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Original article

Vitamin D status among female university students in Pakistan

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Summary. Increased prevalence of hypovitaminosis D and the genesis of a myriad associated maladies among a variety of population fractions have been a focus of the scientific community and health professionals. Present cross-sectional study aimed at assessing the levels of serum 25- hydroxyvitamin D [25(OH)D] among female university students (FUSs) in Pakistan. Students were recruited and evaluated for various socio-demographic parameters including skin color, veil status, previous disease history, sun exposure and dietary condition. Serum levels of 25(OH)D were determined and information on lifestyle variables were gathered to ascertain vitamin D deficiency (VDD) and its correlates. Significant difference in serum levels of 25(OH) D among females with dark skin (22 ng/ml) as compared to white skinned (35 ng/ml) subjects was noted suggesting skin pigmentation as a contributory determinant for vitamin D deficiency. Similarly, the results indicated low vitamin D levels among veil observing FUSs. Though, VDD has not been found to be severe as 25(OH)D levels remained >18.0 ng/ml in all groups, however extreme caution is needed to improve these concentrations to optimal levels. Speculation can be drawn from the results that VDD might be much higher among illiterate population groups suggesting further research to ascertain prevalence of VDD among populations from varied socio-economic tiers in Pakistan.

Key words: vitamin D, South Asia, women, socio-economic, osteoporosis, serum 25-hydroxyvitamin D

Introduction

Significance of 25(OH)D, the circulating form of vitamin D, has been well recognized as a fundamental factor for maintaining musculoskeletal health of people of all ages (1). Evidence is available to suggest vitamin D status of the populations to be linked with several chronic disorders such as diabetes and various types of cancers (2,3). Rickets and osteomalacia among children and adults, respectively, are manifested in severe VDD whereas increase bone loss and fracture risk have been widely reported among individuals with less severe deficiency (4).

Prevalence of VDD has been common among people beyond ethnicity, age, gender, race, geographical boundaries signifying approximately one billion people with low vitamin D worldwide (3,5,6). Levels of serum 25(OH)D have been regarded as the most reliable measure to gauge vitamin D status among all population groups. Several studies substantiated a variability in setting the optimal levels of serum 25(OH)D globally on account of ecological and socio-economic variability, however; consensus of international experts has been arrived at by pronouncing severe deficiency, <12.5nmol/L: moderate deficiency, 12.5-25nmol/L: mild deficiency, 25-50 nmol/L: insufficiency >50nmol/L-75nmol/L and sufficiency >75nmol/L (7,8).

Ecological studies have corroborated the increased prevalence of VDD among females of varied age groups in Middle East and North African region

(9). A recent study from Egypt reported 72% of the females in childbearing age as vitamin D deficient (10). The researchers established observing veil and lack of sun exposure to be the underlying causes for heightened VDD among females. Despite the availability of ample resources and plenty of sunshine, many European populations are at the risk of VDD. In an analogue study, researchers examined 824 elderly people from 11 European countries and found 47% of women having 25(OH)D levels <30 nmol/L (11). South East Asian countries such as China, Japan, Hong Kong, North and South Korea and Taiwan are not the exception with respect to vitamin D deficiency. Populations from Nanjing and Beijing in China were shown to suffer from VDD [25(OH)D <25 nmol/L) in winter and fall, respectively (12).

Food Sources of Vitamin D are not abundant thereby necessitating the consumption of vitamin D fortified foods and supplements (12, 13). Fortification of foods with Vitamin D and supplementation have been widely recognized as means to override vitamin deficiency especially in developing economies (12). Evidence is available to confirm supplementation of vitamin D to be extensively contributing in improved bone mineral density leading to reduced risk of falls and fractures among elderly (14, 15). Available information on the extent of the prevalence of VDD amongst pregnant women in Pakistan signified supplementation of vitamin D, as a viable strategy during pregnancy. Researchers have recommended 400 IU to 600 IU as a reasonable dose during pregnancy (12).

High prevalence rates of VDD have been reported in Pakistani women, but there is no reliable data from Southern Punjab to rule out inadequacy of vitamin D among female literate students. Earlier studies carried out suggested VDD to be pandemic in Pakistan, however the researchers did not find any study typically focusing literate university female students' community. The primary objective of the study in question was to test the hypothesis that VDD {(25(OH)D level, ≤15 ng/mL)} is prevalent among the literate female fraction of Southern Punjab, and to identify correlation with a range of VDD determinants such as skin color, veil status, disease condition, sun exposure and dietary condition.

