Original Article

VITAMIN-D STATUS IN A POPULATION OF HEALTHY ADULTS IN PAKISTAN

Khalid Mahmood[1], Syed Tehseen Akhtar[2], Abu Talib[3], Iftikhar Haider[4]

ABSTRACT

Objective: To determine the prevalence of hypovitaminosis D and its predictors in healthy adult Pakistanis. This study was conducted at various hospitals in Karachi from April 2007 to September 2007.

Methodology: In this study 244 healthy adults 16-62 years of age, visited hospital as an attendant of the patients and fulfilled the inclusion and exclusion criteria has been enrolled. After taking written consent a questionnaire regarding age, gender, occupation, duration of sun exposure, area of skin exposed, type of residence used, clothing and dietary habits were recorded. Serum 25-OH Vitamin D3 levels were determined by electrochemiluminescence method and Vitamin D deficiency was defined as a level <20µg/ml. serum calcium. Phosphorus and Alkaline Phosphatase were also measured in all of these subjects.

Results: Among 244 subjects ranging from 16-62 years, 193(79%) were female. Subjects were predominantly married (72%), mostly residing in apartments (47.5%) and most of them (41.8%) only exposed their face and hands while outdoor. Duration of sun exposure in majority was 1-2 hour /day (42%). Majority used clothes of variable colour (72%) and fabric (41%). One hundred and eighty six (76.2%) subjects had deficiency of Vitamin D and significantly correlated with duration of sunlight exposure, large area of skin exposed, vitamin D in diet consumed and colour of clothes worn. Vitamin D was significantly correlated negatively with serum Phosphorus and Alkaline Phosphatase whereas serum calcium correlated positively.

Conclusion: Prevalence of hypovitaminosis D among healthy Pakistanis is high and duration of sun exposure is the most common predictor of hypovitaminosis D.

KEY WORDS: Hypovitaminosis D, Healthy subjects, Risk factors.

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1. Prof Khalid Mehmood
Prof of Medicine
2. Dr.Syed Tehseen Akhtar
Senior Registrar Medical Unit IV
3. Dr. Abu Talib
Associate Prof of Medicine
4. Dr.Iftikhar Haider
Assistant Prof of Medicine
1-4: Medical Unit IV
Dow University of Health Sciences,
Karachi – Pakistan.

Correspondence

Dr. Syed Tehseen Akhtar
E-mail: drstakhtar@yahoo.com

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INTRODUCTION

Epidemiological studies suggest that lower vitamin-D {25(OH) D} status is associated with a higher risk for osteoporotic fractures[1] and malignancy of breast, colon, ovary and prostate.[2-4] Studies have reported that 25(OH) D deficiency is an unrecognized entity endemic in both children and adults in the United States.[4] Once it was thought that vitamin D deficiency was a rarity in south Asia.[5] In year 2000 a study conducted in healthy subjects in Delhi showed a prevalence of hypovitamin D to be 90 per cent[6] and subsequently a number

of studies from different parts of India showed widespread vitamin D deficiency in Indians of all age groups.[7-9]

Furthermore multiple studies in different countries showed high prevalence of vitamin D deficiency in Asian population.[10-18] Little is known about the status of 25(OH) D in local population. There is no published data regarding the prevalence of vitamin D insufficiency in healthy adult local population. Because of the health risks associated with low levels of vitamin D, the primary objective of this study was to test the hypothesis that vitamin D deficiency (250HD level, <20 ng/mL)[19-22] was prevalent among apparently healthy asymptomatic adult population.

The secondary objective was to identify factors within adult lifestyle that represent predictors for hypovitaminosis D in the study participants.

METHODOLOGY

A cross-sectional multicentre study was conducted in different hospitals of Karachi, Pakistan from April, 2007 to September, 2007. Karachi is located at 24° N latitude and 67° E longitude with abundant sunshine throughout the year. There is no difference in sunshine during the period of field study that could substantially affect vitamin D status. In this study two hundred forty four (244) healthy adult subjects 16-62 years of age who came as attendants to their hospitalized patients were recruited.

The exclusion criteria were:

* Subjects with any signs and symptoms of vitamin D deficiency such as unexplained musculoskeletal pain and aches, generalized bone pain, bony deformities/tenderness, proximal myopathy.
* History of thyroid, parathyroid, adrenal or gonadal disease.
* History of any metabolic bone disease
* Malignancy
* Hepatic and Renal disease
* Malabsorption syndrome or history of gastrointestinal resection, chronic diarrhea.
* Hysterectomy
* Diabetes Mellitus
* History of taking medicines which affect the vitamin D status e.g. calcium supplements, vitamin D supplements, Antiepileptics, estrogen and progesterone.
* Pregnancy and lactation
* Immobility for more than one-week.

