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SM Journal of Endocrinology and Metabolism

Research article

Study of 25-Hydroxyvitamin D Levels and Bone Health Markers in Apparently Healthy Medical and Paramedical Staff of Tertiary Care Hospital

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Article Information

Received date: Oct 15, 2015
Accepted date: Jan 21, 2016
Published date: Jan 27, 2016

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Keywords Health care workers; Vitamin D deficiency; Bone markers

Abstract

Introduction: Vitamin D Deficiency (VDD) in health care workers is thought to be widely prevalent due to poor sunlight exposure due to the nature of their work. However, there is limited information of the vitamin D status in them. Hence this study was undertaken to assess vitamin D status in health care workers based on their nature of work and employment in the hospital.

Material and Methods: The study was carried in 229 healthy health care workers in a tertiary care hospital. These subjects, who were divided in six groups as per their employability: Group-1 (Medical Officers- 37), Group-2 (Nursing Officers-44), Group-3 (O.T. staff-36), Group-4 (Nursing assistant Acute ward- 38), Group-5 (Nursing assistants Non-acute wards-37) and Group-6 (Ambulance assistants-37). They underwent clinical, biochemical and hormonal evaluation for serum 25 (OH) D, iPTH, BsALP and osteocalcin levels.

Results: There were 229 health workers with a mean age of 35.5 ± 7.5 years (range 19-52 years). A total of 45 subjects (19.6%) had VDD [25(OH) D <20 ng/ml], and 151 (65.9%) were vitamin D insufficient [25(OH) D 20-<30 ng/ml] and rest were Vitamin D Sufficient (VDI) [25(OH) D ≥30 ng/ml]. Nurses and operation theatre staff had higher prevalence of VDD and VDI than ambulance assistants. Serum 25(OH) D levels were negatively correlated with PTH (r -0.156, p <0.0001), BsALP (r -0.131, p < 0.001) and osteocalcin levels (r -0.129, p < 0.001). Prevalence of secondary hyperparathyroidism increased from 30.46% to 58.35% from VDI to severe VDD.

Conclusion: Vitamin D insufficiency is universal in health care workers. Absence of a PTH, BsALP and osteocalcin response was observed in more than 40% of individuals with VDD, the cause of which merits further evaluation.

Introduction

Vitamin D Deficiency (VDD) has been documented across all age groups and both sexes from India and different parts of world [1-13]. Few recent studies [7,14] have highlighted this problem to be more in the health care professionals. Exposure to sunlight plays a vital role in maintaining the vitamin D levels in the body. Individuals with optimal nutrition, good sunlight exposure and regular physical exercise have normal bone and mineral biochemical values. The healthcare profession demands the employee to work indoor throughout the day. This places them at risk for VDD. Thus this study was undertaken in health care workers to assess prevalence of Vitamin D deficiency and other bone health markers based on the job profile of the subjects.

Material and Methods

This study was carried in a tertiary care hospital. We recruited 229 apparently healthy hospital staff in a tertiary care hospital belonging to the following six distinct groups: physicians (n=36), nurses and nursing cadets (n=44), Operation Theatre (OT) staff (n=37) and Intensive Care Unit (ICU)/acute medical wards staff (n=37), medical and paramedical staff in non-acute wards (n=38), non-medical hospital staff like Ambulance assistants (n=37). Exclusion criteria were pregnancy, hepatic, renal, dermatological disorders, alcoholism, and those receiving medication likely to adversely affect vitamin D status. Informed consent was taken. Ethical clearance was taken for the study by the hospital ethical clearance committee.

Direct sunlight exposure was assessed by documenting average duration of exposure and percentage of the surface area of the body exposed daily [11]. The average duration of cloud-free sunshine during the year of this study was 3.1 h/d in the summer (April to July 2009 in Kolkata [28.35 N and 77.12 E with zenith angle of 84.5 in peak summer (Meteorology Department, Kolkata)]. The ground surface of Kolkata receives 4 MED (minimal erythemal dose) of ultraviolet radiation/d during the summer [10]. The skin pigmentation of the subjects was classified visually as

Table 1: Clinical characteristics, sun exposure, and biochemical markers in the study groups.

