Original Research Paper

Volume-10 | Issue-1 | January - 2020 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Medicine

CORRELATION OF VITAMIN D LEVELS AND BONE MASS DENSITY IN STUDENTS

Dr Shiv P Mittal* Dept Of Medicine; MM Medical College & Hospital Kumarhatti (Solan)
*Corresponding Author

Dr Kiranjeet Kaur Dept Of Biochemistry; MM Medical College & Hospital Kumarhatti (Solan)

ABSTRACT

INTRODUCTION: Vitamin D and calcium determines the bone health. Peak bone formation in young age is found beneficial in preventing osteoporosis and fractures at later age. Our study is to evaluate correlation between Vitamin D (VD) and bone health in students and likely future risk for diseases at later age because of VD deficiency.

MATERIAL AND METHOD: Students [N=148: Male=67 & female=81] participated in the study. After clinical evaluation, relevant biochemical tests, including serum VD estimation, done and bone mass density (BMD) measured.

RESULTS: Mean age of male and female students was 19.56 ± 1 & 19.52 ± 1 year respectively. Students [(N=139 (93.92%): Male (M)=62 (41.89%) & Female (F)=77 (52.03%)] had low VD levels. Students M=64 (43.24% & F=75 (50.68%)] had normal BMD and 9 students (M=4 & F=5) osteopenia. Many students of either sex with normal or near normal BMD presented on either side of BMD "T score -1". We call them "*Fence Sitter"* because their future improvement or deterioration bone health depends on the lifestyle as they age. Students performing mild physical activities, fast food indulgence or with high blood pressure had significant VD deficiency, and in this subgroup many had normal BMD, but were "Fence Sitters". Sufficient proportion of students of either sex with family history of (F/H/O) chronic metabolic diseases, with Body Mass Index (BMI) of any grade, irrespective of waist circumference and Waist hip ratio (WHR) were also "Fence Sitters" despite normal BMD.

CONCLUSION: VD deficiency, in the participants, not only likely to impact bone health, but may make them vulnerable to various non-skeletal diseases. We view skeptically the normal BMD in most of students, as multiple factors contribute to VD deficiency and bone health among them. Secondly, as they age, environmental factors and life style in face of VD deficiency may impact their bone health and non-skeletal functions. "Fence Sitters" may likely to face this impact most.

KEYWORDS : Vitamin D, BMD, Fence Sitters

***INTRODUCTION*:**

Adequate serum VD levels are essential for the optimal bone health. In VD deficiency, inappropriate bone mineralization occurs due to ineffective calcium metabolism, osteoblastic activity, matrix ossification and induced secondary hyperparathyroidism[1-2]. Secondary hyperparathyroidism further lowers BMD by accelerating bone resorption. Healthy bone is essential to prevent fractures irrespective of age. Optimizing healthy bone formation in childhood not only prevents childhood fractures but also is beneficial determinant in preventing osteoporosis and fractures in later life, because BMD in later life is a function of peak bone mass (PBM) and the rate of bone loss after PBM is achieved [3-5]. PBM is influenced by VD status, calcium intake and physical activity achieved between adolescence and 30 years of age [6]. Our study is to correlate VD and bone health in this young population, and to find out risk factors influencing the bone health and non-skeletal functions due to chronic VD deficiency in long run.

***MATERIAL AND METHOD*:**

This observational and non-randomized was conducted in a Medical College. Students (N=148: M = 67 & F = 81) consented for this study.

DURATION OF STUDY: About One Year.

INCLUSION CRITERIA: All students.

EXCLUSION CRITERIA:

Students taking supplementary VD and / or calcium preparation.

***ETHICAL ISSUE*:**

The research was approved by the Institutional Ethical Committee and conducted as per laid down norms.