A questionnaire was administered to determine the age, sex, occupation, details of duration of exposure to sun light in previous month, area of skin exposed, type of residence used to live, clothing and dietary habits.

Fasting blood samples were drawn in the morning by disposable syringes through venepuncture and a maximum of 5ml of blood was taken. The serum was separated after complete centrifugation of blood samples within two hours of venepuncture, collected in tubes and preserved at a temperature of 20 ^{o}C until analyzed.

The serum 25-OHD levels were used to evaluate the vitamin D status. The serum 25-OHD concentrations were measured by Electrochemiluminescence method from Agha Khan University Lab. The reference range for 25-OHD was = or > 30 ng/ml as normal, vitamin D deficiency was defined as serum 25-OHD levels < 20 ng/ml while a level between 20.1-29.9 ng/ml was defined as insufficiency. The serum levels of calcium, phosphate and alkaline phosphatase activity were measured using routine laboratory methods to get additional information about vitamin D status. The food composition database used in Pakistan does not contain vitamin D. Furthermore study subjects were not able to recall quantity of vitamin D containing foodstuff taken by them. Due to these limitations exact estimation of dietary intake of vitamin D was not possible.

Analysis was performed with SPSS version 15. Continuous variables were expressed as mean ±SD whereas discrete variables were expressed as percentages and analyzed by chi-square test where needed. A p-value <0.05 was considered as statistically significant. A Pearson Correlation analysis between Vitamin D and other variables was done and p-value <0.01 was considered statistically significant.

RESULTS

The prevalence of hypovitaminosis D among healthy subjects was high. Characteristics of subjects and descriptive statistics are shown in Table-I & II respectively. Subjects ranged in age from16-62 years with a mean of 33.62±12.64 years. Among 244 subjects 193(79%) were female. Subjects predominantly were married 177(72.5%), mostly residing in apartments (47.5%) and when outdoor most of them only exposed hands and face (41.8%). Duration of sun exposure in majority was 1-2 hours (42%). Majority of subjects used clothes of variable fabrics (41%) with variable colors (72%).Regarding dietary habits most of the participants consumed food poor in vitamin D content 218(89.3%) whereas 26(10.7%) subjects had adequate amount of food rich in vitamin D content. One hundred eighty six (76.2%) subjects of the study population had deficiency of vitamin D (<20ng), 36 (14.8%) has insufficiency (>20<30) and 22 (9%) has normal level of vitamin D (Table-III). Statistically there was no difference between male and female regarding vitamin D level (Table-IV).

Table-V Shows the correlation between serum 25 (OH) Vitamin D levels and clinical and laboratory variables. Vitamin D levels were not significantly correlated with age, marital status, gender difference, type of residence and type of cloth fabrics whereas there were significant correlation between vitamin D and longer duration of sunlight exposure, area of skin exposed, vitamin D content in diet and color of clothes. Among the laboratory variables vitamin D significantly correlated negatively with serum phosphorus and alkaline phosphatase whereas serum calcium correlated positively.

Table-I: Basic characteristics of study subjects (n=244)

Gender		*n (%)*
	Male	51(20.9)
	Female	193(79.1)
Married	Yes	177(72.5)
	NO	67(27.5)
Vitamin D in diet	Low	218(89.3)
	Adequate	26(10.6)
Residence	Covered Houses	79(32.4)
	Houses with open Space	49(20.1)
	Apartments	116(47.5)
Area of skin exposed	Whole body Covered	30(12.3)
	Face Exposed	85(34.8)
	Face & Hands Exposed	102(41.8)
	Face, Hands & Forearm exposed	27(11.1)
Duration of sun exposure	0 hour / d	13(5.3)
	<1hour /d	88(36.1)
	1 - 2 hour /d	103(42.2)
	>2hour /d	40(16.4)
Color of Clothes	Black	51(20.9)
	White	17(7.0)
	Others	176(72.10
Type of Fabrics	Synthetic	16(6.6)
	cotton	40(16.4)
	Mixed	88(36.1)
	Variable	100(41)

Table-II: Descriptive statistics (n=244)

	Minimum	*Maximum*	*Mean ± S.D*
Age of patient (Years)	16	62	33.62 ± 12.64
Serum Vitamin D (mg/ml)	4	60	15.65 ± 9.91
Serum Calcium (mg/dl)	7.4	10.9	8.82 ± 0.66
Serum Phosphate (mg/dl)	2.3	6.5	3.81 ± 0.69
Serum Alkaline phosphate (1.U/L)	37	375	143.61± 77.13