VariableS	Medical officers (n = 36)	Nursing officers (n = 44)	Operation Theatre staff (n = 37)	Nursing assistants Acute wards (n = 37)	Nursing assistants Non-acute wards (n = 38)	Ambulance assistants (n = 37)
Age (years)	35.67±7.35	27±10.66	32.11±8.55	32.68±6.036	33.75±6.99	34.73±8.15
BMI (kg/m²)	23.2±4.4	21.2±2.0	22.1±2.4	23.4±2.2	21.8±2.7	21.4±2.0
Calcium intake (mg/day)	1140±245[a]	745±434[b]	1050±540[a]	1185±334[a]	1284±165[a]	1274±234[a]
Phosphorus intake (mg/day)	674 ± 17	593 ± 9	651 ± 9	632 ± 18	667 ± 15	667 ± 15
Phytate: Calcium ratio	0.59 ± 0.069[a]	0.79 ± 0.024[b]	0.62 ± 0.016[a]	0.533± 0.053[a]	0.51 ± 0.09[a]	0.52 ± 0.064[a]
Sun exposure (min/day)	20 ± 15[a]	15±10[a]	10±10[a]	25±20[a]	30 ± 20[a]	45±15[b]
Serum total calcium (mmol/l)	2.27±0.15	2.14±0.12	2.34±0.10	2.28±0.10	2.22±0.18	2.24±0.10
Serum Inorganic phosphate (mmol/l)	1.18±0.12	1.31±0.16	1.22±0.10	1.21±0.10	1.20±0.18	1.24±0.13

Values in the same row with different superscript letters are significantly different, *($p < 0.05$)* for that variable among the groups with different superscript.

dark, whitish or fair. In all groups, the proportion of subjects in each pigment category was not significantly different, with about 70% of subjects in each group having a whitish complexion.

The dietary assessment of total energy, calcium, phosphorus and phytates were documented by recalling the diet consumed in the previous 5 to 7 days. From the raw weights, the total energy, calcium, phosphorus and phytate intake were calculated using a published food composition table, detailing the nutritive value of Indian foods [19]. Since the ratio of dietary phytates to calcium is more predictive of the severity of interference of calcium absorption than dietary calcium alone, phytate to calcium ratio was calculated [15]. Dairy products and food fats are not fortified with vitamin D in India.

Blood samples were collected without hemostasis from the different groups under basal conditions for estimations of serum 25(OH) D, i-PTH, total calcium, inorganic phosphate, osteocalcin and bone-specific Alkaline Phosphates (BsALP) activity. The serum was separated in a refrigerated centrifuge, aliquoted, and stored at -20 degree C until analyzed. Serum total calcium and inorganic phosphate, and i-PTH (normal i-PTH range 10-65 pg/ml) were measured by methods described previously [8]. Serum 25(OH) D were measured by a competitive ELISA technique with a selected monoclonal antibody recognizing 25(OH) D and estimated at 450NM. (Immunodiagnostik AG Besheim). Vitamin D sufficiency was defined as levels >30 ng/ml (>75 nmol/l), Vitamin D insufficiency as levels between 20 to 30 ng/ml (50-75 nmol/l), Vitamin D deficiency as levels < 20 ng/ml (< 50 nmol/l) and Severe Vitamin D deficiency as levels < 10 ng/ml (<25nmol/l). BsALP (normal range 15-41 U/L) was measured by an immunoassay in a microtitre strip format utilizing a monoclonal Anti-BsALP Ab coated on a strip to capture BsALP in the sample. The enzyme activity of the captured BsALP was detected with a Pnpp substrate (Quidel Corp, San Diego, USA). Serum Osteocalcin (normal levels 5-25 ng/ml) was measured by immonoenzymatic assay for the in-vitro quantitative measurement of intact human osteocalcin on serum and plasma (Bio-Line SA, Belgium).

