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VITAMIN D STATUS;
THE EPIDEMIOLOGY IN SARGODHA

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ABSTRACT… Objective: Our primary objective was to estimate the vitamin D status among both the rural and urban dwellers of Sargodha. Study Design: A cross sectional population survey. **Place and Duration of Study:** Individuals were selected from six different medical centers in the Sargodha city, Vitamin D measurement was done in Khan Lab Sargodha from January 2013 to June 2013. **Methodology:** After an awareness campaign, healthy individuals and medical patients from six different clinics in Sargodha city were selected after excluding the diseases that interfered with the metabolism of calcium and vitamin D. The initial assessment involved an interview based questionnaire, at the clinic by the treating doctor. It recorded the information regarding age, sex, weight, residence, and co-morbid conditions like, DM, hypertension, ischemic heart disease and kidney disease and medications especially oral vitamin D supplementation. The physical assessments included height, weight record and blood pressure measurements. **The test:** It was performed in Khan Lab Sargodha. Quantitative determination of 25-OH vitamin D was done from the serum by using competitive immunoluminometric assay on Maglumi 1000 fully automated chemistry analyzer. **Results:** Out of total 100 specimens 46 were found to be having low levels of vitamin D levels. Insufficient levels (21-29 ng/ml) were present in 10 individuals. It was less than 10 ng/ml in 11, less than 5 ng/ml in 4, while 21 individuals were having levels between 10-20 ng/ml. Five individuals had levels more than 100 ng/ml. None of the individuals had level in toxic range. Out of 56 females 27(48.22%) had low vitamin D levels, while out of 44 males 19 (43.2%) were found to be having levels less than 30ng/ml. The median age of individuals having levels less than 30ng/ml was 37 years (14-57 years). The relationship of high BMI and vitamin deficiency was statistically non-significant (p-value = .282). **Conclusions:** Although the sample size is small, however our study reveals that a large proportion (46%) of studied individuals representing the asymptomatic general population is having low level of vitamin D. in order to address this public issue concrete measures need to be taken in order to prevent adverse consequences of low vitamin D levels.

Key words: Vitamin D, insufficiency, Sargodha,

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INTRODUCTION

Sufficient levels of vitamin D are required for calcium homeostasis, bone health and development. Vitamin D deficiency leads to rickets and osteomalacia, in pediatric and adults respectively[1]. Its deficiency in adults leads to the development of osteopenia and osteoporosis due to induction of secondary hyperthyroidism[2].

Vitamin D is recognized not only for its importance of bone health and calcium homeostasis in human body, but also for other health benefits. Its deficiency has been associated with fibromyalgia and non specific and unexplained aches and pains[3,4].

Low serum level of vitamin D has been associated with multiple co-morbidities like Infectious diseases[5], otitis media[6] tuberculosis[7], depression in adults and depressive symptoms in youth with cystic fibrosis [8,9,] Alzheimer's disease and Parkinson's disease[10], as a predictor of cardiovascular disease in obese and overweight patients[11]. It has been suggested that hyperinsulinemia and/or insulin resistance are directly responsible for decrease of 25(OH)D levels in obesity[12].

Vitamin D deficiency has been associated with increased risk of common cancer, autoimmune diseases, hypertension, and infectious diseases[13].

Vitamin D receptors are present in various immune cells, such as antigen –presenting cells, T-cells, B-cells and monocytes and have beneficial effects on immune function, particularly autoimmune diseases like type 1 diabetes mellitus[14].

The status of vitamin D is evaluated through the measurement of 25-hydroxy vitamin D [25(OH)D] levels in the body. Its half life is approximately 2-3 weeks. It is a summation of both vitamin D intake and vitamin D that is produced from sun exposure. Although the reference laboratories are able to quantitatively measure 25(OH)D2 and 25 (OH) D3, however for all practical purposes physicians need to know the total 25(OH)D which is a summation of 25(OH)D2 and 25 (OH)D3[15,16,17].

The measurement of its active metabolite vitamin D 1, 25(OH) is not preferred as its circulating levels are a thousand fold less than 25(OH)D. and its levels are influenced by the parathyroid hormone. Further circulating half life of 1, 25 (OH) D is only 4-6 hours[15].

