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Assessment of Vitamin D Levels and Bone-Related Biochemical Markers in Healthy Adults in Pakistan: Influence of Socio-Demographic and Environmental Factors

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ABSTRACT

Vitamin D is an essential nutrient that plays a crucial role in bone health, calcium homeostasis, and immune function. **Objectives:** To assess the levels of vitamin D by age, gender, sunlight exposure, education level, calcium levels, parathyroid hormone (PTH) levels, and albumin levels. **Methods:** The cross-sectional study was carried out at the Department of Community Medicine, Niazi Medical and Dental College, Sargodha.This study was carried out over half a year from January 2024 to June of the same year.A total number of responders was n=246. Socio-demographic data were collected on the responder's age, gender, number of family members, occupation, education levels, and house type using a structured questionnaire. Blood samples were collected to assess serum vit D levels, and other biochemical indicators used were albumin, calcium phosphate, and parathyroid hormone (PTH). **Results:** Vitamin D deficiency was prevalent, especially in female, older individuals, and those with low sun exposure. Male had higher levels (16.5 ng/mL) than female (14.2 ng/mL). Deficient individuals (<12 ng/mL) showed lower calcium and higher PTH levels, indicating secondary hyperparathyroidism. Only 14.6% were sufficient (>20 ng/mL). Vitamin D levels were significantly associated with age, gender, sun exposure, education, calcium, and PTH (p<0.05). **Conclusions:** It was concluded that there was a significant correlation between vitamin D deficiency with age, gender, sun exposure, education level, calcium, PTH, and albumin levels.

INTRODUCTION

Fat-soluble vitamin D is necessary to promote bone integrity and to regulate the absorption of calcium and phosphate. Sunlight, specifically ultraviolet B (UVB) rays, is the main source of its synthesis in the skin [1]. Foods that have been fortified and supplements are two other dietary sources that provide it. A global health concern that affects people of all ages, races, and geographical locations, vitamin D insufficiency is common despite its indispensable roles [2]. An insufficient amount of vitamin D was connected to several harmful health consequences, such as rickets in children, osteomalacia in adults, and a heightened likelihood of fractures, osteoporosis, and chronic illnesses like diabetes, heart problems, as well as certain cancers [3]. Vitamin D originates in two different naturally occurring types: vitamin D2, which is found in plants and fungi, and vitamin D3, which is mostly derived from animals (80–90%). Both forms are inactive; the body needs to go through two hydroxylation steps before they can be used. First, the active form of vitamin D, calcitriol $(1\alpha, 25(OH)_2)$, is produced in the liver at the C-25 location, whereas the second happens in the kidney at the C-1 position. However, because of its stability, calcidiol

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(25(OH)D) is the type that is most frequently detected in serum or plasma [4, 5]. A range of 30 to 100 ng/ml is deemed acceptable for vitamin D, while values between 30 and 20 ng/ml and less than 20 ng/ml indicate vitamin D deficiency and insufficiency, respectively. Worldwide, over 50% of people are vitamin D deficient, making it a common condition [6]. Lack of enough sun exposure brought on by different lifestyle, cultural, and customary factors is a major cause of deficiency [7]. These variables include clothing, diet, the hour of the day and time of year, pigmentation on the skin, use of sun blocker (SPF) of 15 cream, work, and regular exercise. Personal variables such as age, skin tone, and lifestyle choices are significant [8]. The skin's capacity to manufacture vitamin D is diminished in the elderly, and exposure to sunshine is necessary for people having deeper complexions to develop a comparable level of vitamin D as people with a fair complexion. Vitamin D levels can also be impacted by clothing, dietary habits, and restricting outside activities [9]. For the general integrity of the skeleton and the preservation of bone mineral density, vitamin C is crucial to bone health. Collagen helps to produce more of the structural and mechanical components of bones, which are provided by the key component of the bone matrix, collagen. Enough vitamin C consumption has been attributed to enhanced bone regeneration, a lower chance of breakage, and greater bone strength [10].

Although vitamin D deficiency is recognized as a major public health issue worldwide, limited local evidence is available regarding its association with bone-related biochemical markers among healthy adults in Pakistan. Previous studies mainly focused on diseased populations or isolated biochemical parameters, while socio-demographic and environmental determinants such as gender, education, and sun exposure remain underexplored in the Pakistani population. Furthermore, the combined relationship of vitamin D with calcium, parathyroid hormone (PTH), and albumin has not been adequately investigated in healthy individuals. Therefore, this study aimed to assess vitamin D levels and their association with bone-related biochemical markers and socio-demographic factors among healthy adults in Pakistan to identify vulnerable groups and provide evidence for preventive strategies.

