

Original Article

Survey of Vitamin D Deficiency and Associated Comorbidities in LahoreNajiya al-Arifa^{1*}, Noor ul Huda¹ and Samra Atta Ullah¹¹Lahore College for Women University, Lahore

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<https://doi.org/10.54393/mjz.v2i2.5>**Corresponding author:**Najiya al-Arif
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Published: 31st December 2021**ABSTRACT**

Vitamin D is recognized for its importance in bone health along with the reduced risk of cardiovascular, autoimmune and several chronic diseases. **Objective:** Present study was designed to explore the prevalence of vitamin D deficiency and its association with comorbidities (Type 2 Diabetes, Thyroid Disease, Hypertension, Asthma, Heart Disease, Gastritis and Osteoporosis) among the subjects visiting outpatient departments (OPDs) of public hospitals in Lahore. **Methods:** Adult (≥ 18 years) male and female subjects (n = 200) participated in present cross-sectional study. Participating individuals were required to fill questionnaires which recorded their medical history and BMI. Blood samples were taken for laboratory evaluation of vitamin D₃ deficiency. Data was analyzed for evaluation of diverse risk factors. Serum level of vitamin D₃ (25-hydroxyvitamin D [25(OH)D₃]) were measured using standard procedures of measurement using Human Vitamin D₃ (VD₃) ELISA Kit (MyBioSource, Inc. USA). **Results:** Vitamin D deficiency was more prevalent in females than in males. Of the 200 individuals, the highest number of patients suffered from Diabetes Myelitis Type-II (61%) followed by Thyroid Disease (38%). Whereas, the least number of vitamin D₃ deficient subjects were suffering from Osteoporosis (5%). **Conclusion:** It is evident from present study that there are calculable degrees of Vitamin D insufficiency among all age gatherings, sexes, pay levels and areas in Pakistan.

INTRODUCTION:

Vitamin D is recognized for its importance in bone health along with the reduced risk of cardiovascular, autoimmune and chronic diseases including cancers [1]. Bone deformities due to vitamin D deficiency among pediatric and adult populations are commonly referred to as rickets [2]. In early twentieth century rickets were treated with exposure to ultraviolet radiations [3] and sunlight [4]. Phosphorus and calcium absorption is critically dependent upon vitamin D and since its production is inexpensive, vitamin D is widely contained in multivitamin supplements, fortified foods and other pharmaceutical preparations [5]. Natural dietary sources such as fish oils (herring, salmon, cod liver), red meat and eggs are vitamin D rich foods [6]. Vitamin D₁ in humans is synthesized in the epidermis by a non-enzymatic UVB-mediated photolytic reaction which converts the pre-cursor 7-dehydrocholesterol to pre-vitamin D₃ and then conversion to vitamin D₃ by thermal isomerization [7]. Cytochrome P450s converts vitamin D₃ to 25-hydroxyvitamin D₃ (25OHD₃) in the hepatic parenchyma [8]. Serum 25OHD₃ is the best indicator of vitamin D and its qualities are determined by affinity assays. Vitamin D deficiency is associated with seasonal variation [9] and associated comorbidities [10]. Furthermore additional factors such as indoor lifestyles [11], lack of sunlight [12, 13], obesity [14], socio demographic factors [15, 16], milk consumption and breastfeeding [17, 18] also attribute to vitamin D deficiency.

Vitamin D deficiency has been associated with risk for hip fractures [19], low bone mineral density [20] and muscular weakness [21,22]. Vitamin D deficiency in the pregnant women was observed as a consequence in the fetal skeleton as early as 19 weeks of gestation [23]. Clinical research attributes comorbidities and metabolic disorders such as obesity



[24,25], cardiovascular diseases [26], type-2 diabetes [27], insulin resistance [28,29], asthma [30] and hypertension [31] are with vitamin D deficiency. Although vitamin D deficiency is less common in developed Asian and European countries, multiple epidemiological studies carried out in Middle East, Asia and India have reported vitamin D inadequacy [32-34].

