VITAMIN D STATUS IN NORTH INDIAN FEMALES: A CROSS-SECTIONAL STUDY FROM HARYANA, NORTH INDIA

Dr. Ragini Singh Ghalaut[1], Dr. Veena Singh Ghalaut[2], Dr. Aditi Singh[3], Dr. Anuj Chaudhary[4], Dr. Deepika Dalal[5].

Corresponding author: Dr. Aditi Singh

1-Assistant Professor, Blood Transfusion Dept.,Bhagat Phool Singh Govt. Medical College for Women, Khanpur Kalan, Sonepat.

2- Senior Professor and HOD, Dept. of Biochemistry, Pt. B.D.Sharma,PGIMS, Rohtak.

3- Demonstrator, Dept. of Biochemistry, Pt. B.D.Sharma,PGIMS, Rohtak.

4- Consultant, Jeet Hospital, Gohana

5- Demonstrator, Dept. of Biochemistry, Pt. B.D.Sharma,PGIMS, Rohtak

ABSTRACT

Introduction: Vitamin D deficiency is a globally recognized public health problem. Even in a tropical country like India, where there is sufficient sunshine, vitamin D insufficiency/deficiency is widely prevalent. We conducted this study to document vitamin D levels and the prevalence of vitamin D deficiency in a population comprised of Indian females from North India.

Methods: A hospital based cross-sectional study was conducted among 150 women aged ≥10 years in the department of Biochemistry, Pt. B.D Sharma Postgraduate Institute of Medical Sciences, Rohtak, Haryana, India. Serum 25-hydroxyVitamin D (25(OH)D) was estimated by radioimmunoassay method, on fully automated RIA Analyzer SR300 by Stratec Biomedical AG, Germany using Beckman Coulter 25OH vitamin D total RIA kit. Individuals with 25OH vitamin D <20ng/ml were said to have vitamin D deficiency, those with 20-30 ng/ml was considered vitamin D insufficiency (VDI) and those with values>30ng/ml were taken as vitamin D sufficient. Data entry and analysis was done using SPSS version 16.0 and R-software.

Results: A total of 150 females were enrolled in the study and their vitamin D levels were assessed. Out of 150 females, around one-fifth were in the age category of 10-15 years (20.7%), nearly half were aged 15-45 years (52.7%) and the remaining were older than 45 years (26.6%). The mean (SD) vitamin D levels of the study population was 22 (11.9) ng/ml whereas the median (IQR) level was 19.7 (14.5-27) ng/ml. Overall, around half (51.3%) of the study population were deficient in vitamin D and another one-third (33.3%) had insufficient vitamin D levels (i.e. 20-30 ng/ml). Only 15.4% of the participants had sufficient vitamin D levels (>30 ng/ml).

Conclusion: The study found a high prevalence of vitamin D deficiency/insufficiency. The need for improving the levels of vitamin D among the female population is both important and urgent. Emphasis on promoting consumption of vitamin D rich food, vitamin D supplements and outdoor activities to increase sun exposure will help alleviate the problem at large.

INTRODUCTION

Vitamin D is a fat-soluble vitamin and is called as 'sunshine vitamin' or 'anti-rachitic factor due to its pivotal role in calcium homeostasis . [1]From available evidence, we understand that it is involved in maintaining the integrity of the musculoskeletal system owing to its association with parathyroid hormone, calcium and phosphorous metabolism .[1,2] Diet or adequate exposure to sunlight are the two predominant pathways through which human body obtains optimal amounts of vitamin D. Approximately 50–90% of vitamin D is produced by the skin on exposure to sun .[2] Natural diet consumed by most humans contains less amounts of vitamin D. Important dietary sources includes egg, fish and fortified dairy products .[3]