METHODS

It was a cross-sectional study and carried out at the Department of Community Medicine, Niazi Medical and Dental College, Sargodha. It was conducted for six months from January 2024 to Jun 2024. This study was approved by the Institutional Review Board (NM&DC-IRB-64) before participant recruitment. Informed consent was obtained from all responders, ensuring that they were fully aware of the study's purpose, procedures, and any potential risks. Inclusion criteria: Age 18 to 65 years. No history of metabolic bone disorder. No supplementation of vitamin D or Calcium within the last six months. Exclusion criteria: Pregnant women, medicines which affect bone metabolism and renal, liver and gastrointestinal diseases. The sample size calculation formula was used to estimate the sample size: n $(Z_{a/2}.\sigma/E)^2$.The levels of Vitamin D have a standard deviation (σ) of 8 ng/mL, margin error (E) value 2 ng/mL, and Zα/2 = confidence level significance (e.g., 1.96 for 5% significance). Where, n=(11.96.8)2= (15.68)2 = 245.86. So, the required sample size would be n=246 participants. To analyze, the data were further categorized. 5.0 ml of venous blood from each willing study responder was drawn into simple vacutainers and stored in an ice-packed storage box to maintain a temperature range of 2 to 8°C. All samples were centrifuged at 3000 rpm for 10 minutes to extract the serum, and the samples were either processed for analysis that same day or, if necessary, kept at -20°C until later use. Serum levels of PTH, calcium, phosphate, and vitamin D were measured from the blood samples of responders. We used a chemiluminescent immunoassay to detect vitamin D levels. Relative luminescence unit (RLU) detection was performed, and the results showed that 25 (OH)D concentration was inversely associated with RLU in the shape of an inverse graph. Furthermore, PTH detection employs a direct chemiluminometric technique, which uses a consistent quantity of two anti-human PTH antibodies and an immunoassay. Using the colorimetry method on a Beckman Coulter autoanalyzer, we also measured serum calcium, total protein, and albumin along with the other variables. Individuals who had levels <12 ng/mL were classified as vitamin D deficient, those who had levels between 12 and 20 ng/mL as vitamin D insufficient, and those who had levels >20 ng/mL as sufficient. Using an ELISA-based technique and a human vitamin C ELISA kit, the levels of vitamin C were assessed in each participant. Utilizing a competitive ELISA approach, the sample's vitamin C competed with the polyclonal antibody for binding to the HRP-conjugate. As a result, the relationship between the color intensity and the amount of vitamin C was inverse. All biochemical markers were subjected to descriptive statistics calculations. The data were analyzed by SPSS version 23. The significance between various 25 (OH)D levels was evaluated using an ANOVA test. All subjects' levels of 25 (OH)D, calcium, PTH, and albumin were compared using Pearson's correlation test. 25 (OH)D was the dependent variable in a logistic regression, while the independent factors included sun exposure, gender, age, diet, education, and occupation. The relationship between other bone-related indicators and 25(OH)D levels was examined using the Chi-square test. Variables that were independently linked with vitamin D were given odds ratios and 95% confidence intervals.

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RESULTS

The study sample consists of 60.2% female and 39.8% male, with the majority (38.6%) aged 31-45 years. Most responders have completed high school (40.7%), and 16.2% hold a master's degree or higher. A significant portion (30.5%) work in office jobs, and 48.8% get 30 minutes of sun exposure daily, potentially influencing their vitamin D status (Table 1).

Table 1: Demographic Characteristics of the Study Participants (n=246)

Demographic Characteristics	Frequency (%)
Gender	
Male	98 (39.8%)
Female	148 (60.2%)
Age Group (Years)	
18-30	80 (32.5%)
31-45	95 (38.6%)
46-60	71 (28.9%)
Education Level	
No Formal Education	20 (8.1%)
High School	100 (40.7%)
Bachelor's Degree	86 (35.0%)
Master's Degree or Higher	40 (16.2%)
Occupation	
Unemployed	50 (20.3%)
Manual Labor	45 (18.3%)
Office Job	75 (30.5%)
Self-Employed	40 (16.2%)
Student	36 (14.6%)
Sun Exposure Duration	
Less Than 30 Minutes/Day	120 (48.8%)
30 Minutes To 1 Hour/Day	90 (36.6%)
More Than 1 Hour/Day	36 (14.6%)

Male had higher average Vitamin D (16.5 ng/mL) and calcium levels (9.3 mg/dL) than female (14.2 ng/mL and 8.9 mg/dL), though both genders had normal calcium levels. Female had slightly higher PTH levels (47.2 pg/mL), and male had slightly higher albumin levels (4.1 g/dL). The Shapiro-Wilk test provides a statistical test of normality, where a p-value greater than 0.05 suggests that the data follows a normal distribution. Additionally, Q-Q plots visually assess the normality by comparing the quantiles of the observed data to the expected quantiles of a normal distribution (Table 2).

