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Paper

Parathyroid Glands response to Low Vitamin D levels in Healthy Adults: A Cross-Sectional Study

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Abstract

Objective: To assess the correlation of serum parathyroid hormone (PTH) and vitamin D (25-OHD) levels based on different assays for measuring 25-OHD in healthy Saudi Arabians living along the east coast.

Patients and Methods: A cross-sectional study was conducted in 200 patients (150 women and 50 men aged between 18-69 years) between January 2011 and December 2012, attending outpatient clinic at King Fahd Hospital of the University, Al Khobar. The first 200 patients seen without vitamin D supplementation at clinic were enrolled in the study. Serum calcium, phosphorous, alkaline phosphatase, parathormone, and 25-OHD tests were performed.

25-OHD was assessed using:

1. chemiluminescence immunoassay (CLIA)
2. radioimmunoassay (RIA) using Wallac 1470 Gamma Counter
3. HPLC –LC.MS (high performance liquid chromatography-liquid chromatography with mass spectrometry.

The data was collected, entered into a database and analysed using SPSS, Inc., version 14.

Results: The mean age was 45.8±15.8 (18-74) years, and calcium level was 2.27±0.15 mmol/l. (range 2.125 to 2.62 mmol/l). Alkaline phosphatase was 88.91±35.94 (34-302) IU, parathormone 6.7±3.06 (1.35-21.2) (1.3-6.8 pmol/l). Of the participants, 188 were either vitamin D insufficient or deficient as measured by CLIA 11.85±6.14 (2-29.6), and 91 (48.4%) of them had secondary hyperparathyroidism 9.48±4.55 pc/l. Those with normal CLIA-measured 25-OHD levels had normal PTH levels. Of those with insufficiency, 4/21 (19%) had raised PTH levels; and of those with deficiency, 81/166 (48.79%) had raised levels, whereas with HPLC-LC.MS, 156 were shown to be insufficient and 97 deficient (with PTH level of 7.41±4.2). Thirteen of 41 patients (31.7%) with insufficiency were shown, by HPLC-LC.MS, to have raised PTH. All patients with vitamin D deficiency as diagnosed by HPLC-LC.MS had secondary hyperparathyroidism.

Conclusions: The above results suggest that the method of measurement strongly influences vitamin D levels and that previous reports suggesting no association between vitamin D deficiency and secondary hyperparathyroidism should be viewed with caution.

INTRODUCTION:

Hyperparathyroidism is a disease entity that occurs due to increased secretion of parathyroid hormone (PTH) from parathyroid glands and causes hypercalcemia.[1] Secondary hyperparathyroidism is a response to low calcium levels related to hypovitaminosis D. It is well established that there is an inverse relationship between serum 25-hydroxyvitamin D (25-OHD) and serum PTH.[2,3] The levels of 25-OHD that lead to a rise in serum PTH are still a matter of debate.[4-6] Reports suggest that not all who are vitamin D insufficient have increased PTH levels.[7-9] It has been suggested that the variability of PTH levels in hypovitaminosis D may be due to concomitant magnesium deficiency.[9] Patel et al.[10] suggested that glomerular filtration rate is the single most important factor in maintaining PTH levels. Gunnarsson et al.,[11] while supporting the kidney function hypothesis, felt that body mass index may play a role in women by blunting the level of PTH and added that in men, insulin-like growth factor 1, smoking, and testosterone levels may do the same.

A diagnosis of secondary hyperparathyroidism will therefore depend on measurement of an insufficient or deficient amount of 25-OHD in association with a rise of PTH. The World Health Organization (WHO) described a serum level of 25-OHD of 20 ng/ml or 50 nmol/l as deficiency[12] and a level of 30 ng/ml (75 nmol/l) as normal because at this level, PTH drops down to normal levels.[13,14] At present, it is believed

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TABLE I:

Demographic characteristics of the patients

Variables	**Total (n=200)**	
	Male	***Female***
Sex	50 (25)	150 (75)
Age (years)	48.1±17.0 (14-76)	44.8±15.7 (3-80)
Calcium mmol/L	2.287±0.21	2.4±0.065
PO4mg/dl	3.7±0.6 (2.6-4.9)	4.0±2.9 (1.8-39.0)
Parathromone (pmol/L)	7.9±3.5 (3.2-19.8)	6.6±4.2 (1.4-43.9)
25-OHD (ng/mL)	13.3±7.2 (2.0-34.6)	14.0±14.0 (3.3-150.0)
Chemiluminescent immunoassays (ng/mL)	9.2±0.7 (8.3-13.1)	9.6±6.3 (7.9-86.0)
HPLC-D3 (ng/mL)	12.4±4.0 (6.0-25.0)	24.7±14.5 (12.0-150.0)

