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Vitamin D Does Not Affect Intraoperative Parathyroid Hormone Kinetics: A Mixed Linear Model Analysis

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ABSTRACT

Background: Intraoperative parathyroid hormone (ioPTH) monitoring is used to confirm completeness of resection in patients undergoing parathyroidectomy for primary hyperparathyroidism (pHPT). Though there is an inverse relationship between vitamin D and parathyroid hormone (PTH), previous studies have suggested that 25-hydroxyvitamin D (25OHD) level does not affect the likelihood of meeting the Miami criterion. Here, we further investigate whether preoperative 25OHD level affects ioPTH kinetics.

Methods: This is a retrospective case-control study of patients undergoing parathyroidectomy for pHPT at a tertiary referral center. Patients were categorized based on preoperative 25OHD level as vitamin D deficient ($\leq$ 20 ng/mL), insufficient (21-30 ng/mL), or sufficient (>30 ng/mL). Differences in baseline characteristics were analyzed with Kruskal-Wallis H test or chi-square analysis. ioPTH kinetic curves were analyzed using a log-transformed mixed linear model with subject-level random effects. Significance was set at $P < 0.05$.

Results: Among 630 patients who met inclusion criteria, there was a significant difference in ioPTH between groups at baseline ($P < 0.001$), but not at any other time point. As a continuous variable, as well as a categorical variable, in a mixed linear model, vitamin D had no significant effect on ioPTH kinetics.

Conclusions: Despite a difference in preoperative and baseline PTH levels, preoperative 25OHD had no significant effect on ioPTH kinetics. Therefore, ioPTH assays can be used and interpreted uniformly, regardless of patients' vitamin D status.

Introduction

Primary hyperparathyroidism (pHPT) is a disorder that stems from the autonomous overproduction of parathyroid hormone (PTH). This overproduction of PTH leads to disruption of bone and mineral metabolism, usually resulting in elevated total serum calcium levels.[1] pHPT is caused by a single parathyroid adenoma in 80% of cases; less-common causes include double adenomas and four-gland hyperplasia. Surgical excision remains the only definitive treatment for this disease, and has been shown to provide long-term improvement in patient-reported quality of life.[2,3]

Deficiency of 25-hydroxyvitamin D (25OHD) is a common disorder, estimated to affect nearly 30% of US adults.[4] PTH and

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vitamin D form a tightly controlled feedback cycle, in which PTH stimulates vitamin D synthesis in the kidney, and vitamin D negatively regulates PTH secretion.[5] Compared to those without 25OHD deficiency, patients with coexisting 25OHD deficiency and pHPT have a more severe manifestation of the disease, with increased calcium, alkaline phosphatase, and parathyroid gland weight.[6,7] A pre-existing 25OHD deficiency also leads to elevated PTH levels after surgery,[8] and immediate postoperative supplementation with 25OHD reduces the incidence of PTH elevation.

Intraoperative PTH (ioPTH) monitoring allows for real-time assessment of parathyroid function and forms the basis for minimally invasive parathyroidectomy.[9-11] It has become widely adopted to guide parathyroidectomy and has a reported accuracy of approximately 98%.[12-15] Although ioPTH protocols vary by institution, many have adopted the use of the Miami criterion to predict successful resection.[13-16] In a common sampling protocol, a baseline PTH level is obtained after induction but before incision. A "time zero" measurement is obtained after identification of the presumed adenoma, but before ligation of the vascular supply, to control for any possible stimulation of PTH from manipulation of the gland during dissection. PTH levels are then obtained after the ligation of the vascular supply and excision of the gland in 5-min intervals. Based on the knowledge that the half-life of PTH is less than 5 min, the Miami criterion predicts surgical cure (defined as normal serum calcium and parathyroid hormone levels at 6 mo) when the PTH at 5 or 10 min after resection has decreased greater than 50% from the highest pre-excision level.[16-19]

Although ioPTH monitoring is now common, ioPTH kinetics and patient characteristics that may affect it remain poorly understood. Because 25OHD deficiency provides a stimulus for PTH secretion, it may cause persistent PTH elevation intraoperatively. This could lead to a more extensive operation if an inappropriately elevated ioPTH is unable to detect a true surgical cure. The effect of 25OHD deficiency on ioPTH kinetics is unclear, with previous studies differing as to whether 25OHD levels change not only the baseline PTH value, but also the rate of ioPTH decrease.[20,21] The objective of this study is to examine the relationship between 25OHD status and ioPTH kinetics in patients undergoing surgical treatment of pHPT.

