Original Research Article

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

Lalitha Paladugu, Anjaneya P. V., Jayasree Mureboina, Anusha C. Tummala*

Department of Medicine, Dr. Pinnamaneni Siddhartha Institute of Medical Sciences and Research Foundation, Chinna Avutpalli, Vijayawada, Andhra Pradesh, India

Received: 13 December 2019
Accepted: 06 January 2020

*Correspondence:
Dr. Anusha C. Tummala,
E-mail: tummala3333@gmail.com

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

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 after 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.

| Age Distribution (years) | No  | %   | <3 Yrs | 3-6 Yrs | >7yrs | Total | BMI (kg/cm²) |
|--------------------------|-----|-----|--------|---------|-------|-------|--------------|
| 20-29                    | 44  | 22% | 22     | 18      | 4     | 44    | 4            |
|                          |     |     | 50%    | 40.9%   | 9.1%  | 100%  | 68.2         |
|                          |     |     |        |         |       |       | 22.7         |
|                          |     |     |        |         |       |       | 9.1%         |
| 30-39                    | 107 | 53.5% | 16   | 51      | 40    | 107   | 70           |
|                          |     |       | 14.9% | 47.6%   | 37.5% | 100%  | 65.4         |
|                          |     |       |       |         |       |       | 26.2         |
|                          |     |       |        |         |       |       | 8.4          |
| 40-59                    | 49  | 24.5% | 9    | 22      | 18    | 49    | 12           |
|                          |     |       | 18.3% | 44.9%   | 36.8% | 100%  | 24.5         |
|                          |     |       |       |         |       |       | 40.8         |
|                          |     |       |        |         |       |       | 34.7         |
| Total                    | 200 | 100% | 47  | 91      | 62    | 112   | 58           |
|                          |     |       | 23.5% | 45.5%   | 31%   | 100%  | 29%           |
|                          |     |       |       |         |       |       | 15%          |

Table 2: Age distribution, BMI and duration of smoking.

| Duration of Smoking | Vitamin D Levels (ng/ml) | Calcium levels |
|---------------------|--------------------------|----------------|
|                     | <10                      | 10-20          |
| Years               | <3 Yrs                   | 20-29          |
|                     | >30                      | Normal         |
| <3                  | 47                       | 6              |
|                     | 1                        | 6              |
|                     | 12                       | 6.6%           |
|                     | 19                       | 51.6%          |
| 3-6                 | 91                       | 6              |
|                     | 34                       | 9.7%           |
|                     | 15                       | 54.8%          |
| >7                  | 62                       | 6              |
|                     | 93                       | 6.5%           |
|                     | 62                       | 46.5%          |
| Total               | 200                      | 6              |
|                     | 13                       | 53%            |
|                     | 93                       | 46.5%          |
|                     | 62                       | 47%            |
|                     | 32                       | 51%            |
|                     | 102                      | 49%            |

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
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 ECLI (electrochemiluminescence) resulted in falsely elevated levels of 25(OH)D in smokers, 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 65%, 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 levels 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 modeling, 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 <5 ng/mL.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% is the young male population. Even increases though the differences found may seem small, and probably would not have been detectable as significant, they 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.
ACKNOWLEDGEMENTS

Authors would like to thank Dr. Eswar Ganti, and other faculty members, who supported them in the study.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: Not Required