Methods

Study population and design

This prospective observational study was conducted at Bahauddin Zakariya University, Multan, Pakistan. The study was approved by the ethical committee for research on human and animal subjects, Bahauddin Zakariya University, Multan, Pakistan. Apparently healthy female students (n=60) aged between 20-25 years were selected after preliminary screening following predefined inclusion/exclusion criteria. The criteria for inclusion in this study were non-smoker and healthy university graduating female students. Informed consent was solicited from the selected participants and a detailed interview was conducted on skin color, veil status, sun exposure, dietary habits and previous disease history. Questions regarding skin color include dark or white skin. Veil status was inquired for complete body cover including face and unveiled as face and forearm exposed. Information on sun exposure was comprising length of time exposed to sun during peak hours i.e., 11:00 to 13:30 hr with open face and forearm. Dietary habits were evaluated for consumption frequency of dairy products, fish, calcium / vitamin D fortified foods, fast foods, tea, coffee and carbonated beverages.

Serum 25-hydroxy vitamin D analysis

Fasting blood samples (5cc) were drawn from the selected participants by professional nursing staff. Samples were taken in vacutainer coated with clotting activator and stored in refrigerator (4°C). Serum was isolated from the stored samples by centrifugation within an hour of sample collection. Serum 25-hydroxy vitamin D concentration was measured using Enzyme-Linked Immunosorbent Assay (ELISA) technique. Ten microlitre 25(OH)D was pipetted in designated wells of 96-well ELISA plate. Control and serum samples (10 µl) were added to the well plate according to the sampling plan. Freshly prepared working solution i.e., Biotinylated 25 (OH)D reagent was added to each well. The contents of the well plate were manually mixed by gentle shaking for 30 sec and the plate was incubated in dark chamber for 90 min. Washing of the plate contents was performed twice with wash buffer (300 µl). Two hundred microlitre conjugate was pipet-

ted in each well subsequent to washing. Contents of the well-plate were incubated for 30 min at 25°C. Second washing was performed with washing buffer (300 µl). Two hundred microlitre TMB substrate was added in each well and the plate was again incubated for 30 min at 25°C. Stop solution (50 µl) was added to the wells to stop enzymatic reaction. Absorbance was measured on ELISA reader at 450 nm within 10 min of adding stop solution to the well. Concentration of 25(OH)D in each sample was calculated by plotting absorbance values in standard curve. Cut-off values of 25 (OH) D were established as severe deficiency, <12.5 nmol/L: moderate deficiency, 12.5-25 nmol/L: mild deficiency, 25-50 nmol/L: insufficiency >50 nmol/L-75 nmol/L and sufficiency >75 nmol/L (7,8).

Statistical analysis

The Statistical Package for Social Sciences, SPSS (release 25.0, standard version, copyright© SPSS), was used for data analysis. Descriptive analysis was done for demographic variables and proportions were determined. Univariate analyses and ANOVA tests, were performed among subjects for skin color, veil wearing, sun exposure, dietary conditions and pathological conditions. The association of serum 25(OH)D concentrations between these 5 groups were studied by linear regression and correlation. *p*-value less than 0.05 (2-sided test) was considered statistically significant.

Results and Discussion

Data presented in Table 1 depict a mean concentration of 25(OH) D level ranging from 25 to 27 ng/ml among five groups (Table 1). Skin pigmentation has been shown to play a seminal role in raising serum 25(OH)D among population belonging to non-western regions. Evidence is available to suggest higher prevalence of VDD among dark skinned populations especially from South Asian and African regions (16-18).

Our study confirmed the results of foregoing studies reporting a significant difference in serum 25(OH)D levels among female university students i.e., 22 and 35 ng/ml among dark skinned and white skinned female students, respectively (Table 2). These finding are a proxy of skin pigmentation to be one of the contributory factors for increased 25(OH)D levels among adolescents and girls of child bearing age and are supported by evidence from literature (19).