METHODS:

Study Design

Adult (≥ 18 years) male and female subjects ($n = 200$) participated in present cross-sectional study. Participating individuals were required to fill questionnaires which recorded their medical history and BMI. Blood samples were taken for laboratory evaluation of vitamin D₃ deficiency. Data was analyzed for evaluation of diverse risk factors. Following study was approved by the Board of Studies (BOS) at Lahore College for Women University, Lahore as well as from the ethics committees of all the hospitals from which the samples were obtained. All participants of the study signed written informed consent allowing the use of their data and biological samples for scientific purposes. Individuals visiting public and private hospitals in Lahore were recruited for following study. Total 200 subjects were included. All participants were referred by physicians to pathology laboratories for vitamin D₃ deficiency test. Present study included adult male and female subjects ≥ 18 years old who had been referred by the physician to pathology lab for vitamin D₃ deficiency test.

Vitamin D₃ Immunoassay

Total 3ml blood was extracted in serum clot activator tubes via venipuncture technique by a trained lab technician. The tubes were transported to lab in icebox to prevent hemolysis. The collected blood was centrifuged within one hour of the collection at 3000 rpm for 10 minutes. Clear serum was siphoned off using micropipette then stored at 4°C. All serological testing was completed within 3 days of collection. Serum level of vitamin D₃ (25-hydroxyvitamin D [25(OH)D₃]) were measured using standard procedures of measurement using Human Vitamin D₃ (VD₃) ELISA Kit (MyBioSource, Inc. USA). Vitamin D₃ cutoff values were defined as severe deficiency (0-8 ng/mL or ≤ 20 nmol/L), deficiency (10-15 ng/mL or ≤ 37.5 nmol/L), insufficiency (15-20 ng/mL or ≤ 50 nmol/L), optimal (30-100 ng/mL or 75-250 nmol/L) and toxic hypervitaminosis D (>150 ng/mL or ≥ 375 nmol/L). All the collected data was checked and corroborated with lab results on daily basis.

Serological Tests:

Quality control and Quality Assurance

Laboratory tests were performed by strictly following standard operating procedures and protocols. All reagents were prepared at room temperature and stored in refrigerator (4°C). Control samples were run once before and together with patient samples in well polystyrene plates. Patient samples were given unique code numbers which coincided with their laboratory specimens. Standards and samples were added into corresponding pre-coated (25-hydroxyvitamin D₃ antigen) wells. About 300 μ l of releasing reagent was added to all wells after pre-dilution of standards and samples and allowed to diffuse for 5-10 minutes. The plates were covered with foil and incubated for 60 minutes at room temperature. About 150 μ l of anti 25(OH)-vitamin D antibody was added into each well, covered in foil and incubated for 45 minutes at room temperature. The contents were discarded and plates were washed 5 times with buffer. The conjugate (200 μ l) antibody was added into each well, covered in foil and incubated for an additional 45 minutes at room temperature. The contents were discarded and then second washing was carried out 5 times with wash buffer. About 200 μ l substrate was added to the wells and incubated at room temperature in dark for 10-15 minutes. Optical density (OD) was measured with ELISA reader at 450nm. Data was recorded and arranged in MS EXCEL. All measurable variables were analyzed and results were correlated.

RESULTS

Population Characteristics

Population characteristics are provided in table 1. A total of 200 male 94(47%) and female 106(53%) subjects testing positive for vitamin D₃ deficiency were included in present study. The average age of participants was 42.8 ± 11.7 (mean \pm S.D). The male participants were older (43.6 ± 13.3) than the females (42.1 ± 10.1) as shown in figure 1. The mean BMI was 22.2 ± 3.5 in males and 22.3 ± 3.3 in females (figure 2).

Vitamin D₃

The 25(OH)D₃ was determined to be 24.6 ± 11.4 ng/mL for males and 24.1 ± 13.3 ng/mL for females (Figure 3). The normal range for 25(OH)D₃ is 25-80 ng/mL. The difference between the mean values for male and female subjects was found to be insignificant ($p = >0.5$).