STUDY DESIGN:

Students informed about the study design, those who consented were included. Symptoms suggestive of DM or other endocrine disorders, history of hormonal therapy / drugs (those influencing weight), and F/H/O diabetes mellitus (DM), hypertension (HTN) and coronary artery disease (CAD) were recorded. Clinical evaluation included measurement of Weight (Kg), Height (m), BMI, Waist Circumference (WC), Hip circumference (HC), and WHR. Cut-off BMI for Indians for overweight was considered as 23Kg/m². Increased WC was considered as ≥ 90 cm in males and ≥ 80 cm in females, and WHR ≥ 0.90 for male and ≥ 0.85 for female students [7-8]. Relevant laboratory tests were done. Bone mass density gm/cm² (BMD) measured by *Multi-site Ultrasound – based Bone Mineral Densitometer* and recorded as an average of two separate readings taken one minute apart. Students were grouped as: Normal: BMD T-score = or ≥ -1, Osteopenia: BMD T-score ≤ -1 & up to -2.5, and Osteoporosis: BMD T-score ≤ -2.5 [9-10]. VD {[25 (OH)] was estimated by (by *DIAsource 25OH Vitamin D Total ELISA 90 kit standard*). VD levels 30ng/ml and above was taken as sufficient (normal), between 20-29 ng/ml as insufficient and below 20 ng/ml as deficient.

***STATISTICAL ANALYSIS*:**

The data is analyzed based on mean ± SD. Statistical analyses were performed by one way analysis of variance (ANOVA) followed by T. Test for comparison between control and subject groups. *P* value of ≤ 0.05 was taken as significant.

***RESULTS*:**

Students [N=148: (M = 67 & F = 81)] completed the study. Mean age of male students was 19.559 ± 0.997 and of female 19.524 ± 1.016 years.

***VITAMIN D STATUS*:**

Students [N=139 (93.92%): [M=62 (41.89%) & F=77 (52.03%)] had low VD levels: *deficiency* in 126 (85.14%) [M=56 (37.84%) & F=70 (47.30%)], *insufficiency* in 13 (8.72%) [M=6 (4.02%) & F=7 (4.5)] and *normal* in 9 (6.08%) (M=5 & F=4). For purpose of analysis and convenience, we have considered both VD deficiency and insufficiency data as VD deficiency.

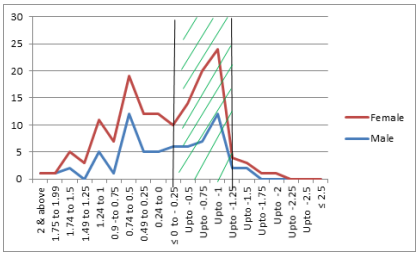
BONE MASS DENSITY:

Students [N=139 (93.92%): M=64 (43.24%) & F=75 (50.68%)] had normal BMD (Table–1) and osteopenia in 9 (M=4 & F=5), and none with osteoporosis. Fractionation of BMD T Score (Fig-1) have revealed significant number of students of either sex *crowded* on either side of BMD score -1 (normal or near normal BMD). We called them as "*Fence Sitter"* because their improvement to normal bone or deterioration to osteopenia depends on their future lifestyle.

Table – 1 (Bone Mass Density in Students)

BMD T-Score	Students		Total
	Male	Female	
Normal (equal to or ≥ -1	64	75	139

Osteopenia (≤ -1 to -2.5)	4	5	9
Osteoporosis (≤ -2.5)	0	0	0
Total	68	80	148



(Fig – 1: Depicting BMD in participants. Horizontal axis shows BMD T – score and Vertical axis number of students. Though BMD T-score -1 and above is taken as normal, but this Figure shows maximum conglomeration of participants on either side of "T Score -1" i.e. between -1.25 and - 0.25. These participants, we call them "*Fence sitter*").

PHYSICAL ACTIVITY, AND VITAMIN D & BMD:
This research center located in hilly terrain had about 750 feet road gradient between residential and teaching zones. Majority of the students walk across this slope, constituting daily physical activity, some use other modes of exercise also.

Mild exercise (30 min walk) performed by 125 (84.46%) students [M=55 (37.16%) & F=70 (47.3%)]. In this subset: 50 (90.91%) males were VD deficient and 5 had normal levels, and normal BMD in 52 and osteopenia in 3. Female students [N=67 (95.71%)] were VD deficient and 3 had normal levels, and normal BMD in 68 and osteopenia in 2.