Table-III: Vitamin d levels (n=244)

Vitamin D Level	*Frequency(%)*
Insufficiency	36(14.8)
Deficiency	186(76.2)
Normal	22(9.0)
Total	244(100.0)

DISCUSSION

Prevalence of Hypovitaminosis D was surprisingly high (76%) among healthy subjects in Pakistanis residing in Karachi despite abundant sunlight throughout the year. This result is endorsed by many previous studies[10-12,18,23-28] in countries having adequate sunshine. Turkey, India, Lebanon, Jordan, China, Saudi Arabia, Iran and Tunisia have high prevalence ranging from 44-95%. In some of these studies the high prevalence of hypovitaminosis was attributed to dietary factors,[12,18,24] multiparity,[24,28] clothing factor,[12,24] limitation and avoidance of sunlight exposure[28] and air pollution[10] which prevents enough Ultra Violet B rays penetration.

In local population avoidance of sun light due to fear of darkening of skin and covering of whole body religiously or only exposing face and hands traditionally especially in female subjects when going outdoor were the main attributing factors. Male subjects also avoid sun exposure not only due to high temperature in summer season in this part of the world but also have misconception regarding harmful effects of sunlight and unawareness regarding the source of Vitamin D. Effect of sunlight on Vitamin D status has been well documented and confirms the importance of sunlight exposure in the synthesis of vitamin D[29,30]

Table-V: Correlation of serum 25(OH) Vitamin D with clinical and lab variable (n=244)

Variable	*r*	*p*
Age	0.023	0.725
Gender	-0.104	0.105
Duration of sun Exposure	0.34	<0.001
Area of skin Exposed	0.564	<0.001
Vitamin in diet	0.565	<0.001
Type of fabrics	0.090	0.161
Colour of fabrics	0.333	<.001
Type of Residence	0.057	0.372
Serum Calcium	0.28	<0.001
Serum Phosphorus	-0.24	<0.001
Serum Alkaline Phosphatase	-0.32	<0.001

Sunlight exposure was the most important determinant of Vitamin D levels in the study population. Area of skin exposed and duration of sunlight exposure strongly correlated with Vitamin D levels in this study as shown similarly by other studies.[10,12,27,28,31] As dark skin requires more sun exposure than less pigmented skin to produce similar amount of vitamin D[30] it is not surprising that hypovitaminosis D is so prevalent in this country.

Dietary factor was another determinant of the study regarding high prevalence as most of our participant were consuming low amount of vitamin D rich food True estimation of vitamin D intake was not possible owing to unawareness regarding quantity of consuming diet by participants and unavailability of food composition database for Vit D used in Pakistan. Furthermore there is no Vitamin D food fortification in Pakistan and no history of use of calcium and Vitamin D supplements among study subjects were also contributing in high

Table-IV: Vitamin D level. Male and Female difference. (n=244)

		Gender		*P-value*
		Male	*Female*	
Vitamin D level	Insufficiency	10 (19.6%)	26 (13.5%)	0.267
	Deficiency	36 (70.6%)	150 (77.7%)	0.283
	Normal	5 (9.8%)	17 (8.8%)	0.826
	Total	51 (100%)	193 (100%)	

prevalence but some previous studies[10,25] showed no such association whereas other studies[27,28] showed significant correlation.

Type of fabric has shown no significant correlation in study population whereas colour of the cloth has. It has previously been found that Ultra Violet rays are attenuated most by black wool and least by white cotton.[36] In this country a significant percentage of women wear black clothes traditionally for pardah while outdoor.

Air pollution is another attributing factor[10]of hypovitaminosis D. Karachi is one of the highly polluted city of the world so the pollution could be an attributing factor in study subjects but this was beyond our study scope to evaluate pollution effects.

Finally it is concluded that local healthy population also has a high prevalence of vitamin D deficiency and the main contributing factor is limited sun exposure due to avoidance of sunlight and covering of the whole body except face and hands while outdoor. Other factors may also play a minor role. To combat this situation it is recommended to create awareness to increase sunlight exposure and high intake of vitamin D rich food at mass level and starting of Vitamin D food fortification program at government level.

Limitation of the study: We had several limitations. Study was done at one point of time. We were not able to calculate the daily dietary intake of vitamin D due to several reasons. Duration of sun exposure was based on recall rather than actual. We also ignored the overcast and rainy days during study period. Furthermore we did not check the serum Paratharmone levels due to limitation of resources.

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