Vitamin D deficiency is a global problem and it is prevalent even in a tropical country like India where there is plentiful sunshine.[4] Studies from different parts of India have underscored that vitamin D insufficiency or deficiency across different age groups range from 70–100%, which is quite high and an issue of concern.[5-7] It is quite ironical that even though vitamin D deficiency has increasingly been recognized as an important public health issue, it is not a part of the existing national health program.[8] Deficiency of vitamin D is ubiquitous and affects an individual irrespective of the baseline socio-demographic characteristics such as sex, age, race and geography. Chronic deficiency of vitamin D in adults results in musculoskeletal problems such as osteomalacia, osteoporosis and muscle weakness.[9-11] Recent evidence also links vitamin D deficiency with the enhanced risk of developing chronic infections such as tuberculosis, ear infections such as otitis media and upper respiratory tract infections/influenza.[12-14] Deficiency of vitamin D has also been linked with the increased risk of chronic health conditions such as hypertension, diabetes, obesity and hypertriglyceridemia, thereby, leading to increased risk of cardiovascular morbidity and mortality.[15-17] There is growing epidemiological evidence linking vitamin D deficiency with type1 diabetes. Findings of a recent meta-analysis showing a 30% reduction in risk of type 1 diabetes mellitus in children receiving vitamin D supplementation is quite remarkable.[17]

In India, the lifestyle of individuals is highly influenced by existing cultural and social taboos. Vegetarian diet is most prevalence and that could play an important role in the existing vitamin D deficiencies. Also, Indians prefer being fully clad and therefore experience less exposure to direct sunlight, thereby, aggravating the vitamin D deficiency. Ironically, although vitamin D supplements are available in Indian markets, many are unaware and therefore do not use them. Since vitamin D under-nutrition is largely silent and sub-clinical, but its long term effects on health are profound, we conducted this study to document vitamin D levels and the prevalence of vitamin D deficiency in a population comprised of Indian females from North India.

MATERIAL AND METHODS

The study was conducted among 150 women, who came for vitamin D analysis in the department of Biochemistry, Pt. B.D Sharma Postgraduate Institute of Medical Sciences, Rohtak, Haryana, India. The females were divided into three groups based on the age: 10-15 years, 15-45 years and >45 years.

Inclusion criteria

Inclusion criteria were females aged 10 years onwards.

Exclusion criteria

Exclusion criteria were age <10 yrs; women with parathormone hormone (PTH) deficiency; women with premature ovarian failure; women with chronic liver disease; women with renal disease; women on anti-tubercular or antiepileptic treatment in the previous 3 months.

Vitamin D estimation

Serum 25OH vitamin D was estimated by radioimmunoassay method, on fully automated RIA Analyzer SR300 by Stratec Biomedical AG, Germany using Beckman Coulter 25OH vitamin D total RIA kit. The assay is based on the principle of competitive binding. At first, calibrators, controls and samples are incubated with the incubation buffer, in coated tubes, to release 25OH vitamin D_3 and 25OH vitamin D_2 from vitamin D Binding Protein (DBP). Then, without washing steps, a fixed amount of ^{125}I labelled 25OH vitamin D is added to compete with the 25OH vitamin D_3 and 25OH vitamin D_2 from samples, controls or calibrators, for a fixed amount of specific monoclonal antibody sites immobilized to surface of plastic tubes. After incubation, the content of tubes is aspirated, washed and bound radioactivity is measured. A calibration curve is plotted and the total 25OH vitamin D (D_3 and D_2) concentrations of the samples are determined by interpolation from the calibration curve.[18]

Statistical analyses

Vitamin D deficiency was considered at a level of <20 ng/ml; insufficiency at level of 20-30 ng/ml and sufficiency at a level of >30 ng/ml.[19] Data entry and analysis was performed using the statistical software SPSS (IBM SPSS Statistics for Windows, Version 16.0) and R-software. Descriptive statistics (mean ± Standard Deviation [SD] and median (Inter-quartile Range; IQR) for the continuous variables and frequency in percentage for the categorical variables was used to describe the data. The age of the participants was categorized into 10-15 years, 15-45 years and >45 years. The mean and median values of Vitamin D were compared across these three age categories. Similarly, the proportion of females with vitamin D deficiency, insufficiency and sufficiency were compared across the three age categories. Chi-square test was used to compare proportions and ANOVA was used to compare means. Generalize additive model (GAM) plots were generated using R statistical package to show the association between age and vitamin D levels. Results were considered significant at $P < 0.05$ level.