The data has been analyzed using standard protocols and statistical packages and softwares like Epi-Info and Win-Pepi. As the data involved multiple comparisons between various categories, the statistical test ANOVA (Analysis of Variance) has been used. Once this test was found to be significant, then various tests for multiple comparisons among different categories have been applied, like Games Howell multiple comparison, etc. Certain other important statistical tests like Chi Square test, Mann-Whitney/Wilcoxon Two-Sample Test (Kruskal-Wallis test for two groups) and Bartlett's Test for Inequality of Population Variances, etc have been used in analyzing the data.

Results

Total of 229 apparently healthy health care workers' of a tertiary care hospital were evaluated during the study. The study groups' mean ages, sunlight exposure, and serum values total calcium, inorganic phosphate, and alkaline phosphatase are summarized in (Table 1). Subjects were working inside the hospital for 07-09 hrs daily starting from 0800hrs except the ambulance assistants group, who were also required to work outdoors. All the groups were normal nutritionally in terms of their body mass indexes. The diet of subjects in all groups constituted of 2200 kcal/day approximately except in nursing officers and cadets group (1800 kcal/day). The dietary energy, carbohydrate, fat, and protein intakes of these groups were normal according to Indian normative data published by the Indian Council of Medical Research (ICMR) [12]. The mean dietary calcium contents of nursing officers' group were significantly lower when compared to other groups. The mean ratio of phytate to calcium in the daily diet of all the groups was significantly higher in nursing officers group as compared to other groups.

The mean direct sunlight exposure of the ambulance assistant group was significantly higher (because of longer duration of daily outdoor work/activities) than that of the physician, nursing officers, nursing assistants and OT staff. The mean duration of direct sunlight exposure in the all groups was 25 ± 5 min. except in ambulance assistant group were it was 45 ± 5 min. The skin surface area exposed to direct sunlight during this time was 20% as a result of summer dress.

Mean 25(OH) D concentrations varied among the different study groups (Table 2). Out of total 36 Medical Officers, 07 (19.3%) had severe VDD, 08 (22.1%) had VDD, 15 (41.7%) had VDI and only 06 (16.7%) were Vitamin D sufficient. The highest mean 25(OH) D concentration was measured in the ambulance assistant (non-nursing staff) group. Of all the groups, OT staff had significantly less sunlight exposure than did the other groups and also had significantly lower 25(OH)D concentrations as had the nursing officers' group . Nurses

Table 2: Vitamin D status according to various groups.

Category	Sufficient	Insufficient	Deficient	Severely Deficient
Medical officer	06(16.7%)	15(41.7%)	08(22.1%)	07(19.3%)
Nursing officer / nursing cadets	03(6.8%)	24(54.5%)	09(20.5%)	08(18.2%)
OT staff	01(2.7%)	30(81.1%)	01(2.7%)	05(13.5%)
Nursing assistant Acute ward	01(2.7%)	32(86.5%)	02(5.4%)	02(5.4%)
Nursing assistant Non Acute ward	01(2.6%)	36(94.8%)	00(0.0%)	01(2.6%)
Ambulance Assistant	21(56.8%)	14(37.8%)	01(2.7%)	01(2.7%)
TOTAL	31	151	21	24

to normal individuals (Table 4). However, as a whole group those with VDI/VDD about 65% were unable to mount secondary PTH response. These findings are in concert with a similar observation study from elderly population in Delhi [12].

Mean serum BsALP and osteocalcin levels varied significantly among the different study groups. OT staff had significantly high BsALP and osteocalcin levels when compared with Medical officers' (p < 0.05), Nursing assistants (Non-acute ward) group (p < 0.01) and Ambulance assistant groups (p < 0.01). Also during comparison of means between other groups BsALP and osteocalcin was significantly different between Nursing Assistant Acute ward vs Ambulance Assistant (p <0.01) and Nursing Assistant Acute ward *vs.* Nursing

Table 3: Vitamin D and bone markers in the study groups.

