Surrogate markers for diagnosis of vitamin D deficiency

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Background/objectives
Vitamin D deficiency is becoming a pandemic problem. Hypovitaminosis-D is diagnosed by measuring 25-hydroxyvitamin D in blood. In Egypt this costs 500 EGP, whereas one ampoule containing 200 000 units of vitamin D costs 5 EGP. Therefore, we need markers for vitamin D deficiency that are affordable.
Materials and methods
We conducted a cross-sectional study on 90 healthy patients aged 20–60 years during spring and summer. Participants underwent history taking, clinical examination, and measurements of hemoglobin, creatinine, calcium (Ca) (total and ionized), phosphorus, magnesium, parathyroid hormone (PTH), and 25-hydroxyvitamin D.
Results
The prevalence of vitamin D deficiency (<20 ng/ml) was 73.33%, that of insufficiency (21–30 ng/ml) was 25.56%, and that of vitamin D sufficiency (>30 ng/ml) was only 1.11% in the samples tested. PTH had a significant inverse correlation with vitamin D level (r=–0.2), whereas serum Ca (total and ionized) and phosphorus had a positive correlation. By receiver operating characteristic curve the predictive accuracy of PTH was 70%, whereas that of total Ca was 38%, ionized Ca was 43%, and phosphorous was 60.7%.
Conclusion
Measurements of PTH, Ca, and phosphorus can be used as markers for vitamin D deficiency; these tests cost less than 200 EGP, resulting in 60% savings in the cost of diagnosis of this widely prevalent condition.

Keywords:
calcium, Egypt, phosphorous, parathyroid hormone, vitamin D

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Introduction

Vitamin D is a hormone that modulates a wide range of cellular functions like musculoskeletal effects [1,2]. Extraskeletal benefits on the immune system and cardiovascular system have also been described [2,3], as well as decreased mortality [4]. Hypovitaminosis-D defined as 25-hydroxyvitamin D [25(OH)D] level below 30 ng/ml is prevalent worldwide [5]. Predictors include older age, female sex, winter season, darker skin pigmentation, and less sun exposure [5]. Asia and the Middle East are at great risk [6,7]. Discrepancy exists between the cost of diagnosis and treatment of this condition. Because of the high prevalence of vitamin D deficiency and high cost of diagnosis, markers are needed to identify this condition.

Aim

The aim of this study was to investigate markers for vitamin D deficiency that can be used in large sectors of society at low cost to diagnose this widely prevalent condition with a good cost–benefit ratio.

Materials and methods

This cross-sectional study was conducted on 90 (34 men and 56 women) healthy adults aged 20–60 years. Participants were recruited from relatives of patients, workers, and medical staff in the Endocrinology and Diabetes Department of Ain Shams University Hospital. Samples were collected from participants living in Cairo (30.0500 N°, 31.2333 E° latitude) during spring and summer from April 2013 to August 2013. This study was approved by the local ethical committee and written consent was obtained from all participants. All participants underwent medical history taking, with emphasis on sun exposure, clothing pattern, dietary intake, and supplement intakes of calcium (Ca) and vitamin D. General clinical examination was performed, including measurement of blood pressure, pulse, temperature,

weight, and height. Patients with chronic systemic diseases or those taking drugs affecting Ca levels, such as antiepileptic drugs and glucocorticoids, were excluded from the study.

Laboratory studies

Laboratory studies included tests for hemoglobin (Hb), serum creatinine, Ca (total and ionized), phosphorus, magnesium (Mg), intact parathyroid hormone (iPTH), and 25(OH)D levels. 25(OH)D was measured using radioimmunoassay, whereas iPTH was assayed using immunoenzymetric assay.

Sampling and analysis

All patients who completed the assessment had fasting blood samples drawn in the morning. Blood samples were collected by venipuncture in a 5 or 10 ml evacuated glass tube. They were allowed to clot at room temperature (15–25°C) and then centrifuged for 15 min and stored at −20°C.

We classified our results regarding vitamin D status according to the Endocrine Society guidelines for 2011 into the following:

Group I: vitamin D-deficient group, with vitamin D level below 20 ng/ml.
Group II: vitamin D-insufficient group, with vitamin D level between 20 and 29 ng/ml.
Group III: vitamin D-sufficient group, with vitamin D level above 30 ng/ml.

In our study only one person had vitamin D level above 30 ng/ml.

