ORIGINAL ARTICLE

VITAMIN D DEFICIENCY AND PARATHORMONE LEVELS IN PUNJABI PAKISTANI POPULATION

Hamid Shafiq, Asifa Majeed

Department of Biochemistry & Molecular Biology, Army Medical College, National University of Sciences and Technology, Islamabad, Pakistan

Background: Vitamins are organic compounds that play significant role in management of metabolic processes. Vitamin D is considered a nutrient associated with a bio-signalling action. It has vital participation in constant maintenance of calcium level in both intra and extracellular body fluids. The present study analyzed the vitamin D and PTH levels in Punjabi population of Pakistan. **Methods:** This was a cross-sectional observational study. Sample collection was done from Oct 2017 to Oct 2018 from various local health facilities of Punjab, and blood samples of 400 subjects were collected. The quantitative measurement of vitamin D was obtained by automated competitive chemiluminescence immunoassay method. PTH levels were calculated by direct Chemiluminescence kit. The values of vitamin D thus obtained were compared with levels of parathyroid hormones along within different age groups. **Results:** Overall 77.5% of studied population was deficient/insufficient of vitamin D. Thirty-five percent individuals in age group up to 20 years, and 88.8% in age group 21−40 years were deficient/insufficient. In age group 41−60 years, 115 out of 120 (95.8%) people showed deficiency/insufficiency. On comparison of vitamin D with parathyroid hormone levels a significant (p<0.001) negative association was observed. Our study is in agreement with others that demonstrate a high prevalence of cholecalciferol deficiency in many populations. **Conclusion:** Cross-sectional study measurement of Vitamin D is of paramount importance in our population. Timely correction of its deficiency can ward off many complications resulting from its direct and indirect effects.

Keywords: Vitamin D, Parathyroid hormone, PTH, Age factor, Deficiency, Bone Mineral, Pakistan

Pak J Physiol 2019;15(2):7−10

INTRODUCTION

Vitamin D commonly known as cholecalciferol plays an important role to maintain the level of calcium in body by acting as a paracrine and autocrine hormone. The activation of vitamin D regulates the autocrine response of human bronchial epithelial cells to *Aspergillus fumigatus*. Existence of an autocrine/ paracrine vitamin D system also exists in the neurovascular unit.[1] The main function of active cholecalciferol is to raise the absorption of calcium through intestine by contact with vitamin D receptor (VDR) found in proximal and distal part of intestine.[2] More than 90% synthesis of cholecalciferol takes place under the skin but physiological maximum is limited. Biological inactive Vitamin D converts into active form by absorbing solar energy. It also can be taken through diet as fish oil and fortified dairy product. Depending upon dietary patterns, major food sources for dietary vitamin D intake are different for different countries.[3] Active form of vitamin D enters the circulation where it is transported towards liver to be converted into hydroxyl form which, though considered biologically inactive, is used as marker of vitamin D status. Such biological inactive form of vitamin D becomes metabolized and produces most biological active form known as calcitriol. Production of nitric oxide and microbicidal capacity of macrophages is increased by Calcitriol.[4]

Various studies conducted in the last decade have revealed that the role of cholecalciferol is not only to maintain the homeostasis of calcium but also regulates various cellular mechanisms. Whole blood taken from very sick dogs showed that calcitriol can induce an anti-inflammatory phenotype *in vitro*.[5] Malabsorption due to celiac disease, inflammation of ileum and prolonged use of steroids, less solar exposure and nutritional factors are usually responsible for vitamin D deficiency in most tropical areas of the world.[6]

Deficiency of vitamin D decreases absorption of calcium through intestine by enhancing the synthesis of parathyroid hormone (PTH).[7] Parathyroid glands mainly secrete PTH. Once blood calcium levels go down PTH increases calcium reabsorption from kidneys, activates vitamin D production (to increase intestinal calcium absorption) and mobilizes calcium from bones.[8] The PTH also helps mainly in trabecular bone formation rather than the cortical, and is used as anabolic in osteoporosis.[8] Primary hyperthyroidism can cause severe bone resorption leading to bone pains and pathological fractures.[9]