Materials and methods

This is a retrospective case-control study of patients undergoing parathyroidectomy for primary hyperparathyroidism at a single, tertiary referral center. This study was approved by our Institutional Review Board, Protocol AAAL3823. Demographic, preoperative laboratory studies, ioPTH measurements, and postoperative laboratory data were collected. Patients were excluded if they underwent surgery for secondary hyperparathyroidism, tertiary hyperparathyroidism, had prior parathyroid surgery, or if they had incomplete collection of preoperative and postoperative calcium, intact parathyroid hormone, and 25OHD values. It is our practice to request 6-month postoperative calcium and intact parathyroid hormone laboratory values from patients' primary care physicians or endocrinologists, and final analysis was performed on only those patients who had normalization of their serum calcium and/or serum parathyroid hormone (in normocalcemic patients) at least 6 mo postoperatively, confirming surgical cure. We analyzed patients with both single- and multi-gland disease stratified by preoperative 25OHD levels. However, poststratification of patients with multigland disease resulted in very small patient cohorts with insufficient power to detect a statistical difference. Therefore, we present only the linear mixed models of patients with single-gland disease.

All ioPTH monitoring was performed using a standard institutional protocol. A "baseline" measurement was obtained after induction but before surgical incision. Once the adenoma was identified and dissected free of all surrounding tissue except for its vascular pedicle, a "time 0," pre-excision measurement was obtained. The vascular pedicle was then ligated and the gland excised. Additional measurements were then taken at 5 and 10 min after excision, then at additional intervals as needed. ioPTH monitoring was performed using the Stat-IO-I-PTH Assay (Future Diagnostics, Netherlands).

Univariable analyses of demographic, preoperative serum laboratory tests, baseline differences in ioPTH levels, and postoperative serum laboratory tests were performed using the Kruskal-Wallis H test and chi-square tests. To normalize the distribution of ioPTH serum measurements, the log-transformation of repeated ioPTH serum measurements was used. To account for patient-level variance in these measurements, repeated ioPTH serum measurements were modeled using a linear mixed model with 1) vitamin D levels as a continuous variable and 2) vitamin D as a categorical variable, with preoperative 25OHD level categorized as deficient ($\leq$ 20 ng/mL), insufficient (21-30 ng/mL), or sufficient (>30 ng/mL). To analyze whether vitamin D modified the change of ioPTH measurements over time, time was treated as a categorical variable and an interaction term between vitamin D and time was modeled. Both models only included the higher value between the first two ioPTH measurements (baseline or time 0), in addition to measurements at 5 and 10 min after gland excision. A third model comprising vitamin D as a categorical variable and preoperative serum calcium and PTH values, age (continuous variables), sex, and race/ethnicity (categorical variables) as covariates was also performed. Individual subjects were set as the random effects, covariates were set as the fixed effects parameters, and an unstructured covariance-variance matrix was used. Significance was set at $P < 0.05$. Statistical analyses were performed with SAS (SAS, Cary, NC) and STATA (StataCorp, College Station, TX) software.

Results

From April 2006 to October 2014, a total of 821 patients with primary hyperparathyroidism who underwent parathyroidectomy at Columbia University Medical Center were identified. One hundred ninety one patients were excluded because of missing data, secondary or tertiary hyperparathyroidism, or surgical failure. A total of 630 patients were included in the study. Table 1 summarizes the demographic and preoperative variables of the patient cohorts. Preoperative PTH levels were statistically different between vitamin

Table 1 – Patient demographics, preoperative, and postoperative variables.

