Hypovitaminosis D: Are Medical Students at Risk?

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ABSTRACT

Background: Vitamin D deficiency is a pandemic problem mostly diagnosed in elderly. Few studies are available exclusively done on the topic among young adults. Specific professions such as medical students may have higher risk for developing hypovitaminosis D. We aimed to assess the vitamin D status in medical students of Iran University of Medical Sciences; and to define a cut-off point for 25-hydroxyvitamin-D (25(OH)D) level based on secondary hyperparathyroidism.

Methods: This was a cross-sectional study on 100 medical students conducted during October 2012. Serum 25(OH)D, intact parathyroid hormone (iPTH), and calcium were measured. Age, sex, body mass index, daily dietary fish and egg consumption, sun exposure, and sunscreen usage were recorded. The association between serum 25(OH)D and iPTH was assessed. Receiver operating characteristics curve analysis was performed.

Results: 25-hydroxyvitamin-D level was <30 ng/ml in 99% of all participants, and <20 ng/ml in 77%. Mean serum 25(OH) D level was 16.8 ± 4.7 ng/ml. iPTH level in the group with 25(OH)D level of <10 ng/ml was significantly higher than in those with serum 25(OH)D level of 10 to <20 ng/ml and 20 to <30 ng/ml (109 ± 47 pg/ml, 47 ± 27 pg/ml and 46 ± 19 pg/ml, respectively; P = 0.0001). There was a significant linear inverse correlation between serum iPTH and 25(OH)D (r = -0.36, P = 0.0001). 25(OH) D level of 15.4 ng/ml was determined as the optimal cut-off point in detecting possible secondary hyperparathyroidism.

Conclusions: To improve the community vitamin D status, in addition to population-based food fortification programs, educational programs seem essential; not only for general population, but also for the more educated groups.

Keywords: Cut-off, hypovitaminosis D, medical students, parathyroid hormone, vitamin D deficiency

INTRODUCTION

Vitamin D deficiency is a common and important worldwide medical problem, which causes rickets in children and may precipitate or exacerbate musculoskeletal pain, fibromyalgia,

osteopenia, osteoporosis, and fractures in adults. It has been associated with increased risk of common cancers, autoimmune diseases, hypertension, infectious diseases, and even depression.[1-6] Besides inadequate exposure to sun light as the major cause, wearing sunscreen and using natural vitamin D depleted foods are other important factors of the deficiency. Even vitamin D food-fortifying programs may be inadequate to satisfy vitamin D requirements and based on recent studies the prevalence of the problem is high and increasing in sunny countries including Saudi Arabia, United Arab Emirates, India, and Australia.[7-10] Fortunately this important health problem may be preventable and if occurred, simply treated.[11,12]

Definition of desirable vitamin D level is still a matter of debate. Using serum 25-hydroxyvitamin-D (25(OH)D) level, measured by a reliable assay, is the standard test for vitamin D status. Many experts currently classify serum 25(OH)D as three different categories: Deficiency, a 25(OH)D of <20 ng/ml; insufficiency, a 25(OH)D of 20-29 ng/ml; and sufficiency, a 25(OH)D of 30-100 ng/ml;[11,13] still the Institute of Medicine insists on a 25(OH)D level of >20 ng/ml as sufficiency, mainly based upon evidence related to bone health.[14] There are different ways to set cut-off points for vitamin D deficiency. Optimal vitamin D stores are necessary for achieving peak bone mass, which is usually attained at about third decade of life. It is also well-known that serum parathyroid hormone (PTH) and intestinal and urinary calcium absorption play an important role in getting optimal bone mass. Considering the inverse relation between 25(OH)D and serum intact PTH (iPTH), at least from the bone health point of view, it seems logical to accept the level of serum 25(OH)D that would be enough for maintaining PTH at normal level, as a probable suitable cut-off value for vitamin D deficiency.[15]

Vitamin D insufficiency is mostly diagnosed in elderly, in residents of nursing homes, and in hospitalized individuals.[16] Young adults especially in countries with cultural background that preclude adequate sun exposure, like veil wearing, may be even at more risk for vitamin D deficiency.[17,18] In contrast to many studies existing on vitamin D deficiency in adults aged over 65, there are few studies published on the vitamin D situation among young adults who are still at critical age for developing peak bone. Considering presence of multiple risk factors, specific professions may have higher risk for developing hypovitaminosis D. Based on some recent reports medical professionals seem to be such a subgroup.[19-24]

This study was designed to determine the vitamin D status in young population of medical students. It also examined the relation between their serum iPTH and 25(OH)D, and aimed to define a cut-off level of vitamin D deficiency based on secondary hyperparathyroidism.