Vitamin D₃ and Comorbidities

The comorbidities in vitamin D₃ deficient subjects are provided in table 2. Vitamin D deficiency was more prevalent in females than in males. Of the 200 individuals, the highest number of patients suffered from Diabetes Myelitis Type-II (61%) followed by Thyroid Disease (38%). Whereas, the least number of vitamin D₃ deficient subjects were suffering from Osteoporosis (5%).

Characteristics	Males %(n=94)	Females %(n=109)	Total %(n=200)
Age			
≥18-20	1(1)	0	0.5(1)
21-30	13.8(13)	14.6(16)	14.5(29)
31-40	28.7(27)	35.7(39)	33(66)
41-50	22.3(21)	25.6(28)	24.5(49)
51-60	12.7(12)	17.4(19)	15.5(31)
≥61	11.7(11)	3.6(4)	7.5(15)
BMI	22.2±3.5	22.3±3.3	22.3±3.4

Table 1: Population characteristics

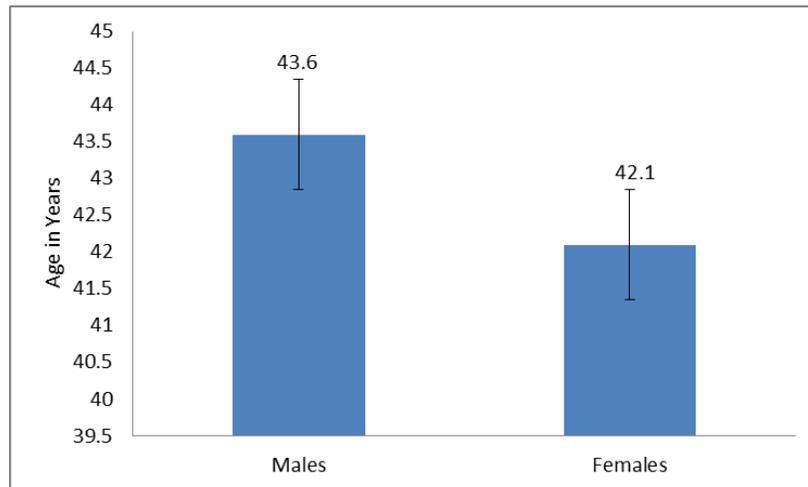


Figure 1: Age of male and female subjects

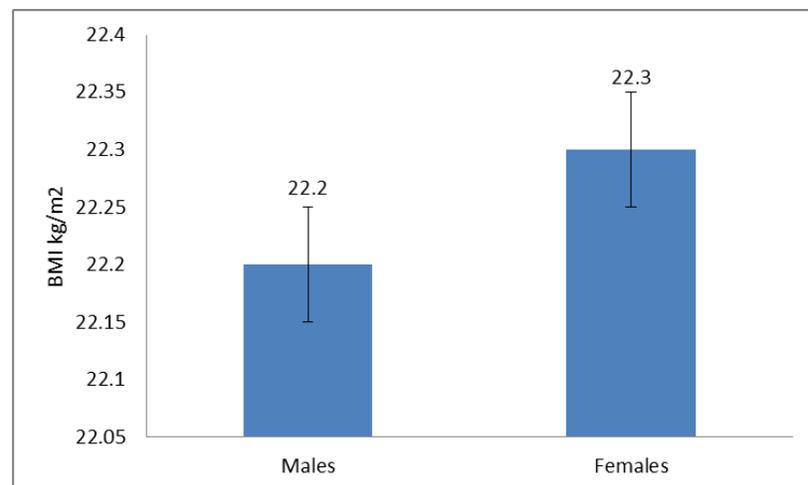


Figure 2: BMI of male and female subjects

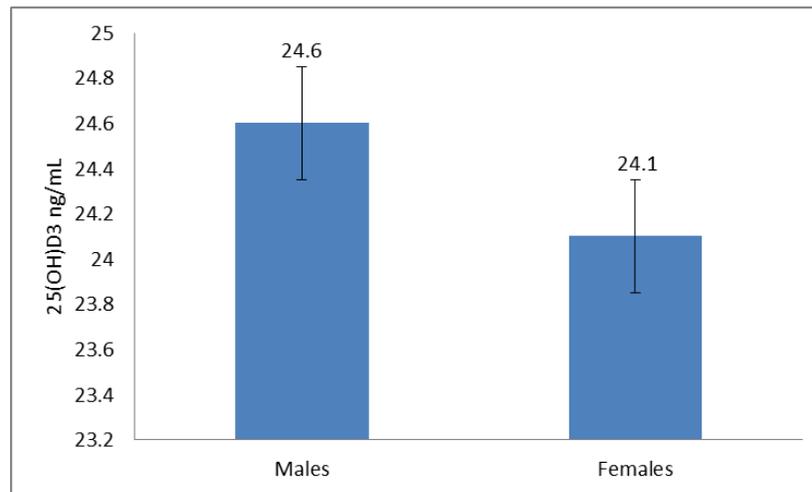


Figure 3: 25(OH)D₃ levels of male and female subjects

Co-morbidities	Male %(n=94)	Female %(n=109)	Total %(n=200)
Type 2 Diabetes	29.5(59)	31.5(63)	61(122)
Thyroid Disease	17.5(35)	20.5(41)	38(76)
Hypertension	16.5(33)	20.5(41)	37(74)
Asthma	18.5(37)	16(32)	34.5(69)
Heart Disease	8.5(17)	9.5(19)	18(36)
Gastritis	7.5(15)	8.5(17)	16(32)
Osteoporosis	0(0)	4.5(5)	2.5(5)

Table 2: Comorbidities in vitamin D₃ deficient subjects

DISCUSSION

Vitamin D deficiency (VDD) has been previously reported in Pakistan [35] in correlation with various comorbidities. VDD has been known to play a major role in a variety of diseases like cardiovascular diseases, neurological disorders, autoimmune disease, depression and cancer. It is deemed as a risk factor for certain birth defects such as rickets in children and osteoporosis, osteomalacia and osteoarthritis in adults. It is also known to cause muscle pain and chronic pain. There are a very few sources of vitamin D in food. Absence of satisfactory daylight exposure and indoor lifestyles are the significant reasons for nutrient D inadequacy [18].