Variables	Medical officers (n= 36)	Nursing officers (n = 44)	Operation Theatre staff (n = 37)	Nursing assistants Acute wards (n = 37)	Nursing assistants Non-acute wards (n = 38)	Ambulance assistants (n = 37)	P Value Kruskal-Wallis H (equivalent to Chi square)
25(OH)D	49.08±28.03 (45.10)	43.80±17.84 (44.14)	47.00±16.06 (43.05)	51.61±14.49 (54.20)	55.07±8.39 (55.50)	85.15±23.99 (87.10)	< 0.0001
PTH	49.62±40.60 (34.6)	80.87±40.05 (58.65)	95.00±60.98 (73.85)	76.68±53.35 (66.10)	65.72±13.88 (68.70)	62.59±14.10 (63.60)	< 0.008
Serum BsALP	31.13±8.46 (44.30)	31.99±13.25 (29.20)	38.88±11.86 (30.25)	33.21±23.38 (27.50)	22.99±5.73 (29.40)	29.36±7.76 (22.80)	< 0.0001
Serum Osteocalcin	17.88±7.69 (16.30)	18.05±4.91 (16.70)	16.34±4.99 (17.15)	14.48±3.66 (15.10)	16.14±1.16 (16.60)	13.53±2.41 (14.20)	<0.0001

Table 4: PTH and bone markers Levels According to Vitamin D Status.

sVitamin D Levels	PTH Levels (% Increase)	BsALP Levels (% Increase)	S. Osteocalcin (% Increase)
Severe VDD (<25 nmol/L)	70.28±51.57 (67.0)	36.88±11.86 (47.8)	18.05±4.91 (33.4)
VDD (25-<50nmol/L)	58.42±28.76 (38.8)	33.21±14.38 (33.0)	16.88±7.69 (24.8)
VDI (50-<75nmol/L)	50.24±26.44 (19.4)	28.36±7.76 (13.6)	14.48±3.66 (7.0)
Normal (>75nmol/L)	42.08±10.32	24.96±5.73	13.53±2.41
P Value Chi-square for trend	< 0.0001	< 0.0001	< 0.001

Table 5: Secondary hyperparathyroidism according to vitamin D status.

Vitamin D Status	Secondary HPT		*p* value
	Present	Absent	
Severe VDD (<25 nmol/L)	14 (58.33%)	10 (41.67%)	< 0.001 (Chi-square for trend)
VDD (25- <50 nmol/L)	09 (42.85%)	12 (57.15%)	
VDI (50-<75 nmol/L)	46 (30.46%)	105 (69.54%)	
Normal (>75nmol/L)	0	33 (100.0%)	

were one of the worst affected groups along with OT Staff with only 2.3% nurses being Vitamin D sufficient, 65.9% being VDI and 31.8% being VDD. 25(OH) D levels were significantly less in females when compared to males (p < 0.0002) but were not significantly different in different age groups (p < 0.172). Out of total 229 health care workers, 24 (10.48%) had severe VDD, 21 (9.17%) were VDD, 151 (65.9%) were insufficient and 33 (14.4%) were vitamin D sufficient.

Mean serum iPTH levels varied significantly among the different study groups (p < 0.008). OT staff had significantly high iPTH levels when compared with Medical officers' (p < 0.01) and ambulance assistant groups (p < 0.05) (Table 3). Serum iPTH levels were not significantly different in various age groups (p< 0.825) and gender (p < 0.537). Intact PTH negatively correlated with vitamin D levels in multivariate analysis (coefficient; r value - 0.156, p <0.0001) (Table 3). Serum iPTH levels progressively increased from VDI to varying severity of VDD. They were within normal range among subjects with serum 25(OH) D levels ≥75 nmol/L. Serum PTH, BsALP, osteocalcin levels progressively increased with reducing Vitamin D levels (Table 4) and prevalence of secondary hyperparathyroidism increased progressively among subjects with vitamin D sufficiency to severe VDD (Table 5). Serum iPTH was higher by 19.39%, 38.83% and 67.01% in those with VDI, moderate VDD and severe VDD compared