RESULTS

A total of 150 females were enrolled in the study and their vitamin D levels were assessed. The mean (SD) age of the participants was 33.7 (16.7 years). Out of 150 females, around one-fifth were in the age category of 10-15 years (31/150; 20.7%), nearly half were aged 15-45 years (79/150; 52.7%) and the remaining were older than 45 years (40/150; 26.6%).

The mean (SD) vitamin D levels of the study population was 22 (11.9) ng/ml whereas the median (IQR) level was 19.7 (14.5-27) ng/ml. Overall, around half (77/150; 51.3%) of the study population were deficient in vitamin D (i.e. vitamin D levels <20 ng/ml) and another one-third (50/150; 33.3%) had insufficient vitamin D levels (i.e. 20-30 ng/ml). Only 15.4% (23/150) of the participants had sufficient vitamin D levels (>30 ng/ml).

Table 1 shows the mean (SD) and median (IQR) across the three age groups i.e. 10-15 years, 15-45 years and >45 years. Table 2 shows the proportion of participants with vitamin D deficiency, insufficiency and sufficiency across the three age categories. Among the study subjects aged 10-15 years, the mean (SD) vitamin D level was 21.4 (9.3) ng/ml and median (IQR) level was 19.1 (14.5-26.2) ng/ml. In this age category, 51.6% were deficient in vitamin D, 38.7% had insufficient levels and only 9.7% had sufficient vitamin D levels. In the age group of 15-45 years , the mean (SD) and median (IQR) levels were 20.8 (13.2) ng/ml and 17.2 (13.5-24.4) ng/ml respectively.

Table 1. Vitamin D levels across the three age categories

	Age category			**P-value**
	10-15 years (N=31)	15-45 years (N=79)	>45 years (N=40)	
Mean (SD) vitamin D levels (ng/ml)	21.4 (9.3)	20.8 (13.2)	24.8 (10.7)	0.104
Median (IQR) vitamin D levels (ng/ml)	19.1 (14.5-26.2)	17.2 (13.5-24.4)	22.2 (16.1-30.6)	0.212

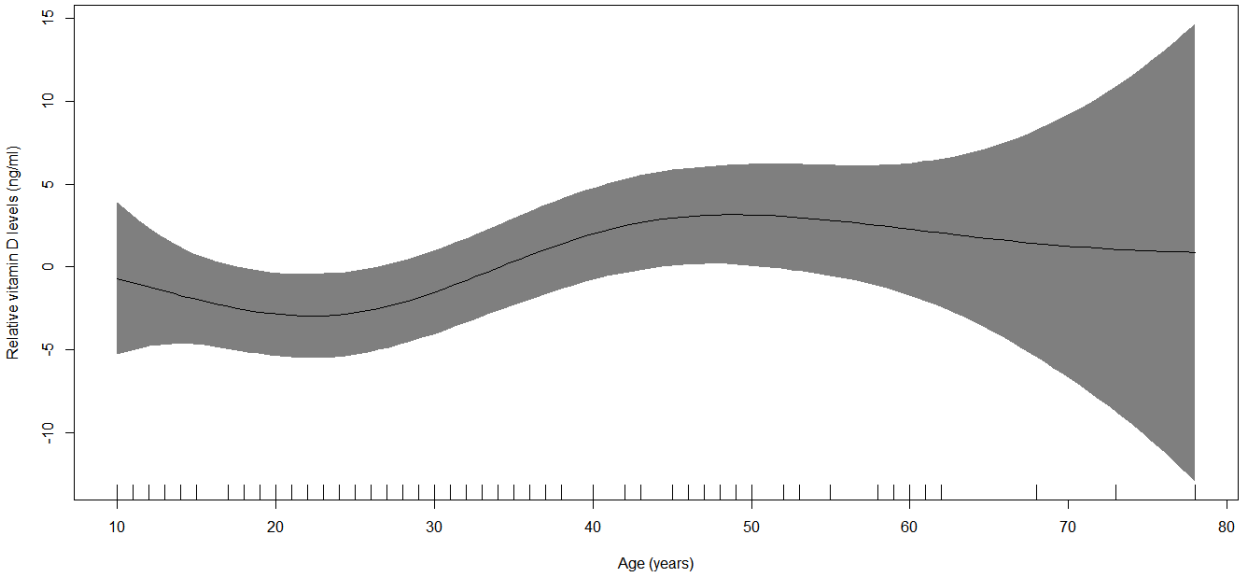
A total of 58.2% had vitamin D deficiency, 29.1% had insufficiency and 12.7% had sufficient vitamin D levels. Among the study participants who were older than 45 years of age, the mean (SD) and median (IQR) vitamin D levels were 24.8 (10.7) ng/ml and 22.2 (16.1-30.6) ng/ml respectively. A total of 37.5% were deficient, similar proportion had insufficient vitamin D levels (37.5%) and only one-fourth (25%) had sufficient vitamin D levels. There was no statistically significant difference in the mean vitamin D levels across the three age categories (P=0.104) and also in the proportion of study participants in each of the three vitamin D categories i.e. deficient, insufficient and sufficient (P=0.160).