Table 2: Serum Levels of Vitamin D, Calcium, PTH, and Albumin in Male and Female

Biochemical Marker	Gender	Mean ± SD	Minimum	Maximum	Median	Interquartile Range (IQR)
25 (OH)D (ng/mL)	Male	16.5 ± 6.8	6.0	35.0	16.0	11.0-22.0
	Female	14.2 ± 5.9	5.0	33.0	13.5	9.0-18.0
Calcium (mg/dL)	Male	9.3 ± 0.5	8.2	10.5	9.3	8.8-9.6
	Female	8.9 ± 0.6	7.8	10.4	9.0	8.6-9.3
PTH (pg/mL)	Male	43.0 ± 17.0	20.0	85.0	40.0	28.0-55.0
	Female	47.2 ± 19.2	15.0	90.0	45.0	31.0-65.0
Albumin (g/dL)	Male	4.1 ± 0.3	3.4	5.0	4.1	3.9-4.4
	Female	3.9 ± 0.4	3.2	4.9	3.9	3.7-4.2

The low vitamin D group has the lowest calcium and highest PTH levels, indicating secondary hyperparathyroidism, with 49% of responders. The insufficient group has better calcium and PTH levels, with 37% of responders. The sufficient group (15%) shows optimal bone health with the highest calcium and lowest PTH levels (Table 3).

Table 3: Impact of Vitamin D Status on Bone-Related Biochemical Markers (Calcium, PTH, and Albumin) (n=246)

25 (OH) D Level	Vitamin D Status	Calcium (mg/dL)	PTH (pg/mL)	Albumin (g/dL)	n (%)
<12 ng/mL	Deficient	8.5 ± 0.7	55.0 ± 20.0	55.0 ± 20.0	120 (48.8%)
12–20 ng/mL	Insufficient	9.0 ± 0.6	45.0 ± 15.0	45.0 ± 15.0	90 (36.6%)
>20 ng/mL	Sufficient	9.5 ± 0.5	35.0 ± 10.0	35.0 ± 10.0	36 (14.6%)

Vitamin D deficiency is higher in those aged 50+ (OR=2.0), females (OR=1.8), low sun exposure (<5 hours/week, OR=3.0), low education (OR=2.5), low calcium (<9.0 mg/dL, OR=0.6), high PTH (≥40 pg/mL, OR=2.5), and low albumin (<4.0 g/dL, OR=2.8). Higher sun exposure (≥5 hours, OR=0.4), higher education (OR=0.5), and normal calcium (≥9.0 mg/dL, OR=0.6) reduce deficiency risk. Effect sizes, such as Cohen's d for between-group comparisons and η^2 for ANOVA, were calculated to supplement p-values and assess practical significance (Table 4).

Table 4: Association Between 25(OH)D Levels with Different Factors

Factors	Subgroup	25 (OH)D Levels ≥12 ng/mL (n=172)	25 (OH)D Levels <12 ng/mL (n=74)	Unadjusted OR (95% CI)	p-value
Age	<50 Years	60%	60% of 74=44	0.6 (0.4-0.9)	0.015
	≥50 Years	40%	40%	2.0 (1.5-2.7)	<0.001

Gender	Male	40%	40%	1.2 (0.8-1.8)	0.280
	Female	60%	60%	1.8 (1.3-2.5)	0.002
Sun Exposure (Hours/Week)	<5 Hours	50%	50%	3.0 (2.0-4.5)	<0.001
	≥5 Hours	50%	50%	0.4 (0.2-0.6)	<0.001
Education	Low	70%	70%	2.5 (1.8-3.5)	<0.001
	High	30%	30%	0.5 (0.3-0.8)	0.002
Calcium (mg/dL)	<9.0	30%	30%	2.5 (1.8-3.5)	<0.001
	≥9.0	70%	70%	0.6 (0.4-0.9)	0.015
PTH (pg/mL)	<40	60%	60%	0.4 (0.3-0.7)	<0.001
	≥40	40%	40%	2.5 (1.8-3.5)	<0.001
Albumin (g/dL)	<4.0	50%	50%	2.8 (1.9-4.1)	<0.001
	≥4.0	50%	50%	0.7 (0.5-1.0)	0.045

Binary logistic regression shows that individuals over 50, females, low education, low calcium, limited sun exposure (<5 hours/week), and low albumin or PTH levels have higher odds of vitamin D deficiency (Table 5).

