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Assessment of vitamin D deficiency level by the physiological response of parathyroid hormone in Turkish adults

Erişkin Türk toplumundaki D vitamini eksiklik sınır değerinin paratiroid hormonun fizyolojik yanıtı ile belirlenmesi

Abstract

Objective: We aimed to contribute to the literature by determining deflection point of parathyroid hormone (PTH) level compared to 25-hydroxy vitamin D3 (25(OH)D3) level for determining the cut-off value of vitamin D deficiency level in Turkish adults.

Methods: The levels of 25(OH)D3 and intact parathyroid hormone (iPTH) which were requested simultaneously in 1 year of 1684 adults were evaluated retrospectively. 25(OH)D3 levels were first classified as 80–50, 50–30, 30–25, 25–20, 20–15, 15–10, 10–5, 5–0 ng/mL and iPTH levels among those groups were compared.

Results: First significant difference in iPTH levels was determined between 25(OH)D3 groups of 80–50 and 50–30 ng/mL (p = 0.007). Second and third significant differences were determined between 25(OH)D3 groups of 15–10 and 10–5 ng/mL and between 10–5 and 5–0 ng/mL, respectively (p = 0.006 and p = 0.035, respectively). There were no differences in iPTH levels among seasons (p = 0.11).

Conclusion: In the light of these findings; we can state that iPTH levels are suppressed when 25(OH)D3 levels in over 50 ng/mL, remains stable when 25(OH)D3 levels in between 50–10 ng/mL and gives the first increase response when 25(OH)D3 falls below 10 ng/mL. We believe that cut-off value for vitamin D deficiency in Turkish adults at all seasons depending on PTH response should be used as 10 ng/mL.

Keywords: Vitamin D; Vitamin D deficiency; Parathyroid hormone; Seasonal; Calcium.

Özet

Amaç: Paratiroid hormon (PTH) düzeyinin, 25-hidroksi vitamin D3 (25(OH)D3) düzeyine göre deﬂeksiyon (sapma) noktasını saptayp, erişkin Türk toplumundaki D vitamini eksiklik sınır değerinin tespit edildiğinde tespit edip literatürü katkıda bulunmayı amaçladık.

Yöntem: Bir yıllık sürede 1684 erişkinin eş zamanlı olarak istenmiş ve çalışılmış olan 25(OH)D3 ve intakt paratiroid hormon (iPTH) sonuçları retrospektif olarak değerlendirildi ve iPTH düzeyleri arasındaki farkların meydana geldiği gruplar belirlendi. 25(OH)D3 düzeyleri ilk olarak; 80–50, 50–30, 30–25, 25–20, 20–15, 15–10, 10–5, 5–0 ng/mL olarak sınıflandırıldı ve bu gruplar arasındaki iPTH düzeyleri karşılaştırıldı.

Bulgular: iPTH düzeylerindeki ilk anlamlı farklılık, 25(OH)D3 düzeyleri 80–50 ile 50–30 ng/mL gruplarında tespit edildi (p = 0.007). İkinci ve üçüncü anlamlı farklılıklar ise sırasıyla; 25(OH)D3 düzeyleri 15–10 ve 10–5 ng/mL ile 10–5 ve 5–0 ng/mL grupları arasında saptandı (sıradaşı; p = 0.006 ve p = 0.035). Mevsimlere göre iPTH düzeyleri arasında anlamlı bir fark bulunmadı (p = 0.11).

Sonuç: Bu bulgular içişinde iPTH düzeylerinin; 25(OH)D3 düzeyleri 50 ng/mL’nin üstüne çıktığında baskılandığını, 50–10 ng/mL arasında iken stabil kaldığini, 10 ng/mL’nin altında indiğinde ise ilk artış yanıtını verdiği ifade edebiliriz. PTH yanıtına bağlı olarak, erişkin Türk toplumundaki
Introduction

As the information on the physiological effects of vitamin D and its roles in other metabolic pathways have increased, it has become more important to determine and evaluate vitamin D levels [1]. Vitamin D deficiency and its effects on human health is still a hot topic which is in the focus of interest of the entire world [2–4]. However, the definition of vitamin D deficiency has not been fully demonstrated yet [5]. The most appropriate parameter indicating the D vitamin status of the individual is accepted as the 25-hydroxy vitamin D (25(OH)D) [5].

