Original Research Article

Study of serum vitamin D2 and calcium in young and middle aged healthy male smokers in rural tertiary care center

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ABSTRACT

Background: Smoking is an essential determinant of various diseases. The study is aimed to understand the influence of smoking on serum vitamin D2/D3 levels and serum calcium levels in healthy young/middle-aged men.

Methods: Prospective observational study was done among young and middle-aged healthy male smokers in a rural territory care center. Two hundred patients were studied and analyzed, who fulfill the inclusion and exclusion criteria.

Results: The prevalence of vitamin D deficiency (25(OH)D <20 ng/ml) was 50.3%. Only 8.8% of the participants had vitamin D sufficiency (25 hydroxyvitamin D ≥30 ng/ml). There is a strong correlation between 25(OH)D and smoking in the participants (p<0.001). 25 hydroxyvitamin D level was lower by approximately 4.3 ng/ml (p<0.001) in a smoker compared to a non-smoker among the total participants, this value increased to 9.2 ng/ml in the 40-50y subgroup (p=0.003). A multinomial logistic regression model demonstrated that a young smoker (20-29y) had a 58% increased likelihood of having vitamin D deficiency compared to a non-smoker of the same age group (p=0.041). Irrespective of age and chronicity of smoking, there was a significantly increased level of serum calcium and significant vitamin D2/D3 deficiency in smokers.

Conclusion: A high prevalence of vitamin D deficiency was identified in young and middle-aged male smokers, which is not likely to be explained by other confounding lifestyle factors. The depression of the vitamin D-PTH system seen among smokers may represent another potential mechanism for the harmful effects of smoking on the skeleton.

Keywords: Bone mineral densitometry, Cigarette smokers, Dual X-ray absorptiometry, Vitamin D deficiency, 25-hydroxy vitamin D

INTRODUCTION

The vital role of vitamin D in bone health has long been recognized.1 Several recent studies have suggested that low serum 25-hydroxyvitamin D (25(OH)D) concentration is associated with bone loss through, among others, secondary hyperparathyroidism and resulting high bone remodeling.

However, most of the studies indicating these relationships have been performed in older people—mainly women while data on healthy young-middle-aged men are limited.2-7

Vitamin D exists in two forms: vitamin D2 or ergocalciferol which is found naturally in foods of plant origin, and vitamin D3 or cholecalciferol which is mainly synthesized in the skin by exposure to ultraviolet-B light and is also abundant in foods of animal origin.

The main source of vitamin D is via exposure of the human skin to sunlight between 10 am and 3 pm in the
spring, summer and fall. It is essential to know that circulating 25(OH)D concentration is the best indicator of whole-body vitamin D status and is used for the classification of vitamin D status into deficient (25(OH)D<20ng/ml), insufficient (25(OH)D<30ng/ml) or sufficient (25(OH)D≥30ng/ml). Although the structural differences between D2 and D3 alter their metabolism, in general, the biologic activity of their active metabolites is comparable.

Hypovitaminosis is a worldwide health problem with the estimated percentages of people suffering from vitamin D deficiency ranging from 31% in Australia to 98% in Mongolia. It is assumed that people living in countries with high amounts of sunlight may have a lower risk of vitamin D deficiency. However, recent studies have indicated that the prevalence of vitamin D deficiency even in tropical countries is as high as that observed in Western populations.

Smoking can trigger increased expression of CYP2A4 in macrophages, which results in increased catabolism and reduced bioavailability of the active compound. In dendritic cells, for instance, the local activation of 25-hydroxyvitamin D is crucial for mediation of the anti-inflammatory effects of vitamin D and is not only defined by absolute 25-hydroxyvitamin D concentrations but also by the concentration and genetic variant of its carrier protein vitamin D-binding protein.