METHODS

Participants

This cross-sectional study was conducted on 100 medical students of Iran University of Medical Sciences (IUMS), Firouzgar General Hospital, Tehran, Iran, during October 2012, and finally analyzed in January 2013. Eligible participants were all medical students aged 20-30 years; but were excluded if they had history of liver or renal diseases, metabolic bone diseases, hypercortisolism, active malignancy, clinically apparent malabsorption syndrome, using drugs known to affect vitamin D metabolism (anticonvulsants, glucocorticoids) or receiving any form of supplements containing calcium or vitamin D in the last 3 months before the study. Pregnant and lactating students were also excluded. Volunteers were fully informed about the study, and written consent was obtained. The study was approved by the IUMS Research Committee.

Methodology

Clinical assessment

Age, sex, height (cm), weight (kg), and body mass index (BMI) (kg/m^2) were recorded for all participants. A checklist was used to assess daily dietary fish and egg consumption, sun exposure, and also sunscreen usage.

Biochemical assessments

Fasting serum calcium, albumin, iPTH, and 25(OH)D levels were assessed. Serum samples were stored at –20°C until final analysis. All of the analyses were carried out at the laboratory of Firouzgar hospital by trained technicians. Serum 25(OH)D was measured using a commercial radioimmunoassay kit (Immune diagnostic systems, Boldon, UK) according to international instructions. Specificity and sensitivity for 25(OH)D

measurements were 100% and 2 ng/ml, respectively. Inter- and intra-assay coefficients of variation were 6.4 and 5.6% at 70 ng/ml, respectively. iPTH was measured by ELISA assay (BIOMERICA, Inc., Hannover, Germany), with sensitivity of 1.6 pg/ml. Inter- and intra-assay coefficients of variation were 3.6 and 3.7%, respectively. Normal range for serum iPTH and albumin adjusted calcium were 10-66.5 pg/ml and 8.2-10.3 mg/dl, respectively. Secondary hyperparathyroidism was defined as serum iPTH level >65 pg/ml. In this study, we categorized participants based on their serum 25(OH)D level as: Group 1 if it was 20 to <30 ng/ml, Group 2 in case of 10 to <20 ng/ml, and Group 3 if it was <10 ng/ml.

Statistical analysis

The data were analyzed using SPSS for windows, version 18 (SPSS Inc., Chicago, IL, USA). Numeric variables were presented as means ± standard deviation. Nominal and categorical variables were illustrated in tables using percentages. The independent sample *t*-test was used to compare the means across sex variable. Chi-square test was used to compare categorical variables across sex variable. Analysis of variance (ANOVA) was used to test the mean of iPTH across three groups of 25(OH)D level (Groups 1-3). To adjust the effect of serum calcium level on iPTH, Analysis of covariance (ANCOVA) was used to compare mean level of iPTH across three groups of 25(OH)D. The association between 25(OH)D and iPTH was tested by Pearson correlation. The optimal cut-off value of serum 25(OH)D to detect iPTH >65 pg/ml as indicator of secondary hyperparathyroidism was estimated from receiver operating characteristics (ROC) curve. The sensitivity, specificity and Youden index were calculated for the determination of the diagnostic value of each cut-off level.[25] All statistical tests were two-tail, with a significance level of 0.05.

RESULTS

Based on the study criteria 100 healthy students aged 20-30 years old, out of total of 120, were involved in the study (83% response rate). The main reasons of exclusion were nonwilling for participation or taking drugs inconsistent with the study protocol. Basic characteristics were shown in Table 1.

Serum values

Mean serum 25(OH)D level was 16.8 ± 4.7 ng/ml (range: 8.8-36 ng/ml) which was significantly higher in males than females (18.6 ± 4.2 vs. 15.6 ± 4.6 ng/ml, respectively; $P = 0.001$). Mean corrected serum calcium level was 9.7 ± 0.5 mg/dl (range: 8-10.3 mg/dl), which was higher in males. Mean serum iPTH was 51.9 ± 31.4 pg/ml (range: 11-192 pg/ml), with no gender difference. Only 26% of students had serum iPTH level >65 pg/ml [Table 1].