Preoperative variable	Sufficient vit D 25-OH ≥30 ($n = 342$)	Insufficient 20 ≤vit D 25-OH<30 ($n = 200$)	Deficient vit D 25-OH < 20 ($n = 88$)	*P* Value
Age, y, median (IQR)	62 (54-70)	59 (50-69)	59 (51-66)	0.01
Female, *n* (%)	274 (80.1)	156 (78.0)	67 (76.1)	0.671
Ethnicity				0.067
White, *n* (%)	276 (90.8)	149 (84.1)	58 (76.3)	
Black, *n* (%)	18 (5.9)	18 (10.2)	10 (13.2)	
Asian, *n* (%)	3 (1.0)	4 (2.3)	3 (4.0)	
Hispanic, *n* (%)	4 (1.3)	5 (2.8)	3 (4.0)	
Other, *n* (%)	3 (1.0)	1 (0.6)	2 (2.6)	
GFR <60, *n* (%)	33 (9.7)	16 (8.0)	3 (3.4)	0.163
Osteoporosis, *n* (%)	97 (29.0)	40 (20.4)	26 (31.0)	0.232
Kidney stones, *n* (%)	65 (19.0)	42 (21.0)	20 (22.7)	0.694
Preop Ca, mg/dL, median (IQR)	10.7 (10.3-11.1)	10.8 (10.4-11.2)	11 (10.5-11.6)	<0.001
Preop 25-OH vit D, ng/mL, median (IQR)	41.9 (±12.7)	24 (22)	15 (11.5-18.0)	<0.001
Preop iPTH, pg/mL, median (IQR)	110 (77-155)	113 (83-158)	133 (92-181)	0.012
Multigland disease, *n* (%)	53 (15.5)	35 (17.5)	3 (3.4)	0.005
Postop Ca, 6 mo, mg/dL, median (IQR)	9.4 (9.2-9.7)	9.5 (9.2-9.8)	9.4 (9.2-9.7)	0.390
Postop iPTH, 6 mo, pg/mL, median (IQR)	37 (27-48)	40 (28-51)	41 (28-52)	0.237
Postop Ca, 1 y, mg/dL, median (IQR)	9.5 (9.2-9.7)	9.5 (9.2-9.7)	9.5 (9.1-9.6)	0.586
Postop iPTH, 1 y, pg/mL, median (IQR)	41 (30-49)	41 (30-50)	37 (28-46)	0.108

IQR = interquartile range.

D–deficient, –insufficient, and –sufficient patients (median 133, 113, and 110 pg/mL, respectively, $P = 0.012$). There was no significant difference in rates of renal insufficiency, kidney stones, or osteoporosis between the vitamin D status groups.

During ioPTH monitoring, there was a significant difference in PTH between groups at the baseline and the highest baseline value, but not at any other individual time points (Table 2). There was no difference in whether patients met Miami criterion at 5 min (68% of sufficient patients, 75% of insufficient patients, and 75% of deficient patients, $P = 0.15$) or 10 min (91% of sufficient patients, 94% of insufficient patients, and 98% of deficient patients, $P = 0.06$) after excision. Among vitamin D–deficient patients, 85 patients (97%) had single-gland disease, with the remainder having two glands removed.

All 630 patients included in the analysis had postoperative labs at least 6 mo after their procedure, confirming surgical cure. Median follow-up was 12 mo (range 6 to 62). There was no significant difference between vitamin D–deficient, –insufficient, and –sufficient patients in postoperative calcium or PTH at 6 mo ($P = 0.39$ and $P = 0.24$, respectively) or at 1 y ($P = 0.59$ and $P = 0.11$) (Table 1).

In a linear mixed model, with 25OHD as a continuous predictive variable of ioPTH measurements (Table 3), time is a significant predictor of change in ioPTH measurements, as expected. However, an interaction between 25OHD and time was not significant, indicating that the effect of time on change of ioPTH measurements is not modified by serum 25OHD levels.

Table 2 – Median intraoperative PTH levels of all patients, pg/mL (interquartile range).

Time	Vitamin D sufficient	Vitamin D insufficient	Vitamin D deficient	*P* Value
Baseline	129 (92-197)	161 (107-231)	178 (125-242)	<0.001
0 min	124 (69-215)	113 (68-202)	143 (78-256)	0.298
Higher value (baseline or time 0)	166 (108-248)	178 (121-271)	201 (130-379)	0.035
5 min	60 (34-113)	55 (35-93)	71 (40-121)	0.245
10 min	40 (25-67)	36 (24-64)	42 (25-70)	0.350

Table 3 – Linear mixed model with vitamin D 25-OH as a continuous variable.

Predictor variable	Estimate	Standard error	P-value
Intercept	5.274	0.085	<0.001
Vitamin D	−0.001	0.002	0.540
Time			<0.001
0	Ref	Ref	Ref
5	−1.151	0.057	<0.001
10	−1.591	0.056	<0.001
Vitamin D*Time			0.167
Vitamin D*0	Ref	Ref	Ref
Vitamin D*5	0.002	0.002	0.151
Vitamin D*10	0.003	0.002	0.070

Time 0 indicates highest value, either baseline (preincision) or immediately preexcision.
*Indicates "interaction with".

Table 4 models 25OHD as a categorical predictive variable of ioPTH measurements, with patients divided by preoperative vitamin D status as sufficient, insufficient, or deficient. Again, the interaction between 25OHD status and time in this model is not statistically significant, indicating that the effect of time on change of ioPTH measurements is not modified by vitamin D status when treated as a categorical variable.