Table 2. Distribution of the females according toVitamin D Status.

	Age category			**P-value**
	10-15 years (N=31)	15-45 years (N=79)	>45 years (N=40)	
Vitamin D levels<20ng/mL	16 (51.6%)	46(58.2%)	15 (37.5%)	0.160
Vitamin D levels20-30ng/mL	12 (38.7%)	23 (29.1%)	15 (37.5%)	
Vitamin D levels>30ng/mL	3 (9.7%)	10(12.7%)	10 (25.0%)	

Figure 1 presents the Generalized Additive Model (GAM) plot and represents the relationship between age (in years) and vitamin D levels. The graph was constructed using generalized additive model in R; the solid line depicts the association of vitamin D level and age (in years). The Y-axis is centered on the mean vitamin D level. The shaded area spans the 95% confidence interval of this association. The plot shows that the level of vitamin D does not vary with age and therefore suggests that all age groups are equally at risk of vitamin D deficiency and/or insufficiency.

Figure 1. Generalized Additive Model (GAM) plot representing the relationship between age (in years) and vitamin D levels among the study subjects



DISCUSSION

We conducted this study among 150 females aged 10 years and above to document the vitamin D status. We found that around half (51.3%) of the study population were deficient in vitamin D (i.e. vitamin D levels <20 ng/ml) and another one-third (33.3%) had insufficient vitamin D levels (i.e. 20-30 ng/ml). Only 15.4% of the participants had sufficient vitamin D levels (>30 ng/ml). The combined prevalence of vitamin D deficiency and insufficiency was 84.6% on our study. This is similar to other studies.[6,7] A study in adult women from rural communities of Ballabgarh development block in Faridabad, Haryana documented the prevalence of vitamin D insufficiency (VDI) to be around 90% (6). Similarly, another study from rural Maharashtra reported a prevalence of VDI to be 88.9% .[7]

It is quite interesting to note that vitamin D deficiency has been observed both in countries which are 'sunshine deficient' and 'sunshine sufficient and is therefore a global public health problem. Particularly in India, even though there is abundant sunshine throughout the year, vitamin D deficiency is a problem of utmost concern. A high prevalence (70-100%) of vitamin D deficiency/insufficiency along with sub-