25-hydroxyvitamin-D level was <30 ng/ml in 99% of all participants. The frequency of Groups 1-3 was 20%, 72%, and 7%, respectively [Table 2]. The

Table 1: Basal characteristics of the 100 participants

Variable	All participants (*N*=100)	Males (*N*=40)	Females (*N*=60)	*P**
Age (years)	23.6±2.7	22.5±2	24.4±2.9	0.0001
BMI (Kg/m^2)	22.5±2.9	23.2±3	22.1±2.8	0.06
Daily sun exposure *n* (%) (min)				
<30	53 (53)	24 (60)	29 (48.3)	0.25
≥30	47 (47)	16 (40)	31 (51.7)	
Weekly fish consumption ≥2 serving *n* (%)	46 (46)	27 (67.5)	19 (31.7)	0.0001
Weekly egg consumption ≥2 serving *n* (%)	25 (25)	16 (40)	9 (15)	0.0001
Daily sunscreen usage *n* (%)	81 (81)	24 (60)	57 (95)	0.0001
Serum 25(OH)D (ng/mL)	16.8±4.7	18.6±4.2	15.6±4.6	0.001
Serum iPTH (pg/mL)	51.9±31.4	45.2±21.3	56.4±36.1	0.08
>65 *n* (%)	26 (26)	8 (20)	18 (30)	0.26
Serum calcium[a] (mg/dL)	9.7±0.5	9.8±0.5	9.6±0.5	0.04

Data denoted as mean±standard deviation, except for numbers, BMI=Body mass index, 25(OH)D=25-hydroxyvitamin-D, iPTH=Intact parathyroid hormone, *Males versus females, a: Albumin corrected serum calcium

Table 2: Characteristics of participants according to their serum 25(OH)D level

Variable	Group 1 (20 to <30 ng/mL) (*n*=20)	Group 2 (10 to <20 ng/mL) (*n*=72)	Group 3 (<10 ng/mL) (*n*=7)	*P**
Age (years)	23.2±2.7	23.7±2.6	24.2±3.9	NS[a]
BMI (Kg/m^2)	23±2.8	22.3±2.8	20.9±1	NS
Gender *n* (%)				
Male	12 (60)	26 (36.1)	2 (28.5)	NS
Female	8 (40)	46 (63.9)	5 (71.5)	NS
Weekly fish consumption ≥2 serving *n* (%)	12 (60)	32 (44)	2 (29)	NS
Weekly egg consumption ≥2 serving *n* (%)	8 (40)	15 (21)	2 (29)	NS
Daily sun exposure >30 min *n* (%)	9 (45)	34 (47)	4 (57)	NS
Daily sunscreen usage *n* (%)	6 (30)	34 (47)	3 (43)	NS
Serum calcium[b] (mg/dL)	9.6±0.6	9.8±0.5	9.5±0.5	NS
Serum iPTH (pg/mL)	46±19	47±27	109±47	0.0001

Data denoted as mean±standard deviation, except for numbers, BMI=Body mass index, iPTH=Intact parathyroid hormone, *Comparison of three groups using analysis of variance, [a]NS=Not significant, b=Albumin corrected serum calcium, 25 (OH) D: 25-hydroxyvitamin-D

three groups were similar regarding age, gender, BMI, serum calcium, sun exposure, sunscreen usage, and vitamin D rich dietary use.

There was no significant difference in serum iPTH level between the Group 1 and the other added Groups 2 and 3 (P = 0.36). Using ANOVA, there was significant difference in mean serum iPTH between the three Groups of 1, 2, and 3, which still remained significant after adjustment for the possible effect of serum calcium using ANCOVA (P = 0.03). The highest iPTH level was found in the 3rd group with 25(OH)D level of <10 ng/ml (P = 0.0001); *post-hoc* analysis using Sheffe test defined that the difference was significant when comparing the 3rd group (109.4 ± 47 pg/ml) with each of the 1st or the 2nd groups (P = 0.0001 and P = 0.0001, respectively). There was no significant difference among mean serum iPTH between Groups 1 and 2 (46 ± 19 vs. 47.1 ± 27 pg/ml, respectively; P = 0.52). Mean corrected serum calcium was similar between the three groups [Table 2].