Values are presented as Mean ± SD or n (%); Range in Parenthesis

that below 30 ng/ml of 25-OHD, the level of PTH should start rising.[15-16]

A review of literature did not reveal any studies correlating PTH levels to the accuracy of 25-OHD levels by different assays; hence, our objective was to determine whether any correlation existed between the true levels of 25-OHD and PTH levels by means of three different assays.

PATIENTS AND METHODS:

A cross-sectional study was conducted in 200 patients (150 women and 50 men) between January 2011 and December 2012, aged between 18-69 years, attending the outpatient clinics at King Fahd Hospital of the University, Al Khobar, Saudi Arabia. The first 200 patients seen who were not taking vitamin D supplementation were included in the study.

TABLE II:

25-OHD levels with ≤20 ng/ml as deficiency, 21-29 as insufficiency and normal level as ≥30ng/ml.

Vitamin D Levels	Normal		Insufficiency		Deficiency	
	PTH levels		PTH levels		PTH levels	
	Normal	Raised	Normal	Raised	Normal	Raised
CLIA	12	0	18	4	80	86
RIA	18	0	6	22	52	102
HPLC	44	0	13	28	0	115

An earlier study had shown that there was wide variation between the three assays tested. -using a 30 ng/ml cut-off, 6%, 9%, and 22% had normal levels of 25-OHD in CLIA, RIA, and HPLC LC-MS, respectively, and showed different levels of hyperparathyroidism.[17] Patients' weight, height and results of renal and liver function tests were extracted from the medical charts. Serum calcium, phosphorous, alkaline phosphatase, PTH, and 25-OHD tests were performed. 25-OHD was assessed using chemiluminescence immunoassay (CLIA), radioimmunoassay using Wallac 1470 Gamma Counter, and HPLC-LC.MS (high performance liquid chromatography-liquid chromatography with mass spectrometry). The data were analysed using the Statistical Package for the Social Sciences (SPSS), version 14.0, Chicago, Illinois. Data is presented as a mean ± standard deviation (SD). Mean serum 25-hydroxyvitamin D values with 95% confidence intervals (CI) for each assay were calculated, and a p value of <0.05 was considered significant. Each was then compared with parathyroid hormone levels and labelled as normal, insufficient, and deficient in 25-OHD as determined by each assay using linear regression analyses for the relationship between age, sex, CLIA, RIA, HPLC-LS.MS and PTH levels and the correlation between PTH and CLIA, RIA, and HPLC-LS.MC.

RESULTS:

The mean age was 45.8±15.8 (18-74) years, and calcium level was 2.27±0.15 mmol/l. (range 2.125 to 2.62 mmol/l). Alkaline phosphatase was 88.91±35.94 (34-302) IU, PTH 6.7±3.06 (1.35-21.2) (1.3-6.8 pmol/l). Table 1 gives the demographic data of all the patients. Table 2 gives the comparison of 25-OHD between the three assays. Of the participants, 188 were either insufficient or deficient as shown by CLIA 11.85±6.14 (2-29.6), and 91 (48.4%) of them had secondary hyperparathyroidism 9.48±4.55 pc/l, whereas with HPLC-LC. MS, 156 were either insufficient or deficient, 97 with PTH level of 7.41±4.2. In patientswith insufficiency as shown by HPLC-LC-MS, 13/41 (31.7%) had raised parathyroid hormone.

All patients with vitamin D deficiency as diagnosed by HPLC-LC.MS had secondary hyperparathyroidism. Linear regression analyses for the relationship between the age, sex, BMI, creatinine level, and alkaline phosphatase, CLIA, RIA, and LC. MS against PTH levels are given in Table 3. It is clear from Table 3 illustrating linear regression that the beta coefficient of the regression of PTH on RIA is significant at beta=0.160, t=2.341, $p<0.05$ and HPLC-LC.MS beta=-0.228,

Table III:

Linear regression analyses for the relationship between the age, sex, BMI, Creatinine level and Alkaline Phosphatase, CLIA, RIA and HPLC-LC.MS against the Parathromone.