optimal dietary calcium intake has been documented in Indian population .[20-22] Several studies conducted to assess hypovitaminosis D in different age groups in India reveal a high prevalence of vitamin D deficiency i.e. 40 to 70 per cent among pregnant women, 70 to 80 per cent among lactating mothers and 30 to 90 per cent among adults.[23-25] This is quite similar to what we have observed in the present study. In India, there is a suggestion that socio-religious and cultural practices may not facilitate sufficient sun exposure, which is essential for 25(OH)D synthesis. The use of fully covered clothing blocks ambient sunlight and decreases cutaneous vitamin D production, thereby, contributing to lower levels of 25(OH)D. Further, Indian cooking styles such as slow cooking and deep-frying could also be aggravating the deficiency of vitamin D. Vitamin D degrades at high temperatures i.e. above 200°C, cooking gas (LPG) reaches a temperature of 1900°C and coal stoves could attain a temperature as high as 300–700°C. During deep frying, 25(OH)D is thermally degraded as it comes out of the cooking medium. This could be a contributing factor to vitamin D deficiency. Moreover, Indians are predominantly vegetarians and vegetarian food is usually sub-optimal in vitamin D content. Hence, such type of food preferences by Indian population may also predispose to vitamin D insufficiency/deficiency.

Vitamin D deficiency among females of reproductive age group has numerous clinical complications . The reproductive organs particularly the cervical epithelia, endometrium, epithelial cells of the fallopian tubes and ovaries are constituted of receptors and enzymes that have a role in vitamin D metabolism . Deficiency of Vitamin D may also lead to infertility due to chronic anovulation and endometriosis.[26,27] In the present study, the prevalence of vitamin D deficiency among women in the reproductive age group (15-45 years) was 58.2% which is very high and an issue of concern. Public health impacts of vitamin D deficiency require urgent attention. It is pretty evident that vitamin D not only has a bearing on bone health, but also plays a crucial role in the overall health of an individual, right from its effect on glycemic control, immunity, cardiovascular diseases, neuromuscular function and other aspects of health. It is therefore imperative that focus should be given to the high burden of vitamin D deficiency among Indian population.

CONCLUSION

The need for improving the levels of vitamin D among the female population is both important and urgent. Emphasis must be given on promoting consumption of vitamin D rich foods and vitamin D supplements. Women should be encouraged to engage themselves in outdoor activities so that they are exposed to adequate sunlight. From a policy perspective, the provision for early diagnosis of vitamin D deficiency, devising population-based programs to provide affordable vitamin D supplements, and vitamin D fortified food will help improve the situation at large.

REFERENCES

1. Holick MF. “Vitamin D deficiency”. The New England Journal of Medicine 2007; 357 (3): 266–81.

2. Lips P, van Schoor NM. The effect of vitamin D on bone and osteoporosis. Best Pract Res Clin Endocrinol Metab. 2011;25:585–91

3. Ritu G, Gupta A. Vitamin D deficiency in India: Prevalence, causalities and interventions. Nutrients. 2014;6:729-75.

4. Mithal A, Wahl DA, Bonjour JP, Burckhardt P, Dawson-Hughes B, Eisman JA, *et al*. Global Vitamin D status and determinants of hypovitaminosis D. Osteoporos Int 2009;20:1807-20

5. Nandini M, Prabhu AK, Kamath AK, Aishwinee VG. Profile Of Vitamin D Deficiency in Mangalore. Int J Pharm Bio Sci 2014;5:179-86.

6. Misra P, Srivastava R, Misra A, Kant S, Kardam P, Vikram NK. Vitamin D status of adult females residing in Ballabgarh health and demographic surveillance system: A community-based study. Indian J Public Health 2017;61:194-8

7. Bawaskar PH, Bawaskar HS, Bawaskar PH, Pakhare AP. Profile of Vitamin D in patients attending at general hospital Mahad India. Indian J Endocrinol Metab 2017;21:125-30

8. Mitra S, Nayak PP, Agrawal S, Sahoo JP, Kamalanathan S, Nanda R. Vitamin D Status and Cardio-Metabolic Risk in Indian Postmenopausal Women. J Clin Diagn Res 2016;10:QC17-20

9. Bischoff-Ferrari HA, Dawson-Hughes B, Willett WC, Staehelin HB, Bazemore MG, Zee RY, et al. Effect of Vitamin D on falls: A meta-analysis. JAMA. 2004;291:1999–2006.