Correlation between serum intact parathyroid hormone and 25-hydroxyvitamin-D

The relation between serum 25(OH)D and iPTH was assessed using a linear regression analysis. There was a significant linear inverse correlation between serum iPTH and 25(OH)D (r = –0.36, P = 0.0001) [Figure 1]. There was also a significant inverse relation between serum calcium and iPTH level (P = 0.02); so ANCOVA analysis was used to assess the possible confounding effect of serum calcium on the relation between serum iPTH and 25(OH)D (P = 0.03).

Cut-off point of serum 25-hydroxyvitamin-D concentration

According to the analysis using the ROC curve and Youden index, the optimal cut-off value of serum 25(OH)D to detect subjects with serum iPTH concentration of >65 pg/ml as indicator of secondary hyperparathyroidism was determined to be 15.4 ng/ml that showed the highest sensitivity, specificity, and Youden index (74%, 69%, and 0.44, respectively) [Table 3]. The area under the curve was 0.77 (confidence interval$_{95\%}$ 0.65-0.89, P = 0.0001) [Figure 2]. Applying this cut-off point, 25(OH)D level was >15.4 ng/ml in 73 participants while it was lower than that in the other 37 students.

DISCUSSION

This study confirmed the presence of 25(OH) D level of <30 ng/ml in nearly all 100 healthy medical students of IUMS (Tehran/Iran). Only one student showed the level of >30 ng/ml, while 77% of the study populations had 25(OH)D level of <20 ng/ml. Although the males demonstrated higher mean serum 25(OH)D level, the severity of hypovitaminosis D was equally distributed between both sexes. Independent to calcium effect, a significant linear inverse correlation between serum iPTH and 25(OH)D level was also shown. Noticeable rise in serum iPTH was found only in

case of 25(OH)D level of <10 ng/ml in comparison to those with a higher 25(OH)D level. Using ROC curve analysis, serum 25(OH)D level of 15.4 ng/ml was the optimal cut-off value to detect secondary hyperparathyroidism (iPTH >65 pg/ml).

Vitamin D deficiency is rather widespread and common in different parts of the world. Although multiple studies have been published on its prevalence, but the majority have only assessed elderly and housebound individuals;[16] still few reports are available on its epidemiology in young and healthy people, especially from different geographic parts of the world. Skin exposure to ultraviolet B radiation is the most important way in vitamin D synthesis and food should provide the rest of its daily need.[26] Based on these facts, it had been theoretically postulated that the tropical zone (latitudes between 23.5°N and 23.5°S) should be rather free of hypovitaminosis D, and also that the economically developed countries might have better situation regarding vitamin D deficiency compared to the developing ones. Although it is still accepted that the latitude and economy may play a general role in epidemiology of hypovitaminosis D, there are increasing evidence on high prevalence of the deficiency even in the tropical zone or in the developed countries, and also on similarity of the temperate (23.5°-66.5°) to the tropics regarding the problem.[27] Reports from Saudi Arabia and Australia are some examples.[7,10] Besides the mentioned traditional risk factors; age, obesity, skin pigmentation, and different styles in clothing are being more recognized as the other possible important modulators in vitamin D status.[28-31] However, the diverse results obtained from studies on prevalence of hypovitaminosis D may be at least in part explained by some other minor risk factors. Special professions should be more evaluated in this regard. We aimed to study vitamin D status in medical students not only because of their homogenous young age, but also because of some of their specific professional life situations; they should usually study for long hours in libraries or stay in medical wards with no sun exposure, have long working shifts in closed environments, have unpredictable meal times and may use more high-caloric food with poor nutritional value. The

Table 3: Sensitivity, specificity and Youden index at some values of serum 25 (OH)D

25 (OH)D level (ng/mL)	Sensitivity (%)	Specificity (%)	Youden index[a]
9.4	100	7.7	0.08
10	100	26.9	0.27
11.2	97.3	34.6	0.32
12.2	87.8	53.8	0.42
14.2	79.7	61.5	0.41
14.8	78.4	61.5	0.39
15.4	74.3	69.2	0.44
15.8	71.6	69.2	0.41
16.6	64.9	73.1	0.38
18.2	46	89	0.35
20.2	24.3	88.5	0.13
22.2	12.2	92.3	0.05
26	2.7	100	0.03