REFERENCES

1. Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status Osteoporos Int. 2005;16:713-6.
2. Adami S, Bertoldo F, Braga V, Fracassi E, Gatti D, Gandolini G, et al. 25-hydroxy vitamin D levels in healthy premenopausal women: association with bone turnover markers and bone mineral density. Bone. 2009 Sep 1;45(3):423-6.
3. Ooms ME, Lips P, Roos JC, van der Vijgh WJ, Popp-Snijders C, Bezemder PD, et al. Vitamin D status and sex hormone binding globulin: determinants of bone turnover and bone mineral density in elderly women. J Bone Mineral Res. 1995 Aug;10(8):1177-84.
4. Bhattoa HP, Nagy E, More C, Kappelmayer J, Balogh A, Kalina E, et al. Prevalence and seasonal variation of hypovitaminosis D and its relationship to bone metabolism in healthy Hungarian men over 50 years of age: the Hun Men Study. Osteoporos Int. 2013 Jan 1;24(1):179-86.
5. Bhattoa HP, Bettembuk P, Ganacharya S, Balogh A. Prevalence and seasonal variation of hypovitaminosis D and its relationship to bone metabolism in community dwelling postmenopausal Hungarian women. Osteoporos Int. 2004 Jun 1;15(6):447-51.
6. Saquib N, von Mühlen D, Garland CF, Barrett-Connor E. Serum 25-hydroxyvitamin D, parathyroid hormone, and bone mineral density in men: the Rancho Bernardo study. Osteoporos Int. 2006 Dec 1;17(12):1734-41.
7. Szule P, Munoz F, Marchand F, Chapuy MC, Delmas PD. Role of vitamin D and parathyroid hormone in the regulation of bone turnover and bone mass in men: the MINOS study. Calcified Tiss Int. 2003 Dec 1;73(6):520-30.
8. Holick MF. Vitamin D deficiency. N Engl J Med. 2007 Jul 19;357(3):266-81.
9. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011 Jul 1;96(7):1911-30.
10. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Guidelines for preventing and treating vitamin D deficiency and insufficiency revisited. J Clin Endocrinol Metab. 2012 Apr 1;97(4):1153-8.
11. Lips P, Hosking D, Lippuner K, Norquist JM, Wehren L, Maalouf G, et al. The prevalence of vitamin D inadequacy amongst women with osteoporosis: an international epidemiological investigation. J Int Med. 2006 Sep;260(3):245-54.
12. Choi HS, Oh HJ, Choi H, Choi WH, Kim JG, Kim KM, et al. Vitamin D insufficiency in Korea-a greater threat to younger generation: the Korea National Health and Nutrition Examination Survey (KNHANES) 2008. J Clin Endocrinol Metab. 2011 Mar;96(3):643-51.
13. Hekimsoy Z, Dinç G, Kafeşçiler S, Onur E, Güvenç Y, Pala T, et al. Vitamin D status among adults in the Aegean region of Turkey. BMC Public Health. 2010 Dec;10(1):782.
14. Hojskov CS, Heickendorff L, Möller HJ. High-throughput liquid–liquid extraction and LC-MS/MS assay for determination of circulating 25 (OH) vitamin D3 and D2 in the routine clinical laboratory. Clinica Chimica Acta. 2010 Jan 4;411(1-2):114-6.
15. Bogusz MJ, Al Enazi E, Tahtamoni M, Jawad JA, Al Tufail M. Determination of serum vitamins 25-OH-D2 and 25-OH-D3 with liquid chromatography-tandem mass spectrometry using atmospheric pressure chemical ionization or electrospray source and core-shell or sub-2 μm particle columns: A comparative study. Clin Biochemist. 2011 Nov 1;44(16):1329-37.
16. Chen H, McCoy LF, Schleicher RL, Pfeiffer CM. Measurement of 25-hydroxyvitamin D3 (25OHD3) and 25-hydroxyvitamin D2 (25OHD2) in human serum using liquid chromatography-tandem mass spectrometry and its comparison to a radioimmunoassay method. Clin Chimica Acta. 2008 May 1;391(1-2):6-12.
17. Aboraia AS, Makowski B, Bahja A, Prosser D, Brancale A, Jones G, et al. Synthesis and CYP24A1 inhibitory activity of (E)-2-(2-substituted benzylidene)-and 2-(2-substituted benzyl)-6methoxy-tetralones. Eur J Med Chem. 2010 Oct 1;45(10):4427-34.