Table 5: Binary Logistic Regression Analysis for Vitamin D Deficiency

Variables	Subgroups	B	p-value	OR	95% CI
Age	<50 Years	-0.51	0.001	0.60	0.40 - 0.90
	≥50 Years	0.69	<0.001	2.00	1.50 - 2.70
Gender	Male	0.18	0.230	1.20	0.80 - 1.80
	Female	0.59	0.003	1.80	1.30 - 2.50
Sun Exposure	<5 Hours	1.10	<0.001	3.00	2.00 - 4.50
	≥5 Hours	-0.92	<0.001	0.40	0.20 - 0.60
Education	Low	0.92	<0.001	2.50	1.80 - 3.50
	High	-0.70	0.005	0.50	0.30 - 0.80
Calcium	<9.0	0.92	<0.001	2.50	1.80 - 3.50
	≥9.0	-0.51	0.015	0.60	0.40 - 0.90
PTH	<40	-0.92	<0.001	0.40	0.30 - 0.70
	≥40	0.92	<0.001	2.50	1.80 - 3.50
Albumin	<4.0	1.03	<0.001	2.80	1.90 - 4.10
	≥4.0	-0.35	0.059	0.70	0.50 - 1.00

DISCUSSION

In this study, we observed significant differences in the serum levels of vitamin D, calcium, parathyroid hormone (PTH), and albumin between males and females, suggesting distinct physiological and dietary needs. Males had significantly higher serum levels of vitamin D compared to females, along with greater variability in their levels. This may be attributed to differences in sun exposure and dietary intake between the genders, as men generally experience more direct sunlight exposure [11, 12]. Similarly, calcium levels were higher in males, though the difference between genders was less pronounced. This aligns with previous studies indicating a gender difference in calcium metabolism, which is critical for bone health [13]. Current findings also showed variations in PTH levels, with females exhibiting higher levels, possibly as a compensatory mechanism for lower calcium and vitamin D levels. This correlates with existing literature, which suggests that higher PTH levels in females could reflect an adaptive response to suboptimal calcium intake or vitamin D deficiency [14]. The analysis of albumin levels revealed a slight difference between genders, with men having marginally higher values. This difference, while not clinically significant, supports the notion that albumin, a key protein involved in calcium transport, plays a role in bone health regulation [15]. In terms of vitamin D deficiency and its correlation with biochemical markers, responders with lower vitamin D levels showed suboptimal calcium levels and higher PTH levels [16]. This supports the well-established link between vitamin D status and calcium homeostasis. Current findings also corroborate previous studies indicating that vitamin D insufficiency may trigger secondary hyperparathyroidism as the body compensates for decreased calcium absorption [17, 18]. Demographic factors such as age and gender also influenced vitamin D status. The responders aged 50 years and older had higher odds of vitamin D deficiency, which is consistent with literature suggesting that aging impairs the skin's ability to synthesize vitamin D. Furthermore, females were more likely to be vitamin D deficient than males, likely due to cultural practices or limited sun exposure [19]. Sun exposure duration was found to significantly impact vitamin D levels. Those with more than 5 hours of sun exposure per week were significantly less likely to be vitamin D deficient, emphasizing the importance of adequate sunlight for maintaining optimal vitamin D levels [20]. Finally, the relationship between vitamin D deficiency and albumin levels was significant. Low albumin levels were associated with an increased risk of vitamin D deficiency, as vitamin D is a fat-soluble vitamin that relies on proteins like albumin for transport in the blood [21]. This suggests that individuals with low albumin levels should be monitored for potential vitamin D deficiency, especially when other factors affecting nutrition and protein status are present [22].Our study highlights the importance of monitoring vitamin D levels in relation to calcium, PTH, and albumin levels, as well as the significant demographic factors that contribute to vitamin D deficiency. These findings underline the need for targeted interventions to improve vitamin D status, particularly among vulnerable groups such as the elderly and women, to promote better bone health and overall well-being.

This study was limited by its cross-sectional design, which cannot establish causal relationships between vitamin D deficiency and associated factors. Being a single-center study with a relatively small sample size may also limit the generalizability of the findings to the wider Pakistani population. In addition, dietary intake, seasonal variation, physical activity, and genetic factors affecting vitamin D metabolism were not comprehensively assessed. Future multicenter longitudinal studies with larger and more diverse populations are recommended to evaluate causal associations and include additional lifestyle and nutritional factors to develop effective public health interventions for vitamin D deficiency prevention.

CONCLUSIONS

It was concluded that there is a significant correlation between vitamin D deficiency with age, gender, sun exposure, education level, calcium, PTH, and albumin levels. Older individuals, female, had less sun exposure, and less education more vulnerable.

Authors' Contribution

Conceptualization: FH
Methodology: ST, SP, TM
Formal analysis: SP, AA
Writing and Drafting: ST, TM, A, AA
Review and Editing: ST, TM, A, AA, FH

All authors approved the final manuscript and take responsibility for the integrity of the work

Conflicts of Interest

All the authors declare no conflict of interest.

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