Second, smoking can inhibit VDR translocation from the nucleus to the cell membrane. In mice, absence of VDR results in an abnormal lung phenotype with characteristics of COPD, including airspace enlargement, a decline in lung function, increased lung inflammatory cellular influx, and formation of immune-lymphoid aggregates. Similar mechanisms of reduced VDR signaling might happen in patients.

Objective of this study to determine the prevalence of 25(OH)D (D2 and D3 independently) inadequacy in healthy young/middle-aged men.

METHODS

All the participants were healthy men aged 20-50 years, having normal blood counts and normal results for liver and kidney function tests. Written informed consent was obtained from all the participants of the survey. All men underwent a general physical examination. Measurements of body weight were obtained to the nearest 0.1 kg using a standard balance beam, and measures of height were obtained to the nearest 0.1 cm using the wall-mounted stadiometer. Body mass index (BMI) was calculated as weight (kilograms) divided by height squared (square meters).

All subjects were medically examined and interviewed using a standardized questionnaire to collect information on smoking habits, dietary calcium intake, and alcohol consumption. Smoking was categorized as a dichotomous variable: non-smokers (never smokers and ex-smokers, i.e., responders who had stopped smoking at least one year before the study) and current smokers.

The consumption of foods representing the significant sources of daily calcium intake, such as typical Greek cheeses (feta cheese and kasseri cheese), yogurt, and milk, was recorded in a weekly food-frequency questionnaire. A fixed range of food containers, i.e., a glass for milk and a cup for yogurt, was used to standardize portion sizes, each containing ≈300 mg of calcium. The number of servings eaten weekly was recorded, and calcium intake per week was estimated and expressed as mg of calcium per week. Questions about the consumption of beer, wine, and spirits were included in each questionnaire, which permitted to evaluate the weekly consumption of ethanol expressed as grams of alcohol per week.

Inclusion criteria

All the participants were healthy men aged 20-50 years, having normal blood counts and normal results for liver and kidney function tests.

Exclusion criteria

- Any treatment or medical complications are known to affect vitamin D and bone metabolism, such as primary hyperparathyroidism, cancer, malabsorption syndrome, hyperthyroidism, diabetes mellitus, pituitary, adrenal, gonadal and rheumatic diseases, as well as a history of immobility for more than one month.
- Besides, participants had not taken vitamin D and/or calcium supplements for the last 12 months.

Biochemical determinations

Venous blood samples were collected in the morning between 0800 and 0900 hours under standardized conditions after an overnight fast. Serum samples were prepared immediately after phlebotomy and stored at -85°C for the measurement of the serum levels of calcium, phosphate, albumin, alkaline phosphatase (ALP), intact parathyroid hormone (iPTH), and 25-hydroxyvitamin D2 (25(OH)D2) and 25-hydroxyvitamin D3 (25(OH)D3).

The levels of 25(OH)D3 and 25(OH)D2 were determined in serum of participants using Liquid-Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) technology. 25(OH)D concentrations were calculated as the sum of 25(OH)D2 and 25(OH)D3.

Bone mineral densitometry

Bone mineral density was measured at the lumbar spine (L2-L4) and proximal femur, using dual-X-ray absorptiometry (DXA). Values for results of DXA
measurements were expressed as BMD (g/cm²) and T score and Z scores of a healthy reference population. Short-term precision for the spine and proximal femur measurements had a coefficient of variation (CV) of 1% to 2%. The same physician recorded age, body weight, height.

RESULTS

Out of 200 population, 53.5% belongs to 30-39 yrs of age group and 22% (20-29), 24.5% (40-59) age groups respectively.

Table 1: Age distribution, BMI and duration of smoking.