Our final model includes 25OHD as a categorical predictive variable of ioPTH measurements, with the addition of age, sex, and race/ethnicity as covariates (Table 5). Again, in this model, time is an independent significant predictor of change in ioPTH, but the PTH and time interaction variable was not a significant predictor. This again demonstrates that preoperative PTH level does not affect the change in ioPTH over time. The predicted ioPTH curves from the model, stratified by vitamin D status, are shown in Figure.

Table 4 – Linear mixed model with vitamin D 25-OH as a categorical variable.

Predictor variable	Estimate	Standard error	P-value
Intercept	5.199	0.046	<0.001
Vitamin D			0.146
Sufficient	Ref	Ref	Ref
Insufficient	−0.004	0.076	0.957
Deficient	0.181	0.096	0.060
Time			<0.0001
0	Ref	Ref	Ref
5	−1.031	0.031	<0.001
10	−1.442	0.030	<0.001
Vitamin D*Time			0.084
Sufficient*time	Ref	Ref	Ref
Insufficient*0	Ref	Ref	Ref
Insufficient*5	−0.092	0.051	0.070
Insufficient*10	−0.106	0.050	0.034
Deficient*0	Ref	Ref	Ref
Deficient*5	−0.105	0.065	0.103
Deficient*10	−0.140	0.063	0.027

Time 0 indicates highest value, either baseline (preincision) or immediately preexcision.
*Indicates "interaction with".

Discussion

Excess secretion of PTH and the disruption of bone and mineral metabolism characterize pHPT. ioPTH monitoring is an essential tool to help guide minimally invasive parathyroidectomy for the treatment of pHPT. Vitamin D deficiency is a common disorder that can coexist with pHPT, but the potential effect of 25OHD level on ioPTH monitoring was previously unclear. In this study, we comprehensively examined the relationship between 25OHD status and ioPTH using a linear mixed model that accounts for repeated measures and patient-level variance.

Despite an expected difference in the baseline PTH level between groups, our analysis shows that, as both a continuous variable and a categorical variable, in both univariate and multivariate models, preoperative vitamin D status had no significant effect on change in ioPTH serum measurements over time. Moreover, this confirms that ioPTH assays can be

Table 5 – Multivariable linear mixed model with vitamin D 25-OH as a categorical variable.

Predictor variable	Estimate	Standard error	P-value
Intercept	4.717	0.162	<0.001
Age	0.007	0.002	0.004
Male sex	0.048	0.081	0.549
Race/ethnicity			0.369
White	Ref	Ref	Ref
Black	0.199	0.121	0.101
Hispanic	0.269	0.217	0.215
Asian	−0.073	0.240	0.762
Other	−0.060	0.321	0.852
Vitamin D			0.252
Sufficient	Ref	Ref	Ref
Insufficient	0.007	0.080	0.932
Deficient	0.190	0.103	0.067
Time			<0.001
0	Ref	Ref	Ref
5	−1.038	0.033	<0.001
10	−1.440	0.032	<0.001
Vitamin D*time			0.111
Sufficient*time	Ref	Ref	Ref
Insufficient*0	Ref	Ref	Ref
Insufficient*5	−0.090	0.054	0.095
Insufficient*10	−0.114	0.053	0.031
Deficient*0	Ref	Ref	Ref
Deficient*5	−0.111	0.069	0.106
Deficient*10	−0.133	0.067	0.048

Time 0 indicates highest value, either baseline (preincision) or immediately preexcision.
*Indicates "interaction with".