Non-observance of veil in most of the Muslim societies is generally grinned and grimaced at as a non-religious practice. Resultantly, Muslim females have to observe veil either by covering the whole body including face or sometimes with only face, arms and feet exposed. This practice generally leads to reduced or sub optimal levels of serum 25(OH)D among females of all age groups in Pakistan. Current study confirms reduced levels of 25(OH)D i.e., 22 ng/ml among FUSs observing veil by covering whole body including face as compared to 25(OH)D (35 ng/ml) among those having some parts of the body exposed to sun (Table 2). Upshots of the present study are in line with the findings of several researchers in terms of veil (*Purdah*) being corollary of increased prevalence of VDD among South Asian populations (20, 21).

Vitamin D serum concentrations have been found to be drastically varying under various disease conditions. Amital et al. (22) demonstrated a significant

Table 1. Descriptive statistics for serum 25(OH)D levels (ng/ml) among female university students aged 20-25 years

	N*	Min.	Max.	Mean	Std. Dev
Skin Color	60	18.00	45.00	26.12	6.66
Veil Status	60	18.00	45.00	26.79	6.93
Disease Status	60	18.00	41.00	25.92	6.38
Sun Exposure	60	17.90	40.00	25.72	6.22
Dietary Condition	60	18.00	39.00	25.00	6.02

** Number of participants*

Table 2. Descriptive statistics for serum 25(OH)D levels (ng/ml) among female university students aged 20-25 years (sub-groups)

		N	Mean	SD	Min.	Max.	Total (%)	p	C I (95%)	
									Upper	Lower
Skin Color	DS	42	22.4	2.73	18.0	29.1	70.0	>0.0001	21.35	23.44
	WS	18	34.8	4.60	29.0	45.0	30.0		33.23	36.43
Veil Status	WV	38	22.1	2.70	18.0	29.3	63.3	>0.0001	21.10	23.17
	NV	22	34.8	3.91	29.8	45.0	36.7		33.47	36.19
Disease Status	WD	39	21.9	2.54	18.0	26.8	65.0	>0.0001	20.94	22.86
	ND	21	34.4	2.80	30.6	39.0	35.0		32.65	35.99
Sun Exposure	Mi.E	42	22.2	2.75	17.9	27.2	70.0	>0.0001	21.26	23.20
	Mx.E	18	33.9	3.97	28.0	40.0	30.0		32.37	35.34
Dietary Condition	PVD	40	22.1	3.05	18.0	28.0	66.7	>0.0001	21.04	23.21
	RVD	20	33.5	4.10	28.4	41.0	33.3		31.98	35.05

** Number of participants: DS=Dark Skin: WS=White Skin: WV=With Veil: NV=No Veil: WD=With Disease: ND=No Disease: MiE=Min. Exposure: MxE=Max. Exposure: PVD=Poor Vit.D: RVD=Rich Vit.D: SD=Standard Deviation: CI=Confidence Interval*

negative correlation between the serum concentration of vitamin D and the standardized values (z-scores) of disease activity scores. Likewise, varying level of vitamin D have been implicated with a variety of diseased conditions and are considered to be a causal factor for the development of CVD (23), statin-associated myalgia (24), breast cancer (25), tuberculosis (26) and asthma (27). Visible difference in serum 25(OH)D levels were noticed among FUSs with diseased and non-diseased heath status (Table 2). Significantly higher concentration of vitamin D levels i.e., 34ng/ml among healthy subjects were observed while vitamin D concentration remained much lower i.e., 22 ng/ml in female subjects who had experienced some disease conditions suggesting the impact of optimal levels of 25(OH)D in maintaining good health.

Several studies demonstrated the role of sun exposure and diet as substantial contributory factor to maintain optimal levels of 25(OH)D, however these studies were not directed to find out vitamin status of the educated females studying at University level where awareness on the role of vitamin D in health were supposed to be higher as compared to the normal population with reduced literacy rates. We concluded that dietary sources of vitamin D and sun exposure have positive impact on avoiding vitamin D deficiency. Present results corroborated the longstanding believe in sun exposure as a fundamental component for acquiring elevated vitamin D levels. Diets rich in vitamin D have also been regarded a good approach to attain sufficient amount of vitamin, however food alone does not meet the increased needs of vitamin D. Therefore, fortification of food has been widely recommended and accepted approach to combat vitamin deficiency in areas and situations where sun exposure does not suffice the needs for vitamin such as genetic factors, age, latitude of the country of residence, life styles and chronic diseases.