[a]: Youden index=Sensitivity+specificity− 1, 25 (OH) D: 25-hydroxyvitamin-D

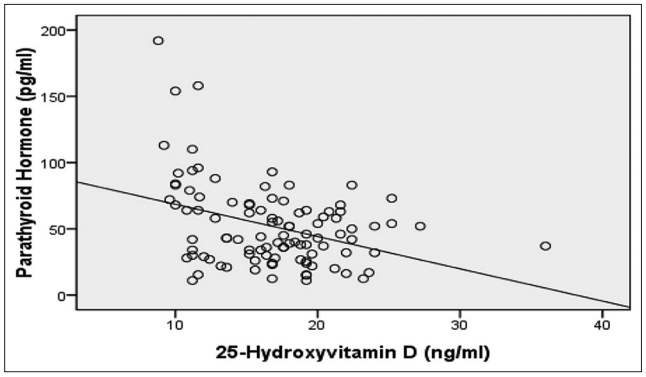

Figure 1: The relation between serum 25-hydroxyvitamin-D (25(OH)D) and intact parathyroid hormone (iPTH) concentrations in medical students. Serum levels of 25(OH) D were inversely correlated with iPTH levels (r = −0.36, P = 0.0001)

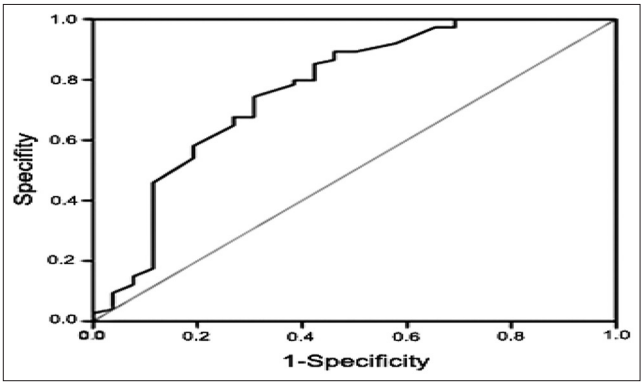

Figure 2: Receiver operating characteristics curve analysis of serum 25-hydroxyvitamin-D for detection of intact parathyroid hormone >65 pgl/ml. Area under the curve was 0.77, P = 0.0001

high frequency of hypovitaminosis D found in the current study was in consistence with those of few other studies published before in this specific group. Using 30 ng/ml as the cut-off value, the frequency of hypovitaminosis D in three studies done on medical students or residents from Saudi Arabia, Boston (USA), and Spain were reported as 100, 69, and 60%, respectively;[24,20,23] three other studies including medical residents from India, Brazil, and Portland (USA) used cut-off level of 20 ng/ml and reached 88, 58, and 50% hypovitaminosis D, respectively.[21,22,19] Adding our results to the other available six studies it may be concluded that the geographic region and not the latitude alone, is a minor risk factor for developing hypovitaminosis D. Although it is a worldwide matter, the problem is more prevalent in Eastern countries than in Europe or North and South America. As discussed above, economy did not have a major distinctive role in the frequency of vitamin D deficiency in the mentioned studies. Based on our knowledge, this is the first study done exclusively on young adult Iranian population. High prevalence of vitamin D deficiency has been shown before in a multicenter study in different parts of Iran.[32] Although it had mainly studied middle-aged and older population, Hashemipour *et al.* found >80% vitamin D deficiency in a heterogeneous age-group, with a significantly more frequency in the younger populations.[33]

In order to define vitamin D normality it is essential to consider all the possible effects of it on the human's health that may be classified as skeletal and nonskeletal actions. Vitamin D plays an inevitable role in attaining and maintaining proper bone architecture so considering the established effect of iPTH in bone physiology, it is rational to consider the serum 25(OH)D level aimed at preventing secondary hyperparathyroidism as one of the criteria of vitamin D normality. During the last decade, many studies have been published on the relation between 25(OH)D and iPTH;[34] although many of them showed an inverse correlation between the two factors, but some few others were unable to find any meaningful association.[35] As discussed earlier the majority of those studies have mainly assessed middle-aged or elderly. The significant inverse correlation found in the current study, $r = -0.36$, was consistent with those prior positive findings. Four studies have previously assessed the relation between 25(OH)D and iPTH in medical students; the Pearson correlation coefficient (r) was −0.33 and −0.58 in the studies from Brazil and Portland area of USA, respectively.[22,19] In the other Indian study, the relation was only significant in males not females, with $r = -0.22$ and the Saudi Arabian study did not show any significance.[21,24] Although 25(OH)D level was <30 ng/ml in 99% of participants, only 26% of our students had secondary hyperparathyroidism; this number was 40% and 45% in Brazilian and Indian studies, respectively.[22,21] Using Youden index based on sensitivity and specificity analysis, this study found 25(OH)D level of 15.4 ng/ml as the optimal cut point of possible secondary hyperparathyroidism. None of the other above-mentioned studies done on medical staffs has tried to set the 25(OH)D cut-off point. We, as well, could not find any clear evidence on that cut-off point individually in young adults to compare with ours, but our finding was in agreement with Sai *et al.*, who assessed 70 studies to define a threshold value of 25(OH)D regarding bone health. They concluded that considering all inconsistencies in the literature the cut-off point would be within the wide range of 10-50 ng/ml.[34]