Traditionally, vitamin D deficiency is defined as serum 25(OH)D level <10 ng/mL (<25 nmol/L) because the values below this level are associated with rickets or osteomalacia [6–8]. World Health Organization (WHO) defined vitamin D levels below 20 ng/mL (50 nmol/L) as insufficiency and vitamin D levels below 10 ng/mL (25 nmol/L) as deficiency [9]. However; Pediatric Endocrine Society, Endocrine Society and the American Institute of Medicine organizations have adopted different vitamin D insufficiency and deficiency values ranging between 10 ng/mL (25 nmol/L) and 30 ng/mL (75 nmol/L) [10–12].

In studies conducted in adults; these values are usually based on association of vitamin D levels with fracture risk, intestinal calcium absorption or bone mineral density [2, 3, 13–16]. In addition to these studies, when the selected values fall below this levels with their definitions varying in the range of 25(OH)D level 15–30 ng/mL (37.5–75 nmol/L), it is supported metabolically to observe increasing serum parathyroid hormone (PTH) levels [14, 16–19].

It is well known that there is an inverse relationship between 25(OH)D and serum PTH levels [14, 20]. Therefore, the deflection point of PTH level compared to the 25(OH)D level will give a good information in terms of sufficiency and deficiency definitions of vitamin D. Some studies in which vitamin D deficiency and insufficiency cut-off values are defined clinically are based on levels maximal suppression of PTH by 25(OH)D as it is the most crucial system providing calcium regulation in the body [1].

Responding to the need of studies on the relationship with vitamin D levels and parathyroid hormone in terms of vitamin D deficiency definition, we have planned this study in adults living in Turkey. We aimed to contribute to the literature by determining the deflection point of intact parathyroid hormone (iPTH) level compared to 25(OH)D level for determining the cut-off value of vitamin D deficiency in Turkish adults.

Materials and methods

Study design and population

The results of the patients between 18 and 65 years of age who had calcium (Ca), inorganic phosphate (P), creatinine, iPTH and 25-hydroxy vitamin D₃ tests at inpatient and outpatient clinics of Okmeydani Training and Research Hospital between the dates of 01/03/2015 and 01/03/2016 which were requested simultaneously and performed in Okmeydani Training and Research Hospital Medical Biochemistry laboratory were scanned retrospectively via laboratory software system (ALIS, Ventura Software Inc., Ankara, Turkey). For our study, ethical approval dated 17.05.2016 with the number 478 has been received from the Ethics Committee of our hospital.

In order to demonstrate the relationship between vitamin D and iPTH more accurately, 1684 (1428 female and 256 male) patients who had iPTH levels within reference ranges together with Ca, P and creatinine which affects this system of the tests requested simultaneously and whose 25(OH)D₃ levels were below 80 ng/mL (200 nmol/L) were included in the study. Thus, a patient with parathyroid diseases and/or kidney functional disorders that may cause misinterpretation of our relationship between iPTH and vitamin D has been excluded. Diseases such as osteopenia/osteoporosis which are caused by vitamin D insufficiency or deficiency have been included as they are not a reason but a result of vitamin D deficiency. We were not able to get the patient’s information about whether they use vitamin D or not due to the retrospective nature of the study.

Analytes

Serum Ca level

It was measured photometrically by using CA2 kit (Roche Diagnostics, Mannheim, Germany) in Cobas c702 (Roche Diagnostics, Mannheim, Germany) analyzer. The reference interval for adults has been accepted as 8.4–10.2 mg/dL (2.10–2.55 mmol/L). Inter-assay and intra-assay CVs for Ca measurement were ≤2.0% and ≤2.5%, respectively.
Serum P level

It was measured photometrically by using PHOS2 kit (Roche Diagnostics, Mannheim, Germany) in Cobas c702 (Roche Diagnostics, Mannheim, Germany) analyzer. The reference interval for adults has been accepted as 2.7–4.5 mg/dL (0.85–1.45 mmol/L). Inter-assay and intra-assay CVs for P measurement were ≤1.0% and ≤1.4%, respectively.