According to sex the patients were classified into group M (men) and group F (women).

According to BMI the participants were classified into group N (nonobese), with BMI less than 30, and group O (obese), with BMI of at least 30.

Statistical analysis

After the collection, revision, and tabulation of data, statistical analysis was performed using the SPSS program, version 21. Continuous data were expressed as mean±SD. The Student *t*-test was used to compare parametric and quantitative variables between groups. All quantitative data were correlated with each other using Pearson's correlation coefficient (*r*). Assessment of the predictive value was performed for Ca, phosphate (PO_4), ionized Ca, Ca×PO_4 product, and PTH level for detection of vitamin D deficiency with estimation of the most powerful cutoff point for each parameter and plotting of receiver operating characteristic curves. Probability (*P*-value) less than 0.05 was considered significant and values less than 0.01 were considered highly significant.

Results

In our study, the proportion of participants with vitamin D deficiency was 25.5%, those with insufficiency was 73.3%, and those with sufficient levels was 1.1%.

On comparing the vitamin D-deficient group with the vitamin D-insufficient group, a highly significant difference was found in PTH level, but there was no significant difference in age, Hb level, creatinine, total Ca, ionized Ca, PO_4, Ca–PO_4 product, Mg, and BMI (Table 1).

On comparing group M (males) with group F (females), a highly significant difference was found in Hb and a significant difference in creatinine, PO_4, and Ca–PO_4 product, but there was no significant difference between

Table 1 Comparison between groups I and II

Items	Group I (vitamin deficient) (*n*=70) (mean±SD)	Group II (vitamin D insufficient) (*n*=19) (mean±SD)	*t*	*P*
Age (years)	30.9±9.8	34.9±12.2	−1.5	0.1
BMI (kg/m^2)	27.6±5.7	26.4±3.9	0.9	0.4
Hb (gm/dl)	13.3±1.4	13.9±1.2	−1.6	0.1
Creatinine (mg/dl)	0.6±0.1	0.6±0.2	−1.1	0.3
Total calcium (mg/dl)	9.8±0.5	9.7±0.4	1.3	0.2
Ionized Ca (mg/dl)	4.8±0.3	4.8±0.3	0.5	0.7
PO_4 (mg/dl)	3.9±0.7	4.1±0.5	−1.2	0.2
Ca–PO_4 product	37.9±7.7	39.3±4.9	−0.8	0.5
Mg (mg/dl)	2.1±0.2	2.1±0.2	−0.4	0.7
PTH (pg/ml)	59.0±9.3	51.5±9.6	3.1	0.003*
Vitamin D (ng/ml)	11.9±4	21±1.4		0.000**

Ca, calcium; Hb, hemoglobin, Mg, magnesium; PO_4, phosphate; PTH, parathyroid hormone; Vit D, vitamin D. *Significant. **Highly significant.

them regarding age, BMI, total Ca, ionized Ca, Mg, PTH, and vitamin D (Table 2).

On comparing group N with group O, a highly significant difference was found in age and significant difference in Ca, PO_4, and Ca–PO_4 Product, but there was no significant difference in Hb, BMI, creatinine, ionized Ca, Mg, PTH, or vitamin D (Table 3).

On correlating different parameters with each other, a significant negative correlation was found between vitamin D and PTH, a highly significant positive correlation between BMI and age and between Ca and ionized Ca, and a significant positive correlation between Hb and creatinine, age, and PO_4 and between BMI and PO_4; however, a nonsignificant correlation was found between other parameters (Table 4).

An receiver operating characteristic curve was plotted to assess the predictive accuracy of PTH, total Ca, ionized Ca, phosphorous, and calcium phosphorous product as markers for vitamin D status (Table 5).

Discussion

In our study 70/90 participants were vitamin D deficient; the prevalence of vitamin D deficiency was 77.8%. Nineteen of 90 participants were vitamin D insufficient; the prevalence was 21.1%. Thus, the prevalence of hypovitaminosis-D among healthy people was about 99% according to our study.

This goes in line with our previous study that investigated the prevalence of vitamin D deficiency among 404 healthy female participants in different age groups in Cairo and Port Said. That study revealed a high prevalence of hypovitaminosis-D: 72.6% in the nursing group, 54% in the pregnant group, 72% in the group in the childbearing age, 39.5% in the elderly group, and 77.2% in the geriatric group [8].