<table>
<thead>
<tr>
<th>Age Distribution (years)</th>
<th>No</th>
<th>%</th>
<th>Duration of smoking &lt;3 Yrs</th>
<th>3-6 Yrs</th>
<th>&gt;7yrs</th>
<th>Total</th>
<th>BMI (kg/cm²) &lt;25</th>
<th>26-30</th>
<th>31-35</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-29</td>
<td>44</td>
<td>22%</td>
<td>22</td>
<td>18</td>
<td>4</td>
<td>44</td>
<td>30</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50%</td>
<td>40.9%</td>
<td>9.1%</td>
<td>100%</td>
<td>68.2</td>
<td>22.7</td>
<td>9.1%</td>
<td></td>
</tr>
<tr>
<td>30-39</td>
<td>107</td>
<td>53.5%</td>
<td>16</td>
<td>51</td>
<td>40</td>
<td>107</td>
<td>70</td>
<td>28</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.9%</td>
<td>47.6%</td>
<td>37.5%</td>
<td>100%</td>
<td>65.4</td>
<td>26.2</td>
<td>8.4</td>
<td></td>
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<tr>
<td>40-59</td>
<td>49</td>
<td>24.5%</td>
<td>9</td>
<td>22</td>
<td>18</td>
<td>49</td>
<td>12</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18.3%</td>
<td>44.9%</td>
<td>36.8%</td>
<td>100%</td>
<td>24.5</td>
<td>40.8</td>
<td>34.7</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>100%</td>
<td>47</td>
<td>91</td>
<td>62</td>
<td>112</td>
<td>58</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Vitamin D levels, calcium levels, and duration of smoking.

<table>
<thead>
<tr>
<th>Duration of Smoking Years</th>
<th>Subjects</th>
<th>Vitamin D Levels (ng/ml)</th>
<th>Calcium levels</th>
<th>Normal</th>
<th>Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;3</td>
<td>47</td>
<td>&lt;10</td>
<td>1</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>3-6</td>
<td>91</td>
<td>10-20</td>
<td>6</td>
<td>47</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20-29</td>
<td>6.6%</td>
<td>51.6%</td>
<td>10</td>
</tr>
<tr>
<td>&gt;7</td>
<td>62</td>
<td>&gt;30</td>
<td>9.7%</td>
<td>54.8%</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>6.5%</td>
<td>46.5%</td>
<td>31%</td>
<td>16%</td>
</tr>
</tbody>
</table>

DISCUSSION

Our results revealed a high incidence (50.3%) of vitamin D deficiency (<20ng/ml), while the mean levels of 25(OH)D were 19.81ng/ml. Participants aged 20-29 years had the highest incidence of vitamin D deficiency (57%). The high incidence of vitamin D deficiency in our study is in line with results of studies from countries at a similar latitude.12,13

This study showed undetectable levels of 25(OH)D2 in the majority of the male population. Studies on the levels of 25(OH)D2 in adults are limited, and the results are mixed; however, the majority have demonstrated higher concentrations than that found in our study.14-16

According to the data, smokers had lower serum 25(OH)D concentrations than non-smokers.

Interestingly, in the totality of participants, smoking was the only significant determinant of serum 25(OH)D levels.
among the tested variables (BMI, age, smoking, alcohol consumption, and calcium intake). Furthermore, 25(OH)D level was expected to be lower by 4.2 ng/dl in a smoker by comparison with a non-smoker for all age-groups, but this value increased to 9.2 ng/dl for the 40-50y subgroup. This suggests the need for young and primarily middle-aged smokers be screened for vitamin D deficiency.

The negative correlation between 25(OH)D levels and smoking could be explained by the fact that smoking is usually accompanied by a less healthy lifestyle (less physical activity, alcohol consumption, and bad dietary habits) leading to reduced sun exposure and thus synthesis of vitamin D. However, a causative role of smoking in vitamin D deficiency could not be excluded; recent studies have in fact shown that metabolic derivatives of naphthalene (a metabolite in cigarette smoke) such as tetralones can inhibit CYP27A1 activity. 17