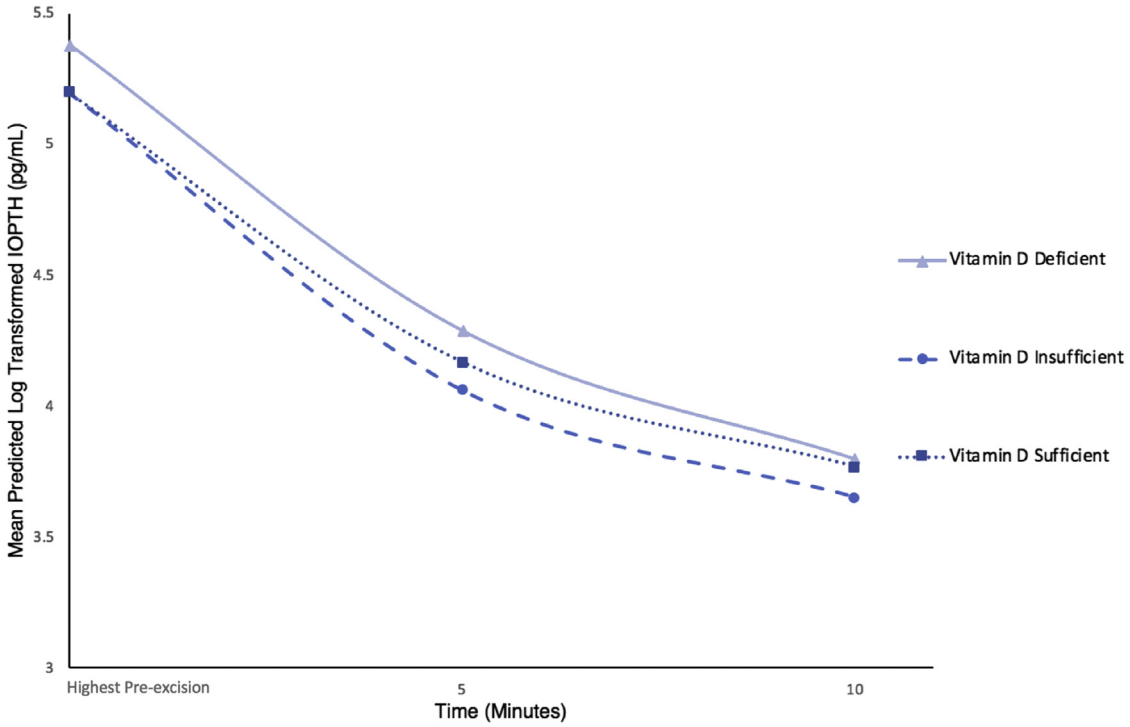

Fig – Predicted ioPTH curves from multivariable linear model. (Color version of figure is available online.)

used and interpreted uniformly, regardless of patients' vitamin D status.

Although the results of our study suggest that repletion of vitamin D is not necessary for use and interpretation of ioPTH monitoring in patients with pHPT undergoing parathyroidectomy, we do not believe this implies that repletion of vitamin D in patients with pHPT is unnecessary for all patients. Certainly, in patients with normocalcemic variant of pHPT, repletion of vitamin D is necessary to confirm the diagnosis and distinguish it from secondary hyperparathyroidism. There is also good evidence that vitamin D repletion helps decrease the rate of symptomatic hypocalcemia after surgery and should be considered for practices that do not routinely prescribe supplemental calcium post-operatively.[1]

Previous studies evaluating the effect of vitamin D status on ioPTH kinetics have focused on ioPTH as a binary outcome: whether or not the Miami criterion could be achieved. Although this provides guidance as to the clinical utility of ioPTH and use of the Miami criterion, these analyses fail to provide more detailed insight into how vitamin D status affects the rate of PTH decline and the shape of the ioPTH curve. The use of a linear mixed model allows for a greater understanding of how vitamin D affects or, as we found, does not significantly affect the change in ioPTH over time. In addition, the model accounts for patient-level variance, as ioPTH measurements are correlated within each patient, rather than being treated as independent observations.

Owing to its retrospective, observational nature, there are inherent limitations to our study. Although we have complete data for most of the data points collected, there were some missing variables, and some continuous variables had minimum or maximum value cutoffs that may have affected the analyses (i.e., <13 ng/mL for 25OHD was analyzed as 13 ng/mL in the continuous model). We were unfortunately unable to include data regarding preoperative planning (i.e., planned focused parathyroidectomy *versus* four-gland exploration), as well as intraoperative decision-making, and whether a change from preoperative plan took place. We attempted to minimize selection bias by including all patients with pHPT over our defined period. Although we analyzed patients with both single- (n = 539) and multi-gland (n = 91) disease, when stratified by preoperative vitamin D status, we found only three patients with multigland disease with preoperative vitamin D deficiency. Although a mixed linear model did not reveal any difference in ioPTH kinetics associated with the three vitamin D cohorts in patients with multigland disease, we lack sufficient power to avoid a type 2 error and therefore did not include this analysis. Finally, this is our single, tertiary referral institution's experience and may not reflect the prevalence and disease pattern of the wider population.

In conclusion, our study shows that preoperative vitamin D status does not affect ioPTH kinetics—the change in ioPTH measurements over time. Therefore, ioPTH assays can be used and interpreted uniformly regardless of a patient's preoperative vitamin D status.

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Disclosure

The authors have no proprietary or commercial interest in any product mentioned or concept discussed in this article.

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