Officer / cadets significant at p< 0.01. BsALP and osteocalcin levels were not significantly different in different age groups (p < 0.180) and gender (p< 0.871). BsALP and osteocalcin markers for bone formation also showed similar trend as observed with serum PTH. BsALP and osteocalcin levels were correlated negatively with vitamin D levels in multivariate analysis (coefficient; r value 0.131, p <0.001 and coefficient; r value – 0.129, p <0.001) (Table 3).

Discussion

There have been very few studies [7,14] which have evaluated the prevalence of VDD in healthcare professionals. The healthcare profession demands the employee to work indoor throughout the day. This places them at risk for VDD. Sun exposure is the most important determinant for endogenous vitamin D synthesis. Among the healthcare professionals sun exposure is determined by the type of duty they perform. The number of hours spent indoors is more in healthcare professionals thus placing them in higher risk for VDD.

Our study from Eastern part of India confirms high prevalence of VDD in Doctors, Nurses and other healthcare professionals working long hours indoors. Mean serum 25 (OH) D levels were 55.46 nmol/L during summer in our study. Various studies in India have shown mean 25(OH)D level ranging from 4.5 ng/ml (11.25 nmol/L) to 20.85 ng/ml (52.13 nmol/L) in general population in various age groups [9]. In contrast, higher serum 25(OH) D levels (63.25±18.5 nmol/L) in females in summer and (46.0±13.25 nmol/L) and in males in winter have been observed in paramilitary personnel. In our study mean 25(OH) D levels though low, were still higher as compared to other studies, likely due to better awareness of VDD state and more sunlight exposure in army doctors and other health workers. Also this study was done in peak summer month which may explain slightly more 25(OH)D levels as compared to that expected levels in winter months.

Serum iPTH levels progressively increased from VDI to varying severity of VDD. Secondary Hyperparathyroidism (SHPT) were present in less than half (41.67%) of subjects with severe vitamin D deficiency (<10 nmol/L). Thus, as a cohort, those with VDI/VDD about 65% were unable to mount secondary PTH response. These findings are in concert with a similar observation study from elderly population in Delhi [12]. The possible explanations for this phenomenon include probable adaptation to vitamin D deficiency or other associated genetic, nutritional or environmental factors which preclude an elevation of serum PTH levels. A study of vitamin D receptor polymorphism from this geographical region did not reveal any abnormality or increase expression explaining adaptability to VDD [18]. However decreased expression of calcium sensing receptor has been observed with vitamin D deficiency in rat and human parathyroid glands [19,20]. This may limit the ability of calcium and calcitriol to regulate PTH secretion in individuals with long standing VDD [21]. Variation in calcium intake and other dietary factor can lead to variation in effect of VDD on PTH [2,22].

Serum 25(OH) D levels were negatively correlated with serum iPTH, BsALP and osteocalcin levels in multivariate analysis. Similar correlation has been observed by others [2,5]. In a recent Indian study among resident doctors [14] BsALP levels were not significantly elevated during winter months however they showed the same to be elevated in winter. BsAP and osteocalcin, seen during summer month, were elevated in our cohort in subjects with VDI or VDD.

Medical officers

Mean Vitamin D concentration in this group was 49.62±40.60 nmol/L. Out of total 36 Medical Officers, 83.1% were either VDD [15] or VDI [6]. Seven (19.3%) had severe VDD. A recent study from India [14] in young resident doctors, reported lower mean 25(OH)D levels (29 nmol/L) during summer as compared to our cohort and higher prevalence of VDD (37.5%) and VDI (62.5%) during summer. This difference could be due to better health awareness and slightly more sunlight exposure in practicing army doctors than resident doctors.