Viewing at the extent of sunshine and weather conditions in Pakistan with latitude 24° 35' North and longitude 61° East to 78° East, Southern Punjab has a tropical climate with low humidity, in summer its temperature rises up to 45-48°C and in winter it has minimum temperature 4-5°C. So, intensity of ultra violet B radiations (UVB) radiations is very high in comparison with the European countries. Despite these favorable weather conditions, high prevalence of vitamin D deficiency has been reported in Pakistan (28). Inadequate exposure of sun (UVB) and or vitamin D supplements may affect vitamin D status (29). Pakistan is among the tropical countries where sun is drenched and possibility for the production of vitamin D through UVB throughout the year is higher. Nevertheless, National Nutritional Survey of Pakistan (30) shows that Pakistan is also a victim of high prevalence of vitamin D deficiency. Sunlight exposure provides 90-95% of vitamin D, but depends on latitude, altitude

and pollution. Our finding confirmed that University students aged 20-25 years who were in habit of exposing their body to sun were able to experience optimum levels of 25(OH)D (34 ng/ml) as compared to those observing veil with little or no exposure to sun having 25(OH)D up to 22 ng/ml (Table 2).

The correlation was tested among the parameters to evaluate the influence of one parameter on the other one. The skin color showed negative correlation with the veil (p<0.123, r=–0.201), dietary conditions (p<0.508, r=–0.087) and sun exposure (p<0.082, r=–0.226) but weak correlation was found with disease conditions (p<0.029, r=0.334). Other groups i.e., veil, dietary and sun exposure have highly significant (p<0.001) positive correlation with each other but disease condition showed the negative correlation behavior with veil (-0.186), dietary habits (-0.337) and sun exposure (-0.256) (Table 3).

Conclusions

VDD remains to be a global issue of public health significance. Resource constrained economies are more inflicted with vitamin deficiency owing to a variety of reasons important being poverty, lack of awareness and poor health facilities. Literature confirm high prevalence of VDD in Pakistan that distresses all population segments. Reports are available to demonstrate VDD to be extremely pervasive among various population groups representing skin pigmentation, sex, age, socioeconomic status, urban and rural folk, veil and non-veil observing females and those consuming vitamin D deficient diets. However, little was known about the magnitude of the prevalence of vitamin D among educated young females in Pakistan. Our study on FUS's validated that awareness might have some role to overpower vitamin deficiency as tested groups for vitamin D status with limiting factors like dark skin, veil observance, diseased conditions, restricted sun exposure and consumption of diet poor in vitamin have insufficient levels of 25(OH)D suggesting that education and awareness might be an instrumental determinant for maintaining optimal vitamin D levels. It seems imperative that public is to be educated on the role of vitamin D in health to avoid the complication of vitamin D insufficiency and deficiency by exploiting optimum sunlight exposure and following life style changes to avert nutritional deficiencies.

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Table 3. Correlation among groups female university students aged 20-25 years based on various parameters to assess serum 25(OH) D levels (ng/ml)

		Skin Color	Veil Status	Dietary Status	Sun Exposure	Disease Status
Skin Color	Pearson Correlation	1	-0.201	-0.087	-0.226	0.290
	Sig. (2-tailed)		0.123	.508	.082	0.069
Veil Status	Pearson Correlation	-0.201	1	0.787**	0.679**	-0.186
	Sig. (2-tailed)	0.123		0.0001	0.0001	0.252
Dietary Status	Pearson Correlation	-0.087	.787**	1	0.712**	-0.337*
	Sig. (2-tailed)	0.508	0.0001		0.0001	.034
Sun Exposure	Pearson Correlation	-0.226	0.679**	0.712**	1	-0.256
	Sig. (2-tailed)	0.082	0.0001	0.0001		0.111
Disease Status	Pearson Correlation	. 0.290	-0.186	-0.337*	-0.256	1
	Sig. (2-tailed)	0.069	0.252	0.034	0.111	

*** Correlation is significant at the 0.01 level (2-tailed).*

** Correlation is significant at the 0.05 level (2-tailed).*

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