Serum creatinine level

It was measured colorimetrically by using CREJ2 kit (Roche Diagnostics, Mannheim, Germany) with Jaffe method in Cobas c702 (Roche Diagnostics, Mannheim, Germany) analyzer. The reference range for adults has been accepted as 0.72–1.25 mg/dL (63–110 μmol/L) in males and 0.57–1.11 mg/dL (50–98 μmol/L) in females. Inter-assay and intra-assay CVs for creatinine measurement were ≤2.5% and ≤3.7%, respectively.

Serum intact PTH level

It was measured by using iPTH kit (Roche Diagnostics, Mannheim, Germany) with electrochemiluminescent immunoassay method in Cobas e602 (Roche Diagnostics, Mannheim, Germany) analyzer. The physiological reference range for adults has been accepted as 15–65 pg/mL (1.6–6.9 pmol/L). Inter-assay and intra-assay CVs for iPTH measurement were ≤2.0% and ≤3.4%, respectively.

Plasma 25(OH)D3 level

By using Zivak 25-OH Vitamin D2/D3 LC-MS/MS analysis kit (Zivak Technologies, Istanbul, Turkey) it was measured in ONH-100 liquid chromatography – mass spectrometry/ mass spectrometry analyzer (Zivak Technologies, Istanbul, Turkey). Inter-assay and intra-assay CVs for 25(OH)D3 measurement were ≤3.4% and ≤4.4%, respectively.

As 25(OH)D3 level of 80 ng/mL (200 nmol/L) and over is accepted as toxicity risk, the values over 80 ng/mL were not included in the study.

Statistical analysis

25(OH)D3 levels were converted to normal distribution by applying logarithmic transformation as they did not show a normal distribution. In all analysis made in order to compare 25(OH)D3 levels, log(25(OH)D3) data were used (as a p value) but they were expressed as 25(OH)D3 data for pellucidity and to reflect the reality better.

Two-way ANOVA and ANCOVA tests followed by Bonferroni test for post-hoc comparisons were used to analyze the effects of 25(OH)D3, iPTH and the season on each other. Pearson correlation coefficient was used to determine the correlation between 25(OH)D3 and PTH.

In order to determine discussed deficiency starting level, 25(OH)D3 levels were first classified as 80–50, 50–30, 30–25, 25–20, 20–15, 15–10, 10–5, 5–0 ng/mL and iPTH levels among those groups were compared by one-way ANOVA test. Afterwards post-hoc comparisons were performed with the Fisher’s Least Significant Difference (LSD) test. In 25(OH)D3 group clusters, each group includes its own lower limit value and does not include its own upper limit value.

All statistical analyses were performed using the SPSS software package, Version 17.0 (SPSS Inc., Chicago, IL, USA). For all analyses, values of p < 0.05 were considered significant.

Results

A significant effect of the season was observed on 25(OH)D3, whereas there was no significant effect of gender in two-way ANOVA analysis which was conducted to analyze the effects of the season and gender on 25(OH)D3 levels (p = 0.048 and p = 0.11, respectively) (Table 1).

When the 25(OH)D3 levels according to the seasons were analyzed in pairwise comparisons with Bonferroni test, the only difference was observed between spring and winter (p = 0.033) (Table 2).

A significant effect of 25(OH)D3 was observed on iPTH, whereas there was no significant effect of the season in ANCOVA analysis which was conducted to analyze the effects of the season and 25(OH)D3 on iPTH levels (p < 0.001 and p = 0.11, respectively) (Table 3).

There was a significant reverse correlation between 25(OH)D3 and iPTH (r = −0.171, p < 0.001). As a result of the one-way ANOVA test which was made to make comparisons of iPTH levels according to 25(OH)D3 levels groupings as 80–50, 50–30, 30–25, 25–20, 20–15, 15–10, 10–5, 5–0 ng/mL, significant difference among groups was determined (p < 0.001).

In Fisher’s LSD tests which were performed post-hoc in order to determine significant differences in iPTH levels; first significant difference was determined between
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25(OH)D₃ groups of 80–50 and 50–30 ng/mL (p = 0.007). No significant difference between 25(OH)D₃ groups of 50–30 vs. 30–25, 30–25 vs. 25–20, 25–20 vs. 20–15 and 20–15 vs. 15–10 ng/mL was determined (p = 0.75, p = 0.74, p = 0.40 and p = 0.67, respectively). Second and third significant differences were determined between 25(OH)D₃ groups of 15–10 and 10–0 ng/mL and between 10–0 and 5–0 ng/mL, respectively (p = 0.006 and p = 0.035, respectively) (Figure 1).