We found that PTH was a good predictor of vitamin D deficiency. Our results agree with those of studies that found a negative correlation between iPTH and 25(OH)D at serum 25(OH)D concentrations less than 30 ng/ml. They also found that for every increase in serum 25(OH)D

Table 2 Comparison between group M (males) and group F (females)

Items	Group M (*n*=34) (mean±SD)	Group F (*n*=56) (mean±SD)	*t*	*P*
Age (years)	31.8±9.9	31.9±10.7	−0.08	0.9
BMI (kg/m^2)	27.0±5.1	27.7±5.6	−0.6	0.6
Hb (gm/dl)	14.3±1.1	12.9±1.2	5.9	0.000**
Creatinine (mg/dl)	0.7±0.2	0.5±0.1	2.8	0.008*
Total calcium (mg/dl)	9.8±0.5	9.7±0.5	0.7	0.5
Ionized Ca (mg/dl)	4.8±0.2	4.8±0.3	−0.3	0.7
PO_4 (mg/dl)	4.2±0.7	3.7±0.6	2.9	0.004*
Ca–PO_4 product	40.8±6.9	36.4±6.9	2.9	0.005*
Mg (mg/dl)	2.1±0.2	2.1±0.2	−0.04	0.9
PTH (pg/ml)	55.4±8.4	58.5±10.5	−1.5	0.1
Vitamin D (ng/ml)	15.1±5.7	13.4±5.4	1.4	0.1

Ca, calcium; Hb, hemoglobin; Mg, magnesium; PO_4, phosphate; PTH, parathyroid hormone. *Means significant. **Means highly significant.

Table 3 Comparison between group N (nonobese) and group O (obese)

Items	Group N (*n*=62) (mean±SD)	Group O (*n*=28) (mean±SD)	*t*	*P*
Age (years)	29.3±9.7	37.6±9.6	3.8	<0.001**
Hb (gm/dl)	13.5±1.2	13.1±1.6	1.3	0.2
Creatinine (mg/dl)	0.6±0.2	0.6±0.2	0.7	0.5
Total calcium (mg/dl)	9.9±0.5	9.5±0.5	2.9	0.004*
Ionized Ca (mg/dl)	4.8±0.3	4.7±0.2	1.0	0.3
PO_4 (mg/dl)	3.9±0.6	3.7±0.7	2.1	0.04*
Ca–PO_4 product	39.4±6.7	35±7.6	2.6	0.01*
Mg (mg/dl)	2.1±0.2	2.1±0.2	0.02	0.9
PTH (pg/ml)	57±9.5	58±10.6	0.6	0.6
Vitamin D (ng/ml)	14.3±5	13.5±6.6	0.6	0.6

Ca, calcium; Hb, hemoglobin; Mg, magnesium; PO_4, phosphate; PTH, parathyroid hormone. *P<0.05, significant. **P<0.001, highly significant.

Table 4 Correlation between different parameters

Parameters	Vitamin D	PTH	Mg	PO_4	Ionized Ca	Ca	Creatinine	Hb	BMI	Age
Age (years)	0.1	0.000	−0.03	−0.2*	0.08	−0.2	0.02	−0.09	0.4**	1
BMI (kg/m^2)	−0.1	0.03	−0.1	−0.3*	0.01	−0.2	−0.1	−0.1	1	*0.001*
Hb (gm/dl)	0.2	0.07	−0.01	0.2	−0.04	0.2	0.2*	1	*0.2*	*0.4*
Creatinine (mg/dl)	0.04	−0.08	−0.1	0.2	0.001	0.08	1	*0.04*	*0.2*	*0.8*
Ca (mg/dl)	−0.04	0.07	0.02	0.2	0.7**	1	*0.4*	*0.06*	*0.08*	*0.1*
Ionized Ca (mg/dl)	−0.09	0.03	−0.08	−0.2	1	*0.000*	*0.99*	*0.7*	*0.9*	*0.5*
PO_4 (mg/dl)	0.2	−0.08	0.09	1	*0.2*	*0.09*	*0.2*	*0.08*	*0.01*	*0.04*
Mg (mg/dl)	0.2	0.1	1	*0.4*	*0.5*	*0.8*	*0.2*	*0.9*	*0.3*	*0.8*
PTH (pg/ml)	−0.2*	1	*0.2*	*0.5*	*0.8*	*0.5*	*0.5*	*0.5*	*0.8*	*0.9*
Vitamin D (ng/ml)	1	*0.045*	*0.1*	*0.051*	*0.4*	*0.7*	*0.7*	*0.2*	*0.3*	*0.3*

Ca, calcium; Hb, hemoglobin; Mg, magnesium; PO_4, phosphate; PTH, parathyroid hormone. *P<0.05, significant. **P<0.001, highly significant. The italic values indicates *P*-value.