Cholecalciferol supplementation along with calcitriol not only improves serum vitamin D levels but also markedly lowers levels of PTH; decreasing secondary hyperparathyroidism.[10] According to recent studies, the deficiency of vitamin D is not only responsible for effects on the metabolism and quality of bone but also causes various autoimmune and neurological disorders.[11] Data on various populations

have revealed that deficiency of vitamin D is not limited to areas of the world where exposure of sunlight is less, but also frequently found in subtropical countries. It is not a must that Vitamin D deficiency is directly related to bone mineral density. Vitamin D deficiency is reported prevalent in Saudi Arabia although it was not found correlated with reduced bone mineral density.[12] Studies up to date are still not sure about the exact normal and abnormal levels of cholecalciferol. Moreover different methodologies are adopted by laboratories to calculate the circulating level of vitamin D. The plasma level of 25-hydroxyvitamin D [25(OH)D] is the best marker for vitamin D estimation. 'Circulating 25(OH)D is a robust and reliable marker of vitamin D and has been used by numerous agencies in the establishment of vitamin D dietary requirements and for population surveillance of vitamin D deficiency or inadequacy'.[13] Though there is a discrepancy in deciding the optimum level of vitamin D yet according to various experts the cut-off values of serum 25(OH)D in Pakistan are: ≤20 ηg /ml, ≥21–29 ηg/ml, and ≥30 ηg/ml defined as deficient, insufficient and sufficient respectively.[14] The adequate level of 25(OH)D is above 30 ηg/mL according to South Asian Society of Endocrinology and Metabolism. Most of the populations are starved of this. Less amount of circulating 25(OH)D in body is associated with high serum levels of PTH leading to increased risk of mortality.[15] Our study correlated the vitamin D deficiency noted in the samples collected from different hospitals of Punjab with age, gender, and parathyroid hormone levels.

MATERIAL AND METHODS

This cross-sectional observational study was done from October 2017 to October 2018. All individuals belonged to different areas of Punjab, Pakistan. Blood samples of 400 individuals referred to different hospital/clinic labs for vitamin D estimation were collected. Apparently healthy male and female subjects aged 0−60 years with normal physical examination were included. All belonged to Northern Punjab Pakistan (in and around Rawalpindi, Faisalabad and Lahore). Subjects having any chronic ailment diagnosed from history or medical examination like tuberculosis, asthma, hepatitis B or C, HIV, hypertension, IHD, Diabetes mellitus, or skin problems were excluded. Smokers and those on vitamin D supplements were also excluded. A questionnaire was prepared to enter the demographic and other data, keeping in mind the inclusion/exclusion criteria.

Parathyroid hormone levels of same individuals were also measured along with serum levels of calcium and phosphorous. The quantitative measurement of vitamin D was obtained by automated competitive chemiluminescence immunoassay method. The reference values of vitamin D for current study were defined[14] as deficient, insufficient, and sufficient. PTH levels were calculated by Direct Chemiluminescence kit with normal range from 10 to 70 pg/mL. The variable data was expressed as Mean±SD. SPSS-20 was used for analysis. Mann-Whitney and Spearman nonparametric test was applied in statistical analysis and $p<0.001$ was considered significant.

RESULTS

Data of 400 people was analyzed after testing for 25(OH)D and PTH levels. Results revealed negative correlation of vitamin D levels with age of subjects. Overall analysis of data showed 310 (77.5%) individuals having deficient or insufficient vitamin D, while 90 (22.5%) had sufficient vitamin D level. Whole population was divided into 3 groups according to age groups. Group I had 100 individuals, aged between 1−20 years. In this group 35 out of 100 were vitamin D deficient/insufficient. In Group II there were 180 individuals aged 21−40 years; 160 (88.8%) of them were deficient/insufficient of vitamin D. In Group III, aged 41−60 years, 115 out of 120 (95.8%) subjects were found vitamin D deficient/insufficient (Figure-1). All 400 subjects also had their PTH levels tested (Table-1).

Significant negative correlation was noted between Vitamin D and parathormone levels ($p<0.001$). The subjects in Group III with overall lowest level of vitamin D had highest values of PTH (88.5 pg/mL), whereas those in Group I with comparatively normal levels of vitamin D had normal value of PTH (Figure-2). Vitamin D levels had significant negative association with both age and PTH level. No significant association was noted concerning gender of individuals.