This study carries some limitations. In addition to small sample size and insufficient information on the participant's calcium intake, serum magnesium was not measured. A checklist method was used to collect the data on consumption of seafood and exposure to sunlight rather than using a validated questionnaire. Besides these limitations we tried to give more strength to our study by doing it in a uniform young population, which still has not been fully assessed in the literature.

CONCLUSIONS

Near all of medical students in this study revealed 25(OH)D level of <30 ng/ml. To prevent iPTH rise, serum 25(OH)D level of 15.4 ng/ml was determined as the optimal threshold. Added to few available results on vitamin D status in medical students worldwide, this study more clarified a pitfall. In fact, the future doctors who should provide medical care to the general population in the near future are not really aware of the common, but latent health matter they suffer from, themselves. Further investigations in

other professional subgroups may be also advisable to reach a comprehensive insight regarding hypovitaminosis D. To improve the community vitamin D status, in addition to population based food fortification programs, more educational activity seems to be essential to increase awareness about the need for vitamin D supplementation.

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REFERENCES

1. Garland CF, Comstock GW, Garland FC, Helsing KJ, Shaw EK, Gorham ED. Serum 25-hydroxyvitamin D and colon cancer: Eight-year prospective study. Lancet 1989;2:1176-8.
2. Grant WB. An ecologic study of dietary and solar ultraviolet-B links to breast carcinoma mortality rates. Cancer 2002;94:272-81.
3. Merlino LA, Curtis J, Mikuls TR, Cerhan JR, Criswell LA, Saag KG, *et al*. Vitamin D intake is inversely associated with rheumatoid arthritis: Results from the Iowa Women's Health Study. Arthritis Rheum 2004;50:72-7.
4. Reis JP, von Mühlen D, Miller ER, Michos ED, Appel LJ. Vitamin D status and cardiometabolic risk factors in the United States adolescent population. Pediatrics 2009;124:e371-9.
5. Ginde AA, Mansbach JM, Camargo CA Jr. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. Arch Intern Med 2009;169:384-90.
6. Mersch PP, Middendorp HM, Bouhuys AL, Beersma DG, van den Hoofdakker RH. Seasonal affective disorder and latitude: A review of the literature. J Affect Disord 1999;53:35-48.
7. Elsammak MY, Al-Wossaibi AA, Al-Howeish A, Alsaeed J. High prevalence of vitamin D deficiency in the sunny Eastern region of Saudi Arabia: A hospital-based study. East Mediterr Health J 2011;17:317-22.
8. Muhairi SJ, Mehairi AE, Khouri AA, Naqbi MM, Maskari FA, Al Kaabi J, *et al*. Vitamin D deficiency among healthy adolescents in Al Ain, United Arab Emirates. BMC Public Health 2013;13:33.
9. Marwaha RK, Tandon N, Garg MK, Kanwar R, Narang A, Sastry A, *et al*. Vitamin D status in healthy Indians aged 50 years and above. J Assoc Physicians India 2011;59:706-9.
10. Boyages S, Bilinski K. Seasonal reduction in vitamin D level persists into spring in NSW Australia: Implications for monitoring and replacement therapy. Clin Endocrinol (Oxf) 2012;77:515-23.
11. 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;96:1911-30.
12. Zabihiyeganeh M, Jahed A, Nojomi M. Treatment of hypovitaminosis D with pharmacologic doses of cholecalciferol, oral vs intramuscular; an open labeled RCT. Clin Endocrinol (Oxf) 2013;78:210-6.
13. Holick MF. Vitamin D status: Measurement, interpretation, and clinical application. Ann Epidemiol 2009;19:73-8.
14. IOM (Institute of Medicine). Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: The National Academies Press; 2011.
15. Steingrimsdottir L, Gunnarsson O, Indridason OS, Franzson L, Sigurdsson G. Relationship between serum parathyroid hormone levels, vitamin D sufficiency, and calcium intake. JAMA 2005;294:2336-41.
16. Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: Consequences for bone loss and fractures and therapeutic implications. Endocr Rev 2001;22:477-501.
17. Gannagé-Yared MH, Chemali R, Yaacoub N, Halaby G. Hypovitaminosis D in a sunny country: Relation to lifestyle and bone markers. J Bone Miner Res 2000;15:1856-62.
18. Tangpricha V, Pearce EN, Chen TC, Holick MF. Vitamin D insufficiency among free-living healthy young adults. Am J Med 2002;112:659-62.
19. Haney EM, Stadler D, Bliziotes MM. Vitamin D insufficiency in internal medicine residents. Calcif Tissue Int 2005;76:11-6.
20. Growdon AS, Camargo CA Jr, Clark S, Hannon M, Mansbach JM. Serum 25-hydroxyvitamin D levels among Boston trainee doctors in winter. Nutrients 2012;4:197-207.
21. Multani SK, Sarathi V, Shivane V, Bandgar TR, Menon PS, Shah NS. Study of bone mineral density in resident doctors working at a teaching hospital. J Postgrad Med 2010;56:65-70.
22. Premaor MO, Paludo P, Manica D, Paludo AP, Rossatto ER, Scalco R, *et al*. Hypovitaminosis D and secondary hyperparathyroidism in resident physicians of a general hospital in southern Brazil. J Endocrinol Invest 2008;31:991-5.