Nursing officers

In our study Nurses were one of the worst affected groups along with OT Staff. Similarly they had significantly higher levels of iPTH, BsALP and Osteocalcin as compared to other groups. This could be explained by poor dietary calcium intake seen in this group coupled with poor sunlight exposure with tendency to avoid sunlight exposure along with remaining indoor for longer durations. Contrary to Indians, the Nurses' Health Study [16] in Americans showed that with proper education majority were able to maintain vitamin D sufficiency.

Nursing assistants

Nursing assistants working in acute wards and non-acute wards didn't show any significant differences in Vitamin D levels and other bone health markers. Mean 25(OH) D levels in group of nursing assistants in acute wards and non-acute wards was significantly lower than compared with ambulance assistant group. This could be explained by lesser duration of sunlight exposure as compared to ambulance assistants due to the nature of their work involving more outdoor activities.

Operation theatre staff

OT staff was showed similar pattern as Nurses. They had comparatively longer duration of indoor activities and least sunlight exposure. The mean 25(OH) D levels in this group were significantly lower as compared to other groups like medical officers and ambulance assistants. They also had highest levels of mean iPTH, BsALP and osteocalcin levels corroborating with low vitamin D levels and suggesting most patients with biochemical osteomalacia in this group. Dietary Calcium intake in this group was not low and dietary Phytate levels were also not significantly high.

Ambulance assistant

This group of healthcare workers was the best of all the groups with highest mean 25(OH) D levels (85.15 nmol/L) and vitamin D sufficiency (56.8%). Of the people with Vitamin D sufficiency, 68% were from Ambulance assistant group and 19.5% were medical officers. They had maximum sunlight exposure of all the groups thus confirming the importance of adequate sunlight exposure. Ramakrishnan et al., [15] showed that with adequate sun exposure 72.5% of individuals were able to maintain vitamin D sufficiency. These values are comparable to the ambulance assistants in our group who have adequate sun exposure. Other bone formation markers were not significantly increased in this group. Also in a north Indian cohort Goswami et al., [17] showed that rural male with prolonged sun exposure were able to maintain vitamin D sufficiency compared to rural women and urban population. The vitamin D levels in rural male were comparable to that of ambulance assistants in our study.

All subjects recruited in this study were though well aware of Vitamin D deficiency states but due to their profession were unable to follow simple preventive measures like adequate dietary calcium (in nurses group only) and adequate sunlight exposure due to remaining indoors for prolonged hours. This study was carried in Kolkata in peak summer months where the weather is usually sultry and humid thus making it a deterrent for the people in these areas to go in sunlight thus making food fortification a good option in solving this problem.

Conclusion

In summary, despite abundant sunlight, majority of the healthy persons in working in hospitals remain vitamin D deficient because inadequate direct sunlight exposure. Adequate exposure to sunlight can prevent VDD as documented by significantly higher serum 25(OH) D and significantly lower i-PTH concentrations in ambulance assistant group than rest of health care workers group. Nurses and the O.T staff were the worst affected groups.

The relative normalization of serum calcium in various groups was likely attained through a PTH-mediated resorptive process of bone mineral. Nonetheless, the absence of an anticipated PTH response to low serum 25(OH) D levels in a significant number of subjects with various levels of vitamin D deficiencies is an observation, which merits further study.

It is said that "Prevention is better than cure". This is true for vitamin D insufficiency and deficiency which are easily preventable. Thus, the current recommendations of taking 1 to 1.5 gm of dietary calcium and 2000 IU of vitamin D per day in the diet should be adhered to avoid Vitamin D deficiency in the health care workers.

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Citation: Brar KS and Garg MK. Study of 25-Hydroxyvitamin D Levels and Bone Health Markers in Apparently Healthy Medical and Paramedical Staff of Tertiary Care Hospital. SM J Endocrinol Metab. 2016;2(1):1007.