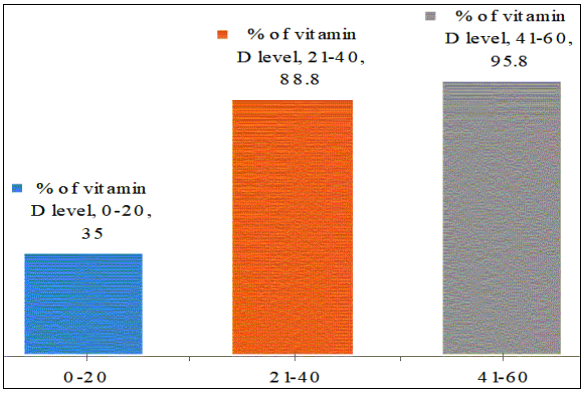

Figure-1: Percentage deficiency of Vitamin D in various age groups

Table-1: Percentage (according to reference value) of serum Vitamin D levels with PTH level (Mean±SD)

RV of Vitamin D (ηg/mL)	N	%	PTH level (ηg/mL)*
<20 (deficiency)	64	16	30.5±17.4
<30 (insufficiency)	246	61.5	29.3±15.6
Up to 90 (adequate)	90	22.5	24.6±8.3

*$p<0.001$

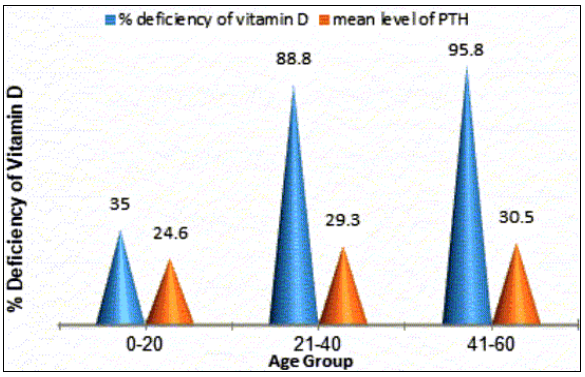

Figure-2: Association between % Deficiency of Vitamin D and mean values of PTH

DISCUSSION

Vitamin D deficiency is an important clinical and nutritional concern all over the world in spite of controversy of the optimum levels of serum 25(OH)D for the maintenance of muscle and bone health. Endocrine Society of International Medical Organizations recommends the level of 25(OH)D be considered insufficient when it is less 29 ηg/mL.[14] Health professionals and scientific community has paid great attention towards the role of vitamin D levels in disease and healthy people during the last 10 years. This is due to better understanding about usefulness of vitamin D not only for bone metabolism but also for other processes like cell signalling and body functions of cardiovascular, immune and nervous system.[16] Deficient vitamin D in the body is being associated with increased risk of autoimmune, cardiovascular and respiratory diseases besides cancer, diabetes, obesity[17], pregnancy and child health[18]. Increased concern towards the deficient levels of vitamin D has led to excessive demands for its laboratory assessment. In the current study there was about 50% increase in the laboratory examination of vitamin D samples (as revealed by lab records). The phenomenal increase in laboratory testing of vitamin D levels in Australian community occurred much earlier after realizing its significance.[19] In some laboratories of Pakistan frequency of vitamins D testing samples has increased by more than 100%. The tests are being requested in elderly, obese, pregnant ladies, patients with liver/renal problems, malabsorption syndrome, lymphomas, hyperparathyroidism, and inflammatory bowel disease (IBS); and IBS further increases the risk of cancer.[20] In Western Europe, deficiency of 25(OH)D is found to be associated with increased risk of hepatocellular carcinoma.[21] Our study showed high prevalence of vitamin D deficiency in Punjab. Low levels of 25(OH)D were observed in 77.5% of population; 16% of total subjects were deficient, 61.5% insufficient. Only 22.5% were found sufficient. These observations are in accordance with the reference values available in literature. Different studies performed abroad also support this data. It was observed that 49.9% of healthy youngsters in Italy have deficient level of vitamin D and that correlated with nationality, sun exposure and weight.[22] Our study also found value of serum 25(OH)D (16% deficiency and 61.5% insufficiency) with seasonal variation. Vitamin D was found interrelated with PTH in Brazilian tropical population with increased vitamin D deficiency in winter compared to the summer.[23] Previous studies also exposed the high occurrence of vitamin D deficiency in people all around the world including those countries having sunny environment. Many other studies show the vitamin D deficiency related to increasing age. This is due to the physiological changes that are related to ageing process. Our study revealed significant vitamin D deficiency in elderly. The high frequency of vitamin D deficiency in old age is associated with decrease in sun exposure, changed mineral bone thickness and in some cases because of hyperparathyroidism. In agreement to previous studies our data also shows higher levels of 25(OH)D among youngsters as compared to elderly. Numerous studies reveal that due to high prevalence of vitamin D deficiency in old age risk of osteomalacia and osteoporosis increases many folds. I hope our government will also consider this issue seriously now and take some substantial measures. Though many studies showed correlation between vitamin D deficiency and old age yet it is pertinent to say that youngsters and children can also have insufficient vitamin D level. One Chinese study[24] showed low levels of vitamin D in young females.