	Unstandardized Coefficients		R-square	F-value	t	P-value
	B	Std. Error				
(Constant)	7.965	1.767			4.509	.000
AGE	.018	.017			1.052	.294
SEX	-.022	.699			-.031	.975
BMI	.013	.015				
Creatinine level	.011	.010				
Alkaline Phosphatase	.011	008	0.151	5.551	1.361	.175
Chemiluminescent immunoassays	-.050	.050			-.995	.321
Radioimmunoassay	.160	.068			2.341	**.020**
High-pressure liquid chromatography-D3	-.228	.061			-3.714	**.000**

Dependent Variable: PARATHROMONE

t=-3.714, p<0.001 with r square of 0.151, and that there is a significant association between PTH and RIA and HPLC-LC. MS.

We further performed Pearson correlation analysis, showing the relationship between the variables, and the table depicts that PTH has a significant negative relationship with RIA (r = -0.247, p <0.001), HPLC-LC.MS (r=-0.322, p<0.001) and that RIA has a positively significant relationship with HPLC-LC.MS (r=0.946, p<0.001). Hence, there is a significant relationship between PTH and RIA, HPLC-LC.MS (Table 4).

Table IV:

Correlation between Parathormone and CLIA, RIA, HPLC-LC.MS.

		CLIA	RIA	HPLC
PARATHROMONE	r-value	-.054	**-.247**	**-.322**
	P-value	.451	.000	.000
CLIA	r-value	1	.045	.032
	P-value		.523	.654
RIA	r-value		1	**.946**
	P-value			.000
HPLC	r-value			1
	P-value			

DISCUSSION

Our study shows that there is a definite association between hyperparathyroidism and 25-OHD levels, but the assay chosen will influence the level of 25-OHD and hence classification of a patient as vitamin D deficient or insufficient. All patients with normal 25-OHD as assessed by all three assays, CLIA, RIA, and HPLC-LC.MS, had normal PTH levels. In patients with deficiency, PTH was raised in 51.8%, 66.23%, and 100% as assessed by CLIA, RIA, and HPLC-LC.MS, respectively.

Our study further supports others which indicate the inverse relationship of 25-OHD and PTH,[2,3,18,19] even though Kilicarslan, Cenoliaslan, and Gezgen[20] reported that in their study, that over 75% of the vitamin D-deficient patients had normal levels of PTH. In another study of a small number of patients by Elsammak et al.[21] PTH did not correlate with serum vitamin D level in either of the genders.

Studies have reported different influencing factors that raise PTH level and are not related directly to the level of 25-OHD. Moreover, Vučeljić et al[22] found that a raised PTH level is not seen in all patients with 25-OHD deficiency. In their study, only 25.3% with 25-OHD deficiency had raised PTH. On the other hand, Arabi et al[23] stated that the negative relationship between 25-OHD and PTH is modulated by age but not gender. Secondly, reports from the Middle Eastern countries indicate that an inverse relationship exists between vitamin D and PTH levels in all age groups.[24,25] In this study, we have noticed that the relationship between PTH and 25-OHD existed in all age groups and that gender does play a role. Our male population had significantly higher PTH levels when compared to the female population (7.9±3.5 versus 6.6±4.2 P<0.039), while the 25-OHD level was 13.3±7.2 versus 14.0±14.0 ng/ml (P<0.723), respectively.

Ardawi et al[26] in their study found that the inverse relationship between 25-OHD and PTH was not influenced by the level of 25-OHD and could not pinpoint the levels of 25-OHD at which PTH levels will plateau. From the studies cited above, it appears that various factors play a role in raising the PTH, but we believe that one important factor that increases PTH is a level of 25-OHD below 20ng/ml. The inconsistency of other reports of the relationship of PTH and 25-OHD may be due to the inaccuracy of the assay used in the assessment.

Our study has limitations as a small cohort group, and differences in the gender comparison were unequal; secondly, we also need to compare results from different PTH assays,

as well. The strength of our study is that we have tried for the first time to check the vitamin D levels with three different assays and simultaneously correlate PTH levels with different assays. The results of this study, we believe, should give a direction for future studies to correlate PTH levels with 25-OHD with a properly calibrated assay.

In conclusion, we found that serum PTH levels are normal with all 25-OHD assays of ≥30ng/ml, while issues lie in the accurate measurement of insufficiency and deficiency of 25-OHD. With HPLC-LC.MS, the gold standard to assess 25-OHD, all deficient patients had a raised PTH, while other assays showed normal PTH.

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