Moderate exercise (60-90 min walk; 30 min jogging; 60 min walk, run & jogging, and 60 in gym & walk) by 19 students (M=10 & F=9). *All students of either sex were VD deficient*, normal BMD present in 9 male and 7 female students, and osteopenia in 1 male and 2 female.

Severe exercise (60 min gym, running, games & walk; 60-180 min exercise & games, and 90 min badminton) by 4 students (M=2 & F=2). *All 4 students were VD deficient*, but normal BMD in both male and 1 female, and osteopenia in 1 female student.

F/H/O CHRONIC METABOLIC DISEASES, AND VITAMIN D & BMD: (TABLE- 2)
(i) Diabetes mellitus: Students with F/H/O DM [N=49 (33.1%): M=17 & F=32. All students, except two female were VD deficient.
(ii) Obesity: Students with F/H/O obesity [N=35 (23.65%): M=13 & F=22]. All students, except 1 student of either sex, were VD deficient.
(iii) Hypertension: Students with F/H/O HTN [N=30: (M=7 & F=23). All students, except 1 student of either sex, were VD deficient.
(iv) CAD: F/H/O CAD in 3 students (M=1 & F=2) and all were VD deficient.

DIETARY PREFERENCES AND VITAMIN D & BMD:
136 (91.89%) students [M=59 (43.38%) & F=77 (56.62%)] accepted indulgence in consumption of packed / unpacked or tinned fast food frequently. VD deficiency was present in 128 (94.12%) students [M=55 (40.44%) & 73 (53.68%)]. Of these, 55 male students had normal BMD, and 32 (58.18%) were "*Fence sitters"*. Female students (N=72) had normal BMD, and 43 (59.72%) were "*Fence sitters.* VD deficiency in female and male "*Fence sitters"* was (97.67%) and (93.75%) respectively.

Other students (M=8 & F=4) denied frequent intake of fast food. Except 1 male, all were VD deficient, but all had normal BMD. Except two male and one female student, all were "*Fence sitters*".

BLOOD PRESSURE, AND VITAMIN D & BMD:
Students [N=4: (M=4 & F=0)] had systolic blood pressure 135 mm Hg and above, all were VD deficient. Normal BMD was in1 and osteopenia in 3.

Students [N=11: M=7 & F=4] had diastolic blood pressure 85 mm Hg and above. All were VD deficient. Except one student of either sex, all were osteopenic.

Table – 2 (Family History of Chronic Metabolic Diseases)

Metabolic Diseases	Family Relatives			Total
	P	M	P & M	
DM				
Male	7	3	7	17 (25.37%)
Female	18	7	7	32 (39.51%)
Obesity				
Male	5	2	6	13 (19.4%)
Female	6	5	11	22 (27.16%)
HTN				
Male	2	1	4	7 (10.45%)
Female	16	4	3	23 (28.4%)
CAD				
Male	1	0	0	1 (1.49%)
Female	2	0	0	2 (2.47%)

(Table – 2: Depicting major chronic metabolic diseases in families of students. Male=male students, Female=female students. P=close relatives of parental side, M=close relatives of maternal side, and P & M= close relatives of both paternal & maternal side).

BODY WEIGHT AND, BMD & VITAMIN D (TABLE – 3):
MALE STUDENTS:
Students (N=6) were underweight (BMI ≤ 18.5 Kg/m²), all were VD deficient, but five had normal BMD, *two* "*Fence sitters"*. Students (N=37) had normal BMI (18. – 22.9 Kg/m²), 35 were VD deficient but with normal BMD and 21 (56.76%) "*Fence sitters"*. Students (N=24) were with BMI 23 and above, and 21 VD deficient, normal BMD in 23 and 15 (62.5%) were "*Fence sitters"*.

FEMALE STUDENTS:
Students (N=14) were underweight, all VD deficient, but with normal BMD and 6 (42.86%) "*Fence sitters"*. Students (N=40) with normal BMI, 36 VD deficient, 37 with normal BMD, and 26 (65%) were "*Fence sitters"*. Students (N=27) were overweight and obese, and all were VD deficient, 25 with normal BMD and 14 (51.85%) "*Fence sitters"*.