Inverse relationship between vitamin D and parathyroid hormone is important for remodelling of bone, mostly in younger people. Significant negative association has been found between 25(OH)D and parathyroid hormone in our study, and low levels of vitamin D were found inversely correlated with high levels of parathyroid hormone. Similar results were found in Australian population. A study at Riga showed that the threshold level of plasma 25(OH)D is 38 ηg/mL (above this no further suppression of PTH occurs). Before treating Vitamin D deficient patients with supplements, one must inquire about their lifestyle including the duration of sun exposure, effects of seasons, application of sun blocks and oral intake of dairy products. All these factors contribute to vitamin D content.

Supplements of vitamin D are available in 2 forms: Vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol). Their usefulness and therapeutic effect depends upon many factor like severity of vitamin D deficiency, dosing procedure, safety and suitability for dosing regimen.[25] Though limited data was evaluated in available time span and resources yet results indicate clear and helpful information. However our study does not explain such a high percentage of vitamin D

deficiency when our country is blessed with so much sunshine and people of Punjab take plenty of dairy products.

CONCLUSION

Our data indicate high frequency of deficiency and insufficiency of 25(OH)D, especially in old age group of Punjabi Pakistani population. The vitamin D levels were inversely proportional to the parathyroid hormone levels. High prevalence of vitamin D deficiency in spite of adequate sunlight and dietary intake of dairy products in Punjab requires further studies especially to look into other causes like genetic polymorphism.