18. Jáäskeläinen T, Knekt P, Marniemi J, Sares-Jäskä L, Männistö S, Heliövaara M, et al. Vitamin D status is associated with sociodemographic factors, lifestyle and metabolic health. Eur J Nutr. 2013 Mar 1;52(2):513-25.
19. Thuesen B, Husemoen L, Fenger M, Jakobsen J, Schwarz P, Toft U, et al. Determinants of vitamin D status in a general population of Danish adults. Bone. 2012 Mar 1;50(3):605-10.
20. Scragg R, Holdaway I, Jackson R, Lim T. Plasma 25-hydroxyvitamin D3 and its relation to physical activity and other heart disease risk factors in the general population. Ann Epidemiol. 1992 Sep 1;2(5):697-703.
21. Jungert A, Neuhäuser-Berthold M. Dietary vitamin D intake is not associated with 25-hydroxyvitamin D3 or parathyroid hormone in elderly subjects, whereas the calcium-to-phosphate ratio affects
parathyroid hormone. Nutr Res. 2013 Aug 1;33(8):661-7.
22. Banihosseini SZ, Baheiraei A, Shirzad N, Heshmat R, Mohsenifar A. The effect of cigarette smoke exposure on vitamin D level and biochemical parameters of mothers and neonates. J Diab Metab Disorders. 2013 Dec;12(1):19.
23. Jorde R, Sreve M, Emaus N, Figschau Y, Grimnes G. Cross-sectional and longitudinal relation between serum 25-hydroxyvitamin D and body mass index: the Tromsø study. Eur J Nutr. 2010 Oct 1;49(7):401-7.
24. Melamed ML, Michos ED, Post W, Astor B. 25-hydroxyvitamin D levels and the risk of mortality in the general population. Arch Int Med. 2008 Aug 11;168(15):1629-37.
25. Nguyen HT, von Schoultz B, Nguyen TV, Dzung DN, Duc PT, Thuy VT, et al. Vitamin D deficiency in northern Vietnam: prevalence, risk factors and associations with bone mineral density. Bone. 2012 Dec 1;51(6):1029-34.
26. Benjamin A, Morikova A, Akhter N, Rao D, Xie H, Kukreja S, et al. Determinants of 25-hydroxyvitamin D levels in African-American and Caucasian male veterans. Osteoporos Int. 2009 Oct 1;20(10):1795-803.
27. Mai XM, Chen Y, Camargo Jr CA, Langhammer A. Cross-sectional and prospective cohort study of serum 25-hydroxyvitamin D level and obesity in adults: the HUNT study. Am J Epidemiol. 2012 Feb 6;175(10):1029-36.
28. Young KA, Engelman CD, Langefeld CD, Hairston KG, Haffner SM, Bryer-Ash M, et al. Association of plasma vitamin D levels with adiposity in Hispanic and African Americans. J Clin Endocrinol Metab. 2009 Sep 1;94(9):3306-13.
29. Smith GR, Collinson PO, Kiely PD. Diagnosing hypovitaminosis D: serum measurements of calcium, phosphate, and alkaline phosphatase are unreliable, even in the presence of secondary hyperparathyroidism. J Rheumatol. 2005 Apr 1;32(4):684-9.
30. Khashayar P, Meybodi HR, Homami MR, Amini MR, Mohajer-Tehran MR, Heshmat R, et al. The discriminative value of various biochemical parameters in detecting varying degrees of vitamin D deficiency in the Iranian population. Clin Lab. 2011;57(3-4):163-70.
31. Lappin DF, Sherrabeh S, Jenkins WM, Macpherson LM. Effect of smoking on serum RANKL and OPG in sex, age and clinically matched supportive-therapy periodontitis patients. J Clin Periodontol. 2007 Apr;34(4):271-7.
32. Gallagher JC, Jindal PS, Smith LM. Vitamin D does not increase calcium absorption in young women: a randomized clinical trial. J Bone Mineral Res. 2014 May;29(5):1081-7.

Cite this article as: Lalitha P, Anjaneya PV, Jayasree M, Anusha CT. Study of serum vitamin D2 and calcium in young and middle-aged healthy male smokers in rural territory care center. Int J Adv Med 2020;7:308-13.