In line with the results, Jaaskelainen et al, study 5714 subjects (47% men) aged 30-79 years found that smokers had lower serum 25(OH)D concentrations than non-smokers. 18 Moreover, Thuesen et al, in a recent large population study showed that odds ratios of vitamin D severe deficiency (25(OH)D <10ng/ml) vitamin D deficiency (25(OH)D <20ng/ml) associated with daily smoking was 1.47 and 1.36, respectively.19

In contrast, Scragg et al, in a sample of 295 men aged 35-64 years found that smoking was not correlated with 25(OH)D levels, 20 while data from recent studies also agreed with the absence of correlation between smoking and 25(OH)D serum concentrations. 21,22 The different way could explain the inconsistency among the various studies that smoking is defined, heterogeneity in smoking intensity as well as by the different methodology used to measure serum 25(OH)D.

Notably, the Tromso study revealed that determination of serum 25(OH)D using ECLIJA (electrochemiluminescence) resulted in falsely elevated 25(OH)D concentrations, which does not occur using LC-MS/MS. 23 An overestimation of 25(OH)D concentration due to the methodology used could overlook detection of a negative correlation between smoking and 25(OH)D levels.

Authors found no significant correlation between serum 25(OH)D concentration and age, although there was a definite 25(OH)D gradient with age. This observation is inconsistent with earlier studies, which have indicated that serum 25(OH)D concentrations decrease with increasing age. 24,25 However, the KNHANES study including 2504 males aged >20 years found that vitamin D deficiency was most prevalent in the age group of 20-29, with a rate of 65g, and least prevalent in the older age subgroups.26 Findings could be explained by increased prevalence of health-promoting physical activity in older subgroups, thus it is possible that they spend more time outdoors. Moreover, age is positively linked to the daily dietary intakes of vitamin D. 27

Data demonstrated no correlation between BMI and 25(OH)D concentration. Findings from previous studies on the association between serum levels of vitamin D and obesity are conflicting. 23,26-28 The Tromso study, although confirming the inverse relationship between BMI and 25(OH)D, noted that this correlation became significant in men with higher BMI levels and more pronounced in subjects with BMI levels greater than 35.29 Although the range of BMI values was wide, the number of obese subjects with BMI >30 was small (n=17), this probably not allowing us to draw statistically significant results.

Similarly, to other studies, 29,30 authors did not find any significant correlation between serum 25(OH)D and calcium, phosphate. Looking at the younger subgroup (20-29y), who had the lower 25(OH)D levels, authors did not find any differences in the indices of bone remodeling between smokers and non-smokers, although smoking has been associated with reduced bone remodeling between smokers and non-smokers, although smoking has been associated with reduced bone remodeling, as shown by Lappin et al. 31

In terms of the routine measurements of calcium, phosphate, ALP, and PTH, studies have demonstrated that these parameters are not adequate to identify patients with hypovitaminosis D and are thus not reliable predictors of hypovitaminosis D. 7,29,30

Authors observed a positive correlation between bone turnover markers and 25(OH)D concentration in the younger age group (20-29y) which cannot be explained. Authors have found no correlation between BMD in either the lumbar spine or proximal femur and 25(OH)D, indicating that other factors (e.g., testosterone levels) may play a more important role in BMD regulation in this age group. Moreover, a recent study by Gallagher et al, conducted in young women suggested that active transport of calcium is saturated at low serum 25(OH)D levels 32. This very efficient calcium absorption at deficient levels of serum 25(OH)D could explain why healthy subjects do not develop osteomalacia.

CONCLUSION

A high prevalence of vitamin D deficiency was identified in the young/middle-aged male population. Data suggest that vitamin D status is not a determinant of bone metabolism and BMD in young/middle-aged men. Smoking is a significant determinant of serum 25(OH)D, while the likelihood of having vitamin D deficiency by approximately 60% in the young male population. Even increases though the differences found may seem small, and probably would not have been detectable as significant, may become clinically crucial if the exposure sustained for decades and may in part account for the decreased bone mass and increased fracture risk seen among smokers later in life.
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