REFERENCES

1. Li P, Wu T, Su X, Shi Y. Activation of vitamin D regulates response of human bronchial epithelial cells to *Aspergillus fumigatus* in an autocrine fashion. Mediators of Inflammation 2015;2015:208491 (14 pages).
2. Reyes-Fernandez PC, Fleet JC. Compensatory changes in calcium metabolism accompany the loss of vitamin D receptor (VDR) from the distal intestine and kidney of mice. J Bone Miner Res 2016;31(1):143–51.
3. Guo J, Lovegrove JA, Givens DI. A Narrative review of the role of foods as dietary sources of vitamin D of ethnic minority populations with darker skin: The underestimated challenge. Nutrients 2019;11(1): pii: E81. doi: 10.3390/nu11010081.
4. Garcia-Barragan A, Gutierrez-Pabello JA, Alfonseca-Silva E. Calcitriol increases nitric oxide production and modulates microbicidal capacity against *Mycobacterium bovis* in bovine macrophages. Comp Immunol Microbiol Infect Dis 2018;59:17–23.
5. Jaffey JA, Amorim J, DeClue AE. Effect of calcitriol on *in vitro* whole blood cytokine production in critically ill dogs. Vet J 2018;236:31–6.
6. G Ritu, Gupta A. Vitamin D deficiency in India: prevalence, causalities and interventions. Nutrients 2014;6(2):729–75.
7. Den Ruijter HM, Franssen R, Verkerk AO, van Wijk DF, Vaessen SF, Holleboom AG, *et al*. Reconstituted high-density lipoprotein shortens cardiac repolarization. J Am Coll Cardiol 2011;58:40–4.
8. Goltzman D. Physiology of Parathyroid Hormone. Endocrinol Metabol Clin North Am 2018;47(4):743–58.
9. Baig MN, Mac Dhaibheid C, Shannon FJ. Hip Fracture in a Patient with Primary Hyperparathyroidism: Medical and Surgical Lessons. Cureus 2018;10(1):e2104.
10. Banon S, Rosillo M, Gomez A, Perez-Elias MJ, Moreno S, Casado JL. Effect of a monthly dose of calcidiol in improving vitamin D deficiency and secondary hyperparathyroidism in HIV-infected patients. Endocrine 2015;49(2):528–37.
11. Wintermeyer E, Ihle C, Ehnert S, Stockle U, Ochs G, de Zwart P, *et al*. Crucial Role of Vitamin D in the Musculoskeletal System. Nutrients 2016;8(6): pii: E319. doi: 10.3390/nu8060319.
12. Alkhenizan A, Mahmoud A, Hussain A, Gabr A, Alsoghayer S, Eldali A. The relationship between 25(OH)D levels (Vitamin D) and bone mineral density (BMD) in a Saudi population in a community-based setting. PloS One 2017;12(1):e0169122.
13. Cashman KD, van den Heuvel EG, Schoemaker RJ, Preveraud DP, Macdonald HM, Arcot J. 25-Hydroxyvitamin D as a biomarker of vitamin D status and its modeling to inform strategies for prevention of vitamin D deficiency within the population. Adv nutr 2017;8(6):947–57.
14. Nadeem S, Munim TF, Hussain HF, Hussain DF. Determinants of vitamin D deficiency in asymptomatic healthy young medical students. Pak J Med Sci 2018;34(5):1248–52.
15. El Hilali J, de Koning EJ, van Ballegooijen AJ, Lips P, Sohl E, van Marwijk HWJ, *et al*. Vitamin D, PTH and the risk of overall and disease-specific mortality: Results of The Longitudinal Aging Study Amsterdam. J Steroid Biochem Mol Biol 2016;164:386–94.
16. Romagnoli E, Pepe J, Piemonte S, Cipriani C, Minisola S. Management of endocrine disease: value and limitations of assessing vitamin D nutritional status and advised levels of vitamin D supplementation. Eur J Endocrinol 2013;169(4):R59–69.
17. Hossein-nezhad A, Holick MF. Vitamin D for health: a global perspective. Mayo Clin Proc 2013;88(7):720–55.
18. Fiscaletti M, Stewart P, Munns CF. The importance of vitamin D in maternal and child health: a global perspective. Public Health Rev 2017;38:19.
19. Quaggiotto P, Tran H, Bhanugopan M. Vitamin D deficiency remains prevalent despite increased laboratory testing in New South Wales, Australia. Singapore Med J 2014;55(5):271–80.
20. Ananthakrishnan AN, Cheng SC, Cai T, Cagan A, Gainer VS, Szolovits P, *et al*. Association between reduced plasma 25-hydroxy vitamin D and increased risk of cancer in patients with inflammatory bowel diseases. Clin Gastroenterol Hepatol 2014;12(5):821–7.
21. Fedirko V, Duarte-Salles T, Bamia C, Trichopoulou A, Aleksandrova K, Trichopoulos D, *et al*. Prediagnostic circulating vitamin D levels and risk of hepatocellular carcinoma in European populations: a nested case-control study. Hepatology 2014;60(4):1222–30.
22. Vierucci F, Del Pistoia M, Fanos M, Erba P, Saggese G. Prevalence of hypovitaminosis D and predictors of vitamin D status in Italian healthy adolescents. Ital J Pediatr 2014;40:54.
23. Eloi M, Horvath DV, Szejnfeld VL, Ortega JC, Rocha DA, Szejnfeld J, *et al*. Vitamin D deficiency and seasonal variation over the years in Sao Paulo, Brazil. Osteoporos Int 2016;27(12):3449–56.
24. Ning Z, Song S, Miao L, Zhang P, Wang X, Liu J, *et al*. High prevalence of vitamin D deficiency in urban health checkup population. Clin Nutr 2016;35(4):859–63.
25. Stokes CS, Lammert F. Vitamin D supplementation: less controversy, more guidance needed. F1000Research 2016;5. doi: 10.12688/f1000research.8863.1.

Address for Correspondence:

Dr. Hamid Shafiq, Department of Biochemistry & Molecular Biology, Army Medical College, National University of Sciences and Technology, Islamabad, Pakistan.
Email: hamidshafiq.amc@gmail.com

Received: 8 Jan 2019 **Reviewed: 25 Jan 2019** **Accepted: 21 Feb 2019**