univariate analysis in demonstrating an association between increasing serum and ionized calcium levels with a decreased risk of BNE vs UNE. This result was not statistically significant for the multivariate model, which was consistent with the univariate analysis, and thus only the results of the univariate model are presented.

### Comments

The main findings of this study suggest that the ionized calcium level is highly correlated with the serum calcium level, but the scatter is much larger than one might intuitively predict (Fig. 1). These findings are consistent with a study reported by Burritt et al.\(^{12}\) Although the ionized calcium level appears to be better correlated with the PTH level than does the serum calcium level, this difference was not statistically significant. Ionized calcium appears to be a more sensitive marker of disease severity in PHPT, because there are increased incidences of relatively elevated ionized calcium levels compared with serum calcium levels than vice versa. Moreover, there appears to be a direct association of higher mean PTH levels for subgroups that have ionized calcium levels relatively higher than serum calcium levels. This result of ionized calcium being a more sensitive marker of disease severity was consistently observed through 2 separate analyses ($P < .0001$).

Both serum calcium and ionized calcium levels were directly correlated with adenoma size, although this direct relationship was more robust for ionized calcium than for serum calcium ($P < .001$). These results are also consistent with other reports in the literature that have demonstrated that preoperative calcium measurements are predictive of adenoma size.\(^{13,14}\)

Overall, our results suggest that there may be additional benefits to measuring ionized calcium levels compared with serum calcium levels because they appear to be well correlated with PTH levels and are potentially a more sensitive marker of disease severity. Ionized calcium is positively associated with adenoma size, and there are significantly more instances of ionized calcium levels being elevated when serum calcium levels are not increased to the same degree. Higher mean PTH levels in instances in which ionized calcium levels are relatively higher than serum calcium levels may suggest that ionized calcium is more

### Table 3 Secondary outcomes

<table>
<thead>
<tr>
<th>Calcium</th>
<th>Simple adenoma (95% CI)</th>
<th>Complex adenoma</th>
<th>$P$ value</th>
<th>Calcium</th>
<th>Adenoma &lt;1.5 cm</th>
<th>Adenoma ≥1.5 cm</th>
<th>$P$ value</th>
<th>Calcium</th>
<th>Odds ratio (BNE vs UNE)</th>
<th>95% CI</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum calcium (mmol/L)</td>
<td>2.75 ± .19 (2.72–2.77)</td>
<td>2.77 ± .19 (2.69–2.85)</td>
<td>.55</td>
<td>Serum calcium</td>
<td>.36</td>
<td>.08–1.50</td>
<td>.16</td>
<td>Ionized calcium</td>
<td>.16</td>
<td>.02–1.50</td>
<td>.106</td>
</tr>
<tr>
<td>Ionized calcium (mmol/L)</td>
<td>1.52 ± .13 (1.51–1.54)</td>
<td>1.51 ± .14 (1.45–1.57)</td>
<td>.61</td>
<td>Ionized calcium</td>
<td>1.48 ± .09 (1.46–1.50)</td>
<td>1.55 ± .15 (1.53–1.59)</td>
<td>.0001</td>
<td>Calcium Odds ratio (BNE vs UNE)</td>
<td>1.55 ± .15 (1.53–1.59)</td>
<td>1.55 ± .15 (1.53–1.59)</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

BNE = bilateral neck exploration; CI = confidence interval; UNE = unilateral neck exploration.
physiologically responsive to elevated PTH levels than is serum calcium. Alternatively, the results do not suggest that ionized calcium levels are significantly better than serum calcium levels at predicting multiglandular disease or an increased risk of BNE compared with UNE.

With respect to PHPT disease severity, the current study relied primarily on objective markers such as the serum and ionized calcium levels. It is possible that a more clinically based definition of disease severity could be more clinically relevant (ie, proportion of patients with symptomatic PHPT). However, there are several symptoms associated with PHPT, such as fatigue, abdominal pain, and neuropsychiatric complaints that are nonspecific and may be caused by other medical conditions. When comparing asymptomatic to symptomatic patients with PHPT, we did not identify any significant association with serum calcium level \((P = .70)\), ionized calcium level \((P = .78)\), or PTH level \((P = .99)\). This observation may be a consequence of the PHPT symptoms either representing a crude marker of disease severity or being confounded by other medical conditions.