Regarding the normal level of serum 25(OH) vitamin D level, we have followed the guide lines of the endocrine society clinical practice[17].

A level of less than 20 ng/ml (< 50nmol / l) is considered to be deficiency where as a level between 21 to 29 ng/ml (52 – 72 nmol / l) is defined as a state of insufficiency. While serum levels greater than 30 ng /dl (> 75 nmol/l) are considered as sufficient (normal) for children and adults. The upper limit of normal has also been questioned. The professional exposed to prolonged sunlight life lifeguards typically have levels of 100-125 ng/ml, however vitamin D intoxication from sun exposure has never been reported. Vitamin D intoxication is defined as a 25 (OH) D > 150 ng/ml that is associated with hypercalcemia, hypercalciuria, and often hyperphosphatemia[15,16,17].

The low level of vitamin D is wide spread in both sexes and in all age groups. There are multiple studies in different countries regarding the prevalence of vitamin D deficiency. These studies showed high prevalence of vitamin D deficiency in Asian countries. Both children and adults in United Arab Emirates, Australia, Turkey, India, and Lebanon have been found to have vitamin D deficiency,[18] and its prevalence appears to be increasing[19]. A high prevalence rate of vitamin D deficiency has been observed in the young otherwise healthy medical students[20].

Prevalence of severe, moderate and mild Vitamin D deficiency was 9.5%, 57.6% and 14.2% respectively in study conducted in Tehran[21]. When serum level of 20ng/ml was used as cut off 78.3% of a local hospital staff in India was found to be deficient[22].

A very high prevalence rate of low vitamin D levels (< 30ng/ml) has been reported in Pakistan. A study conducted in a tertiary care hospital showed deficiency in 69.9% while 21.1% had insufficient levels of 25OHD in apparent healthy individuals[23].

Another study in asymptomatic ambulatory patients of endocrinology outpatient services revealed that in 92% had low serum 25OHD[24].

A cross sectional population survey conducted at two different densely populated areas of Karachi showed vitamin D deficiency in 84.3% of healthy individuals[25].

Most of the published studies in Pakistan had been done in urban areas of Karachi. Our primary objective was to assess the status of vitamin D in both urban and rural areas of Sargodha.

MATERIAL AND METHODS

This was a cross sectional study conducted on randomly selected individuals at 6 clinics located in different parts of the Sargodha city.

Awareness posters, briefly describing the significance of this test were displayed at these selected clinics which were run by different doctors including general practitioners as well as specialists.

Only those individuals who volunteered were enrolled. Informed consent was taken from all those who participated in the study.

The idea was to select the individuals representing all fractions of the population with diverse socioeconomic background of Sargodha city and its surroundings. These included both the healthy persons as well patients suffering from co-morbid conditions not specifically related to vitamin D.

The criteria of exclusion included the non-willing individuals, Patients suffering from diseases that interfered with the metabolism of calcium and vitamin D, taking vitamin D supplements, anticonvulsants, or steroids. Nursing mothers, bed ridden patients and Patients suffering from any malignancy.

After taking the consent, the initial assessment involved the filling of a questionnaire, at the clinics by the doctor. Detailed clinical examination along with the previous investigations was reviewed to evaluate the diseases that interfered with the metabolism of calcium and vitamin D.

Data regarding age, sex, height, weight, resident of, and co-morbid conditions including diabetes mellitus, hypertension, non specific fibromyalgia, backache and sciatica were recorded. Body mass index (BMI) was calculated by dividing the weight in Kg by the square of the height in meters.

The individuals were then referred to Khan lab Sargodha where blood sample was collected and quantitative determination of 25-OH vitamin D was done from the serum by using competitive immunoluminometric assay on Maglumi 1000 fully automated chemistry analyzer.