10. Bischoff-Ferrari HA, Dietrich T, Orav EJ, Dawson-Hughes B. Positive association between 25-hydroxy vitamin D levels and bone mineral density: A population-based study of younger and older adults. Am J Med. 2004;116:634–9

11. Bischoff HA, Stahelin HB, Urscheler N, Ehrsam R, Vonthein R, Perrig-Chiello P. Muscle strength in the elderly: Its relation to vitamin D metabolites. Arch Phys Med Rehabil. 1999;80:54–8

12. Nnoaham KE, Clarke A. Low serum vitamin D levels and tuberculosis: A systematic review and meta-analysis. Int J Epidemiol. 2008;37:113–9

13. Cannell JJ, Vieth R, Umhau JC, Holick MF, Grant WB, Madronich S, et al. Epidemic influenza and vitamin D. Epidemiol Infect. 2006;134:1129–40

14. Martineau AR. Old wine in new bottles: Vitamin D in the treatment and prevention of tuberculosis. Proc Nutr Soc. 2012;71:84–9

15. Park SK, Garland CF, Gorham ED, BuDoff L, Barrett-Connor E. Plasma 25-hydroxyvitamin D concentration and risk of type 2 diabetes and pre-diabetes: 12-year cohort study. PLoS One 2018;13:e0193070

16. Degerud E, Nygård O, Vogel S, Hoff R, Svingen GFT, Pedersen ER, *et al*. Plasma 25-hydroxyvitamin D and mortality in patients with suspected stable angina pectoris. J Clin Endocrinol Metab 2018;103:1161-70.

17. Zipitis CS, Akobeng AK. Vitamin D supplementation in early childhood and risk of type 1 diabetes: a systematic review and meta-analysis. *Arch Dis Child*. 2008;93(6):512-517

18. Bruce WH, John QK, Sandra RS, Jeffrey DL, Joseph LN. Determination of vitamin D status by radioimmunoassay with an I^{125} labelled tracer. Clin Chem 1993;39:529-33.
19. Holick MF . Vitamin D deficiency. N Engl J Med.2007;357:266–281.
20. Sahu M, Bhatia V, Aggarwal A, Rawat V, Saxena P, Pandey A, et al. Vitamin D deficiency in rural girls and pregnant women despite abundant sunshine in northern India. Clin Endocrinol (Oxf) 2009;70:680–4.

21. Marwaha RK, Sripathy G. Vitamin D and bone mineral density of healthy school children in northern India. Indian J Med Res. 2008;127:239–44.

22. Harinarayan CV, Sachan A, Reddy PA, Satish KM, Prasad UV, Srivani P. Vitamin D status and bone mineral density in women of reproductive and postmenopausal age groups: A cross-sectional study from south India. J Assoc Physicians India. 2011;59:698–704.

23. Kalra S, Kalra B, Sachin Kumar, Khandelwal SK. Vitamin D deficiency in healthy postmenopausal women in Haryana. World J Life Sci Med Res. 2011;1:11–5.

24. Sachan A, Gupta R, Das V, Agarwal A, Awasthi PK, Bhatia V. High prevalence of Vitamin D deficiency among pregnant women and their newborn in northern India. Am J Clin Nutr 2005;81:1060-4

25. Jain V, Gupta N, Kalaivani M, Jain A, Sinha A, Agarwal R. Vitamin D deficiency in healthy breastfed term infant at 3 months and their mothers in India. Seasonal variation and determinants. Indian J Med Res 2011;3:267-73

26. Vigano P, Lattuada D, Mangioni S, Ermellino L, Vignali M, Caporizzo E, Panina-Bordignon P, Besozzi M, Di Blasio AM. Cycling and early pregnant endometrium as a site of regulated expression of the vitamin D system. J Mol Endocrinol. 2006;36(3):415–424.

27. Kinuta K, Tanaka H, Moriwake T, Aya K, Kato S, Seino Y. Vitamin D is an important factor in estrogen biosynthesis of both female and male gonads. Endocrinology. 2000;141(4):1317–1324.