Table 5 The predictive accuracy of PTH, total Ca, ionized Ca, PO_4, and Ca×PO_4 product as markers for vitamin D status

	Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Predictive accuracy (%)
PTH	51	85	45	84.5	47.3	70
Total Ca	9.5	31.4	60	73.3	20	38
Ionized Ca	4.6	21	70	75	20	43
PO_4	3.5	30	75	80	23.4	60.7
Ca×PO_4 product	40	71	55	84	35	59.2

Ca, calcium; NPV, negative predictive value; PO4, phosphate; PPV, positive predictive value; PTH, parathyroid hormone.

of 1 ng/ml, there was a 1.03 pg/ml decrease in serum iPTH level after adjustments for sex, race, age, total Ca intake, and duration of Ca intake. This relationship was not observed at 25(OH)D levels of at least 30 ng/ml [9,10].

It was noted that hypovitaminosis-D may coexist with a blunted PTH response and hence not all patients with hypovitaminosis-D develop secondary hyperparathyroidism. The mechanism underlying the blunted PTH response is unclear but may be related to Mg deficiency [11,12].

Although the inverse relationship between PTH and vitamin D is well known, it can be modulated by other variables such as age, sex, and BMI. We investigated the effect of these variables on the 25(OH)D/PTH relation within the same population. We found that there was no significant correlation between age and PTH, nor between age and vitamin D level. Other studies found that 25(OH)D levels were lower and PTH levels were higher in elderly participants compared with young adults [13]. This could be because elderly individuals have lower 25(OH)D levels compared with young participants because of decreased cutaneous production of vitamin D with aging [14].

As regards sex and its effect on 25(OH)D/PTH we found no significant difference between the male group and the female group in PTH or vitamin D levels. This is not in line with other studies, which reported higher 25(OH)D levels and lower PTH in men in comparison with women throughout the year [15]. This interesting phenomenon may be due to differences in adiposity between men and women with the same BMI [16].

As regards BMI, our study showed no significant difference between obese and nonobese groups in PTH and vitamin D levels, and no correlation between PTH and BMI or vitamin D and BMI. This goes in line with studies that reported that PTH was not significantly correlated with any of the indices of obesity [17]. Other studies found that the association of adiposity with serum 25(OH)D is stronger than that with body weight and BMI [18]. The reason for this phenomenon may be that BMI and body weight do not necessarily reflect the percentage of body fat. Athletes may have relatively high BMI and can be considered overweight or obese despite having quite low total fat mass [19].

In contrast, some studies found that obese patients had significantly lower 25(OH)D concentrations and higher PTH concentrations compared with age-matched controls [20,21], which could be due to increased sequestration of vitamin D in subcutaneous adipose tissue [22].

As regards other bone biochemical markers that could reflect vitamin D status, such as total Ca, ionized Ca, and phosphorous, we found no significant difference between the vitamin D-deficient group and the vitamin D-insufficient one in total Ca, ionized Ca, and phosphorous, and on correlating them to vitamin D levels there was no significant correlation between vitamin D and total or ionized Ca.

This is in agreement with a previous study that had three groups: 25(OH)D-deficient group [25(OH) D<50 nmol/l] (90% of the sample), 25(OH)D-insufficient group (5% of the sample), and vitamin D-sufficient group (5% of the sample). It found no significant difference in mean ionized Ca results among the three groups and there was poor negative correlation between ionized Ca and 25(OH)D levels [23]. In a study conducted on clinical patients low serum Ca levels were found only with severe vitamin D deficiency; the levels remained normal in patients with mild and moderate deficiency [24].

Conclusion

Our results revealed that PTH can be used as a surrogate marker for vitamin D to reflect its status (hyperparathyroidism despite normal Ca and creatinine) after taking into consideration the blunted PTH response that may coexist with hypovitaminosis-D due to Mg deficiency. Age and BMI also should be taken into consideration as they can modulate the relationship between PTH and 25(OH)D.

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Conflicts of interest

There is no conflict of interest.

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