At this stage; groups which had no significant differences in between were combined and the second and third points where iPTH increase response was seen were combined because they were consecutive. Thus, three different groups which had 80–50, 50–10 and 10–0 ng/mL of 25(OH)D₃ levels and which did not remain 95% confidence intervals of each other were formed according to the response of iPTH. Significant differences were observed between these groups (p < 0.001 for each) (Figure 2).

Discussion

Today, vitamin D insufficiency and deficiency is regarded as epidemic all over the world [3]. There is an increasing interest in the physiological, pathological, therapeutic and laboratory dimensions of 25(OH)D all over the world. Herewith, there has been a dramatic increase of 25(OH)D test requests in the recent years up to 80–90% [21, 22]. Therefore, the cost increase has also been a matter of issue [23]. For these reasons, it is highly crucial to make correct diagnosis for vitamin D insufficiency or deficiency.

As 25(OH)D level in the blood has a longer half-life (~15 days) and a more loose regulation compared to vitamin D (1,25(OH)₂D), it is accepted as the best indicator indicating the vitamin D depots in the body [24, 25]. As well as 1,25(OH)₂D have a tighter regulation with the stimulating effects of iPTH, calcium and phosphorus, it also has a relatively shorter biological half-life (~15 h) in

| Source        | Sum of squares   | df | Mean square | F   | p-Value |
|---------------|-----------------|----|-------------|-----|---------|
| Corrected model | 7197.95ᵃ        | 4  | 1799.49     | 14.12 | <0.001  |
| Intercept     | 235717.84        | 1  | 235717.84   | 1848.98 | <0.001  |
| Season        | 6700.43          | 1  | 6700.43     | 52.56 | <0.001  |
| Gender        | 759.92           | 3  | 253.31      | 1.99  | 0.114   |
| Error         | 214048.16        | 1679 | 127.48     | 0.114 |
| Total         | 3242820.50       | 1684 |           |      |         |
| Corrected total | 221246.11       | 1683 |            |      |         |

ᵃR² = 0.033 (adjusted R² = 0.030).
the blood [24, 25]. The PTH response occurs via the calcium-sensing receptor due to calcium absorption under the control of active vitamin D in bloodstream [26].

Levels of 25(OH)D have been shown to vary according to gender and season in some studies [27–30]. However, in some studies, two of them in Turkey, there was no difference between genders [31–33]. We have also found that gender has no effect on vitamin D when assessed with seasons. When we compare the 25(OH)D, levels according to the seasons; we found that the average 25(OH)D, levels in winter were higher than in spring. Choi et al. [34] also found the lowest 25(OH)D season as spring. Unlike other studies [27, 30, 31, 35], we could not find any significant differences between summer or autumn and winter or spring seasons. Although we do not know the exact cause of this finding, it could be a factor that the winter months had

![Figure 1: iPTH levels in accordance to 25(OH)D₃ level grouping *p < 0.05. Values were given as mean and the error bars represent 95% CI.](image1)

![Figure 2: iPTH deflection points formed by regrouping 25(OH)D₃ levels according to iPTH levels *p < 0.05. Values were given as mean and the error bars represent 95% CI.](image2)
the lowest population (n = 257) in seasons. It should also be noted that the 15 days of the first and last months of the seasons actually reflect the previous and next months due to the half-life of 25(OH)D is 15 days. In our study, it may also be due to the inadequate or excess number of patients in the 15-day periods of the first and last months of the relevant seasons. The lack of seasonal effect on PTH levels in our study also supports the lack of seasonal vitamin D level differences.