WAIST CIRCUMFERENCE AND WHR, AND VITAMIN D & BMD:
Students [N=46: (M=26 & F=20)] declined consent for WC and hip measurement. Remaining 102 (M=41 & F=61) were evaluated.

MALE STUDENTS (N=41):
Waist Circumference: Students (N=31) had normal WC (≤90 cm), normal BMD in 30 and were "*Fence sitters"* were 15. Students (N=10) had increased WC (≥ 90 cm), normal BMD in 8 and "*Fence sitters"* were 5*, and a*ll these students, except 2 were VD deficient.

Waist-Hip Ratio (WHR): Students (N=26) had normal WHR (≤ 0.90), normal BMD in 25 and 13 were "*Fence sitters"*. Students (N=15) had increased WHR (≥ 0.90), normal BMD in 13 and 7 were "*Fence sitters"*. All were VD deficient except 2 students with increased WHR.

FEMALE STUDENTS (N=61):
Waist Circumference: Students (N=43) had normal WC (≤ 80 cm), normal BMD in 39 and "*Fence sitters"* were 22. Students (N=18) had increased WC (≥ 80 cm), all with normal BMD but *"Fence sitters"* were 9. All these, except one with normal WC, were VD deficient.

Waist-Hip Ratio (WHR): 35 students had normal WHR (≤ 0.85), normal BMD in 32 and "*Fence sitters"* were 22. Students (N=26) had increased WHR (≥ 0.85), normal BMD in 25 and "*Fence sitters"* were 9. All students were VD deficient except one each with normal and increased WHR.

(Table -3) (Searching *"Fence Sitters"* in Students with Normal BMD)

Parameters	Male			Female		
	N	BMD (Normal)	Fence Sitters	N	BMD (Normal)	Fence Sitters
BMI (kg/m²)						
≤ 18.5	6	5	2	14	14	6
18.5 – 22.9	37	35	21	40	37	26
23 and above	24	23	15	27	25	14

Waist						
≤90 cm	31	30	15	—	—	—
≥ 90 cm	10	8	5	—	—	—
≤80 cm	—	—	—	43	39	22
≥ 80 cm	—	—	—	18	18	9
WHR						
≤0.90 cm	26	25	13	—	—	—
≥0. 90 cm	15	13	7	—	—	—
≤0.85 cm	—	—	—	35	32	22
≥ 0.85 cm	—	—	—	26	25	9

DISCUSSION:

Activated Vitamin D [1,25(OH)D] is essential for healthy bone formation and prevention of bone demineralization through *Classical Pathway*. VD deficiency has negative impact on calcium metabolism, matrix mineralization and ossification, and bone remodeling and results in low bone mass (low BMD). It is accompanied with secondary hyperparathyroidism induced bone resorption and associated with increased incidence of falls and fracture due to defective bone strength.

VD deficiency is a global concern and its causes are multifactorial – extent of body area and duration of exposure to sun (UVB rays), sessional variation, latitude [11], dietary supplementation, low education level [12], physical inactivity [13-15], obesity and high BMI [16]. Role of alcohol and tobacco use in VD deficiency remains debated [17]. In our study, 93.92% students, especially female, were VD deficient i.e. female (52.03%) vs male (41.89%). Our findings were not in accordance with other studies which observed male to be VD deficient [18-19]. Factors responsible for VD deficiency in our students were inadequate sun exposure, because of indoor academic activities, physical inactivity in 84.46%, Indulgence in fast / junk food in 136 (91.89%), high BMI and increased WC / WHR, especially in female. Students declined alcohol and smoking habits – another lifestyle factor lowering VD levels and hence low BMD [20].

VD deficiency leads to decreased calcium absorption and bone matrix mineralization, whereas serum calcium level is maintained by development of secondary hyperparathyroidism, thereby, increasing the fracture risk [21]. Thus, chronic persisting VD deficiency decreases bone health. It is observed that development of bone disease in later life is related to maximum peak bone mass (PBM) developed and the maintained in adulthood [22], which is the function of genetics, physical activity, nutrition and lifestyle factors [20,23].