Current study was carried out to evaluate the comorbidities in deficiency of vitamin D. In present study the total of 200 male (47%) and female (53%) subjects testing positive for vitamin D₃ deficiency. The average age of participants was 42.8±11.7 (mean±S.D). These findings are in accordance with another study who has also detailed that a high pervasiveness of nutrient D insufficiency in people in grown-up age. The explanation of high pervasiveness of nutrient D insufficiency related for comorbidities that includes the sun exposure, atmospheric pollution, the degree of physical activity and dietary habits [34].

A study reported that a relation between high BMI measurements and lower vitamin D concentration. Results from current study support a relationship between Vitamin D deficiency and obesity. The 25(OH)D₃ was determined to be 24.6±11.4ng/mL for males and 24.1±13.3ng/mL for females. The normal range for 25(OH)D₃ is 25-80ng/mL. Mean values between the two genders were found to be insignificant ($p = 0.5$) [36].

Vitamin D inadequacy found in current investigation concurs with previously reported studies in Pakistan and in its adjoining nations. An undeniable degree of Vitamin D inadequacy (40%) was likewise noted among kids in the 2011 National Nutrition Survey conducted by Aga Khan University's Division of Women and Child Health, Pakistan's Ministry of Health and UNICEF with restricted contrasts among metropolitan and rustic regions [37].

CONCLUSION:

Present study revealed that 65% men and 91.7% of women with vitamin D deficiency had BMI was 22.2 ± 3.5 in males and 22.3 ± 3.3 in females.

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