In our study, when we evaluated iPTH responses given to the reduction in 25(OH)D$_3$ levels; we determined significant iPTH increases firstly when 25(OH)D$_2$ levels decreased under 50 ng/mL, secondly decreased under 10 ng/mL and thirdly decreased under 5 ng/mL. In the light of these findings; we can state that iPTH levels are suppressed when 25(OH)D$_2$ levels in between 50 and 10 ng/mL and gives the first increase response when 25(OH)D$_2$ falls below 10 ng/mL. If vitamin D deficiency and insufficiency definitions should be made for adult Turkish population, we believe that it is not appropriate to make definition of insufficiency without having clinical information and risks. However, we think that it is not possible for metabolic homeostasis to respond to vitamin D deficiency which is mostly synthesized endogenously. Thus, we can say that 10 ng/mL is the cut-off level of 25(OH)D$_2$ deficiency that metabolism gives reaction to it. However, in order to be able to define vitamin D insufficiency, we think that the results of prospective studies which evaluate future disease risks should be compiled.

It should be noted that; it has been revealed that there are differences between study methods of measured 25(OH)D levels. In a study conducted by Sadat-Ali et al. [36], when the 25(OH)D levels were measured by HPLC-LC/MS, all of the vitamin D deficient participants had an increased PTH. Nevertheless, when the same participants’ 25(OH)D levels were measured by CLIA and RIA, vitamin D deficient participants showed normal PTH at a high ratio [36]. As LC-MS/MS method -the gold standard- was used for the measurement of 25(OH)D$_3$ levels in our study, it can be said that the levels which found in our study are more accurate and reliable when compared to studies using other methods.

Another difference of our study from other studies is that we gave only the 25(OH)D$_3$ levels as a result not total 25(OH)D levels. However, this does not constitute a limitation for the population of Turkey. The most substantial vitamin D compounds are vitamin D$_2$ (ergocalciferol) which found in plants and consumed as a supplement or in fortified foods, and vitamin D$_3$ (cholecalciferol) which is synthesized in the human skin under the influence of sunlight or consumed in oily fish, fortified foods or as a supplement [37]. Although there are no vitamin D$_2$ supplemented foods in Turkey, the main source of vitamin D is vitamin D$_3$, which is synthesized endogenously via sunlight exposure. When we take a look at the literature, there are not many studies which distinguish 25(OH)D$_2$ and 25(OH)D$_3$ measurements. In the study conducted by Schleicher et al. [38] in American population over 15,652 people, it was indicated that only 19% of people included to the study had a detectable level (> 0.8 ng/mL) of 25(OH)D$_2$ levels. They stated that most of these people, especially those over 60 years of age, were the ones who had been prescribed vitamin D$_2$ supplementation. They found the mean level of 25(OH)D$_2$ of people who were under the age of 60 and whose 25(OH)D$_2$ were detectable as 1.9 ng/mL [38]. Again, in a recent study mean level of 25(OH)D$_2$ of 1068 people was determined as approximately 0.7 ng/mL [39]. The results of these studies support us for the fact that 25(OH)D$_2$ levels will not change the results of our study.

A limitation of our study was that it was not known whether the patients included in the study received vitamin D supplements or not. However, the majority of patients had 25(OH)D$_3$ levels of < 50 ng/mL and the average of 25(OH)D$_3$ levels of all patients was low (179 ng/mL), which suggest that most of the patients have not received vitamin D supplements. In addition, although the low number of individuals in the group with 25(OH)D$_3$ levels of 50–80 ng/mL seems to be a limitation in terms of statistical analysis, we think this is not a disadvantage because this group constitutes only 2.8% (n = 47) of all patients and it is suitable for population distribution.

Another issue to be considered in the studies examining relationship between 25(OH)D and PTH is, the active form 1,25(OH)$_2$D$_3$ which has a much shorter half-life has an effect on the iPTH which has a ~4 min of half-life [40] while 25(OH)D levels with a half-life of 15 days are still unchanged. The low correlations between 25(OH)D and PTH in our study and in many other studies [41] may also be a result of this situation. This situation should be considered when evaluating the physiological PTH response to vitamin D deficiency. This, in fact, indicates that the use of personal reference intervals for PTH will be more accurate.

In conclusion, we believe that cut-off value for vitamin D deficiency in Turkish adults at all seasons depending on iPTH response should be used as 10 ng/mL. For the cut-off value required to be used in the diagnosis of vitamin D insufficiency, the results of clinical studies in which risk factors are evaluated should be taken into consideration.
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