Many studies had established the relationship between VD and BMD [24-25] and positive effect on BMD when VD was given either alone [26-27] or with calcium [28-29]. Genetic constitution also has some impact on bone health [23]. It is well established that chronic metabolic diseases, especially DM, associated with strong genetic components, *except obesity*, were found to be associated with low BMD. We have observed also significant number of students with higher BMI, higher WC and / or WHR had normal BMD. In these students with obesity (higher BMI), the significance of normal or high BMD is underscored not by quantity but by the defective quality of bone formation. Whether VD deficiency adversely impact directly and through genetic modulation? It has been shown that, VD plays a very significant role in maintaining epitome by preventing hypermethylation of diabetes-related genes via *Autocrine pathway*. 25(OH) D is a robust and reliable marker of VD status [30] and main substrate for a*utocrine pathway* which is mediated through VD receptor (VDR) widely distributed in the body tissues [31-32]. In VD deficiency, the functions of these genes start declining – setting the stage for onset of DM [33]. Secondly, VD deficiency contributes to β-cell death, insulin resistance and hence onset of DM. DM has been shown to be associated with low BMD. VD in addition to preventing hypermethylation of diabetes–related genes, also maintains many other cellular processes [33]. In VD deficiency, these cellular processes also start declining resulting in manifestation of diseases**.** Therefore, BMD in other chronic metabolic diseases is also influenced by these declining cellular processes due to VD deficiency. Role of genetic in the maintenance of bone health (BMD) in our students stems indirectly from F/H/O chronic metabolic diseases. As chronic metabolic diseases are under varying degree of genetic association, students with F/H/O these diseases must have acquired this genetic susceptibility. As students age, they under the acquired genetic constitution compounded by adverse environmental factors and chronic VD deficiency not only prone to chronic metabolic diseases but also to low BMD and defective bone formation and maintenance of bone health.

Vulnerability of our participants to various non skeletal systemic diseases also can be anticipated in long standing VD deficiency. As mentioned above, VD also influences various cellular functions through *autocrine and paracrine pathway* mediated through 25(OH) D. Studies have shown that 25(OH) D modulates and regulates large number of genes (about 3065 genes constituting 0.8 – 5 % of the total genome) through this pathway [34]. Thus, VD is important for growth regulation, DNA repair, cell differentiation, apoptosis, membrane transport, metabolism, cell adhesion and oxidative stress [35-36]. So, long standing VD deficiency has been linked with many pathological disorders and dysregulation of metabolic pathways i.e. infections, autoimmune diseases, chronic metabolic diseases, multiple Sclerosis, sarcopenia, and the synthesis, regulation and release of hormones (e.g., insulin, rennin-angiotensin-aldosterone system), cancer, and altered cognition and depression [37]. If timely suitable corrective and preventive measures are not implemented to replenish and maintain adequate VD body store, these vulnerable young population not only develop defective bone and muscle health, but also vulnerable to develop other diseases as they age.

In our study, most of students 139 (93.92%), especially the male, were observed to have BMD within the normal T score. Only 9 students (M=4 & F=5) had osteopenia. Taking into the account of highly prevalent VD deficiency and other contributory factors affecting bone health of these students, it was skeptical to accept if this measured *normal BMD* could reflect as true healthy bone in real sense. Yes, students were in growing age and bone matrix mineralization occuring, even at VD levels ≤ 20 nmol/L, at rapid pace. But as they age, chronic VD deficiency and inadequate calcium supplements compounded by unfavorable life style factors may likely to impact their bone health. In view of this unforeseen future situation, we have arbitrarily identified an area on either side of BMD T score -1. This area covers BMD - T score between 0.25 to -1.25 (Lowest points of curve). We call students falling within this area "*Fence Sitter"*, because under the influence of various factors i.e. genetic, environmental, nutritional and life style they may shift on either side i.e. further improvement in BMD score towards healthy bone or deteriorate to osteopenia as they age.