23. González-Padilla E, Soria López A, González-Rodríguez E, García-Santana S, Mirallave-Pescador A, Groba Marco Mdel V, *et al*. High prevalence of hypovitaminosis D in medical students in Gran Canaria, Canary Islands (Spain). Endocrinol Nutr 2011;58:267-73.
24. Al-Elq AH. The status of vitamin D in medical students in the preclerkship years of a Saudi medical school. J Family Community Med 2012;19:100-4.
25. Schisterman EF, Perkins NJ, Liu A, Bondell H. Optimal cut-point and its corresponding Youden Index to discriminate individuals using pooled blood samples. Epidemiology 2005;16:73-81.
26. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. Am J Clin Nutr 2004;80:1678S-88.
27. Arabi A, El Rassi R, El-Hajj Fuleihan G. Hypovitaminosis D in developing countries-prevalence, risk factors and outcomes. Nat Rev Endocrinol 2010;6:550-61.
28. MacLaughlin J, Holick MF. Aging decreases the capacity of human skin to produce vitamin D3. J Clin Invest 1985;76:1536-8.
29. Snijder MB, van Dam RM, Visser M, Deeg DJ, Dekker JM, Bouter LM, *et al*. Adiposity in relation to vitamin D status and parathyroid hormone levels: A population-based study in older men and women. J Clin Endocrinol Metab 2005;90:4119-23.
30. Matsuoka LY, Wortsman J, Haddad JG, Kolm P, Hollis BW. Racial pigmentation and the cutaneous synthesis of vitamin D. Arch Dermatol 1991;127:536-8.
31. Sedrani SH. Low 25-hydroxyvitamin D and normal serum calcium concentrations in Saudi Arabia: Riyadh region. Ann Nutr Metab 1984;28:181-5.
32. Heshmat R, Mohammad K, Majdzadeh SR, Forouzanfar MH, Bahrami A, Ranjbar GH, *et al*. Vitamin D deficiency in Iran: A multi-center study among different urban areas. Iran J Publ Health 2008;1:72-8S.
33. Hashemipour S, Larijani B, Adibi H, Javadi E, Sedaghat M, Pajouhi M, *et al*. Vitamin D deficiency and causative factors in the population of Tehran. BMC Public Health 2004;4:38.
34. Sai AJ, Walters RW, Fang X, Gallagher JC. Relationship between vitamin D, parathyroid hormone, and bone health. J Clin Endocrinol Metab 2011;96:E436-46.
35. 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;32:684-9.

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