There are specific advantages to measuring ionized calcium levels rather than serum calcium levels. Compared with serum calcium measurements, ionized calcium measurements account for physiologic fluctuations, particularly when corrections are made for pH. However, a typical patient with PHPT is usually a stable outpatient with incidentally discovered hypercalcemia without major physiologic stressors that would greatly impact pH and calcium levels. The ionized calcium level may be a more sensitive marker for diagnosing PHPT because it may detect more than half of patients with asymptomatic PHPT and normal or fluctuating serum calcium levels. There may be further incremental value in measuring the ionized calcium level as a recent study suggests that the combination of serum calcium and ionized calcium measurements for diagnosing PHPT achieves a sensitivity of 99% compared with 96% for either measurement alone. Our results are consistent with the findings reported in the literature. The specialized assay for ionized calcium measurement is faster than serum calcium measurement and may be collected as either an arterial or venous sample. Although ionized calcium measurements require dedicated instruments, specialized storage, and immediate processing, the test is no more technically challenging than serum calcium measurements in our hospital laboratory. At our center, ionized calcium measurements are $0.26 (Canadian dollars) more expensive than serum calcium measurements. This calculation takes into account analyzer depreciation, number of tests run on the analyzer, cost of receiving specimens, cost of reagents, and the cost for the operators involved. In addition to its lower cost, in the outpatient setting the serum calcium level may be transported and processed several hours after blood collection and thus may represent the more attractive elective screening test for PHPT.

There are several strengths and limitations of the current study that must be reviewed. The study limitations include its retrospective design, relatively small patient population, a lack of long-term follow-up data to assess disease recurrence, and diminished generalizability because of its single-institution bias. However, conducting the study at a single institution may also be considered a strength because there is minimized measurement error and bias with a single laboratory processing all samples under uniform biochemical protocols. In a study that compares biochemical tests, measurement protocol consistency is of critical importance. Another strength of the current study is that we derived our data analysis from a database with parameters that allow for examination of biochemical, pathologic, and clinical outcomes of interest in a population with surgically and pathologically confirmed PHPT.

In summary, there appear to be certain advantages to using ionized calcium measurements compared with serum calcium measurements in the evaluation of individuals diagnosed with PHPT. In addition to being a more rapid test, ionized calcium measurement is also highly correlated with PTH levels and adenoma size and are possibly a more sensitive diagnostic marker of disease severity. As a result, there may be additional benefits in using the ionized calcium level as an adjunct to the serum calcium level in specific clinical scenarios that may include borderline hypercalcemia, the need for rapid inpatient analysis of calcium levels, and for evaluation of patients experiencing significant physiologic stress. Further research into the added value of ionized calcium measurement ultimately may lead to improvements in the diagnosis, treatment, and outcomes of PHPT patients.

References


Discussion

Mark MacFarlane, M.D., Spokane, WA

Primary hyperparathyroidism is a relatively rare disease, but the diagnosis is straightforward based on corresponding elevated parathyroid hormone (PTH) and serum calcium levels. Despite this, the determination of when to operate, size of the tumor, and severity of symptoms are rather unknown before operation. The authors did a retrospective analysis of 268 patients with primary hyperparathyroidism at a single institution to determine if there was a difference between serum and ionized calcium with regard to PTH levels, multiglandular disease, adenoma size, severity of disease, or extent of exploration. Ionized calcium requires a dedicated machine, specialized handling of samples, and rapid analysis and is more expensive in our hospital. On the other hand, serum calcium is routine to measure. In this study, the authors found that ionized calcium was a better predictor of primary hyperparathyroidism when the discordance between serum calcium and PTH was greater. There was also a more linear association between adenoma size for ionized calcium as opposed to serum calcium. Both serum and ionized calcium correlated equally with PTH elevation, multiglandular disease, severity of disease, and extent of neck exploration. I have several questions for the authors.

1. Was ionized calcium a better predictor for the severity of disease, eg, osteoporosis, kidney stones, or other physical manifestations of the disease? How did you define severity of disease, since I do not see it specifically addressed in the manuscript?

2. Is there a difference between serum and ionized calcium with regard to recurrence or persistent primary hyperparathyroidism?

3. Does the increased cost to measure ionized calcium outweigh the benefits of its use? How do you propose to use ionized calcium in primary hyperparathyroidism in the future?

4. Were there instances in which you could not make a diagnosis of primary hyperparathyroidism based on serum calcium, but could on ionized calcium?