CONCLUSION:

In our study significant numbers of students, especially female, are VD deficient. If VD deficiency persists longer in these participants, it will not only impact calcium metabolism and the bone health but also may make them vulnerable to various non-skeletal diseases. We view skeptically the *normal BMD* in most of our students because multiple factors contribute to VD deficiency and impact bone health. Secondly, as they age, different environmental and life style factors in face of chronic VD deficiency are going to impact their bone health and non-skeletal functions. "Fence Sitters" may have to face this impact, unless corrective measure at this age not taken.

LIMITATIONS OF THE STUDY:

(i) Short duration of the study (ii) BMD T Score estimation and its confirmation on bone histopathological examination neither feasible nor possible. (iii) Selection and definition of BMD area i.e. "Fence Sitters" area is arbitrary, based on lowest points taken on rising and falling curves of BMD "T score -1" graph.

ACKNOWLEDGMENT:

The authors are thankful to all students who consented and participated in this study. We also acknowledge every concerned department of MM Medical College and Hospital for providing valuable support to the study.

CONFLICT OF INTEREST:

None to disclose.

AUTHORS' CONTRIBUTION:

All authors participated in conceptualization, study design, methodology, study administration and supervision, collection and collating relevant medical literature, preparation of manuscript and editing and final revision.

REFERENCES:

1. Villareal DT, Civitelli R, Chines A, et al. Subclinical vitamin D deficiency in Postmenopausal women with low vertebral bone mass. J Clin Endocrinol Metab 1991;72:628–34.
2. Khaw KT, Sneyd MJ. Compston J. BMD, parathyroid hormone and 25-Hydroxyl vitamin D concentrations in middle aged women. BMJ 1992;305:273–7.
3. Alkhenizan A, Mahmoud A, Hussain A, et al. (2017) 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 12(1): e0169122. https://doi.org/10.1371/journal.pone.0169122
4. Grant WB, Holick MF. Benefits and requirements of vitamin D for optimal health: a review. Altern Med Rev 2005 Jun 1;10(2):94–111.
5. Ghannam NN, Hammami MM, Bakheet SM, et al. Bone mineral density of the spine and femur in healthy Saudi females: relation to vitamin D status, pregnancy, and lactation. Calcif Tissue Int 1999 Jul 1;65(1):23–8.
6. Stagi S, Cavalli L, Iurato C, et al. Bone metabolism in children and adolescents: main characteristics of the determinants of peak bone mass. Clin Cases Miner Bone Metab 2013;10(3):172–9.
7. WHO - Waist circumference and waist–hip ratio: report of a WHO expert consultation, Geneva, 8–11December 2008.
8. World Health Organization. Obesity and overweight: fact sheet N°311. Geneva: World Health Organization; 2015.
9. WHO Scientific Group on the Assessment of Osteoporosis at the Primary Health Care level. Summary Meeting Report Brussels, Belgium, 5-7 May 2004.
10. National Osteoporosis Foundation. Bone Density Test, Osteoporosis, Screening & T – Score Interpretation. https://www.nof.org › Patients › Diagnosis Information
11. Burgaz A, Akesson A, Oster A, Michaelsson K, Wolk A. Associations of diet, supplement use, and ultraviolet B radiation exposure with vitamin D status in Swedish women during winter. Am J Clin Nutr 2007;86(5):1399–404.
12. Jaaskelainen T, Knekt P, Marniemi J, Sares-Jaske L, et al. Vitamin D status is associated with sociodemographic factors, lifestyle and metabolic health. Eur J Nutr 2013;52(2):513–25.
13. Wanner M, Richard A, Martin B, Linseisen J, et al. Associations between objective and self-reported physical activity and vitamin D serum levels in the US population. Cancer Causes Control 2015;26(6):881–91.
14. Constantini NW, Dubnov-Raz G, Chodick G, Rozen GS, et al. Physical activity and bone mineral density in adolescents with vitamin D deficiency. Med Sci Sports Exerc 2010;42(4):646–50.
15. Larose TL, Chen Y, Camargo CA, et al. Factors associated with vitamin D deficiency in a Norwegian population: the HUNT Study. J Epidemiol Community Health 2014;68(2):165–70.
16. Cheng S, Massaro JM, Fox CS, Larson MG, et al. Adiposity, cardiometabolic risk, and vitamin D status: the Framingham Heart Study. Diabetes 2010;59(1):242–8.
17. Skaaby T, Husemoen LL, Thuesen BH, Pisinger C, et al. Longitudinal associations between lifestyle and vitamin D: a general population study with repeated vitamin D measurements. Endocrine 2015 May 30. [Epub ahead of print]
18. Oberg J, Jorde R, Almas B, Emaus N, et al. Vitamin D deficiency and lifestyle risk factors in a Norwegian adolescent population. Scand J Public Health 2014;42(7):593–602.
19. Rune Tønnesen, Peter Hambak Hovind, Lars Thorbjørn Jensen, and Peter Schwarz. Determinants of vitamin D status in young adults: influence of lifestyle, sociodemographic and anthropometric factors. BMC Public Health 2016; 16: 385.
20. Seeman E. The effects of tobacco and alcohol use on bone. In: Marcus R., Feldman D., Kelsey J., editors. Osteoporosis. 1st. Calif Academic Press Inc; San Diego, USA: 1996. pp. 577–598.
21. Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocrinology Rev 2001;22:477–501.
22. Slemenda C.W., Hui S.L., Longcope C., et al. Predictors of bone mass in perimenopausal women. Ann Intern Med 1990;112:96–101.
23. Heaney R.P., Abrams S., Dawson-Hughes B., Looker A., Marcus R., Matkovic V., Weaver C. Peak bone mass Osteoporos Int 2000;11:985–1009.
24. Khaw K.T., Sneyd M.J., Compston J. Bone density, parathyroid hormone and 25-hydroxyvitamin D concentrations in middle-aged women. Br Med J 1992;305:273–277.
25. Bischoff-Ferrari H.A, Dietrich T., Orav E.J., 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;116:634–639.
26. Sato Y., Manabe S., Kuno H., et al. Amelioration of osteopenia and hypovitaminosis D by 1a-hydroxyvitamin D3 in elderly patients with Parkinson's disease. J. Neurosurg. Psychiatry 1999;66:64–68.
27. Gallagher J.C., Fowler S.E., Detter J.R., et al. Combination treatment with estrogen and calcitriol in the prevention of age-related bone loss. J. Clin. Endocrinol. Metab 2001;86:3618–3628.
28. Chapuy M.C., Arlot M.E., Duboeuf F., Brun J., et al. Vitamin D3 and calcium to prevent hip fractures in elderly women. N. Engl. J. Med 1992;327:1637–1642.
29. Dawson-Hughes B., Harris S.S., Krall E.A., Dallal G.E. Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. N. Engl. J. Med 1997;337:670–676.
30. Seamans KM, Cashman KD. Existing and potentially novel functional markers of vitamin D status: a systematic review. Am J Clin Nutr 2009;89:1997S–2008S.
31. Jones G. Expanding role of vitamin D in chronic kidney disease: importance of blood 25-OH-D levels and extra-renal 1α-hydroxylase in the classical and nonclassical actions of 1α,25-dihydroxyvitamin D3. Seminars in Dialysis 2007;20(4):316–324.
32. Heaney RP. Vitamin D in health and disease. Clin J Am Soc Nephrol 2008;3(5):1535–1541.
33. Berridge MJ. Vitamin D deficiency and diabetes. Biochem J 2017 Mar 24;474(8):1321-1332.
34. Wang TT, Tavera-Mendoza LE, Laperriere D, Libby E, et al. Large-scale in silico and microarray-based identification of direct 1,25-dihydroxyvitamin D3 target genes. Mol Endocrinol 2005;19:2685–95.
35. Bouillon R, Carmeliet G, Verlinden L, van Etten E, et al. Vitamin D and human health: lessons from vitamin D receptor null mice. Endocr Rev 2008;29:726–76.
36. Howard A Morris and Paul H Anderson. Autocrine and Paracrine Actions of Vitamin D. Clin Biochem Rev 2010; 31(4): 129–138.
37. Wimalawansa S. (2016) Extra-Skeletal Benefits, Endocrine Functions, and Toxicity of Vitamin D. J Endocrinol Diab 2016; 3(3): 1-5.