DATA ENTRY AND ANALYSIS

The data collected was entered in a predesigned proforma indicating age and sex of the patient, BMI, serum levels of Vitamin D. Depending their residential address they were divided in two groups urban and rural. Similarly depending upon

Sample characteristics	Serum 25(OH) Vitamin D status Deficiency (n = 36)	Insufficiency (n = 10)	Sufficiency (n = 56)	Toxic levels (n=0)	p-value
	< 20 ng/ml	21-30ng/ml	>30 ng > 30 - <150 ng/ml	> 150ng/ml	
Age (years)	35(19-54)	37(14-57)	38 (20-56)		.147
Gender					.312
Male (n=44)	14 (31.8%)	5(11.4%)	25 (56.8%)		
Female(n=56)	22(39.3%)	5(8.92%)	29 (51.78%)		
Median vitamin D levels (ng/ml)	12.705 (1.83-19.48)	25.105 (22.95-29.04)	55.375 (30.24-126.54)		.084
BMI(Kg/m2)	24.943 (19.975-33.302)	25.246 (16.326-32)	24.818 (20.829-33.563)		.282
Residence				0%	.312
Rural (n=44)	14 (31.8%)	5(11.4%)	25 (56.8%)		
Urban (n=56)	22(39.3%)	5(8.92%)	29 (51.78%)		
Working class				0%	.121
House wife/ unmarried girls (n= 47)	19(40.42%)	5(10.64%)	23(48.94%)		
Office worker(n=27) Male (n=12) Female (n=15)	12(44.44%) 5(18.52%) 7(25.92%)	5(18.52%) 2(7.41%) 3(11.11%)	10(37.04%) 7(25.93%) 3(11.11 %)		
Field worker(n=26)	5(19.23%)	0%	21(80.77%)		

Table-I. Vitamin D groups and other characteristics their profession they were divided into groups as office workers, field workers or house wives. The data was analyzed using SPSS (Statistical Package for Social sciences) version 20.

The variable of serum vitamin D level was divided into categories of deficiency (< 20 ng/ml), insufficiency (21 to 29 ng/ml), sufficiency (> 30 ng/ml) and toxic levels (> 150 ng/ml). Chi Square test was used to find out any associations between groups of vitamin D levels and other categorical variables. A P-value of <0.05 was taken to be statistically significant.

RESULTS

Analysis of our sample size of 100 reveled 56 females and 44 males. The median age of the sample was 36 years (median age 36.8 years). The minimal age in the sample was 14 years while the maximum age was 58 years. The individuals came from diverse socioeconomically background, 44 % were from rural suburbs of Sargodha while 56 % were dwellers of the urban area of the Sargodha. The majority of females (47%) were house wives/unmarried girls, while out of 27 (27%) office workers 15 were females. The field workers were 26%, they were all males. 12 males were office workers.

The median serum level of 25 (OH) vitamin D was 32.27 with sample vitamin D levels ranging from 1.83 to 126.54 ng/ml. A total of 46 % had low (< 30ng/ml) levels of vitamin D. Vitamin D deficiency, insufficiency and sufficiency have been summarized in table-I. Deficiency group comprises of 36 individuals (36%), 14 males and 22 females and had level less than 20ng/ml. The insufficiency level of vitamin D, between 21-29ng/ml, was found in 10 individuals (10%) with equal male to female ratio. A total of 54 individuals (54 %) had level more than 30 ng/ml, males were 25 and 29 females. None of the individuals were found to be in toxic range (more than 150ng/dl).

In term of gender, the median serum vitamin D level in males was 32.27 ng/ml, while in females it was 30.24ng/ml.

While considering the deficiency state, taking cut off level of vitamin D less than 30 mg/ml, the difference in the males 19 (43.2%) and females 27 (48.22%)was statistically non significant (p value = .312). The effect of the residence, rural 19 (43.2%) and urban 27(48.22%) was also not significant (p value = .312). those who were found to be deficient the effect of the job was also not statistically significant, 24(51.06%) house wives or unmarried girls, 17(62.96%) office workers while only 5 (19.23%) field workers (p value = .121).

	BMI		**(p-value = .282)**
	< 25	25-30	>30
Deficiency (n = 36)	19(36.53%)	14(35%)	3(37.5%)
Insufficiency (n = 10)	4(7.7%)	5(12.5%)	1(12.5%)
Sufficiency (n= 54)	29(55.77%)	21(52.5%)	4(50%)

Table-II. BMI and vitamin D level

N	Valid	100
	Missing	0
Mean		40.4973
Median		32.2700
Std. Deviation		30.46217
Minimum		1.83
Maximum		126.54

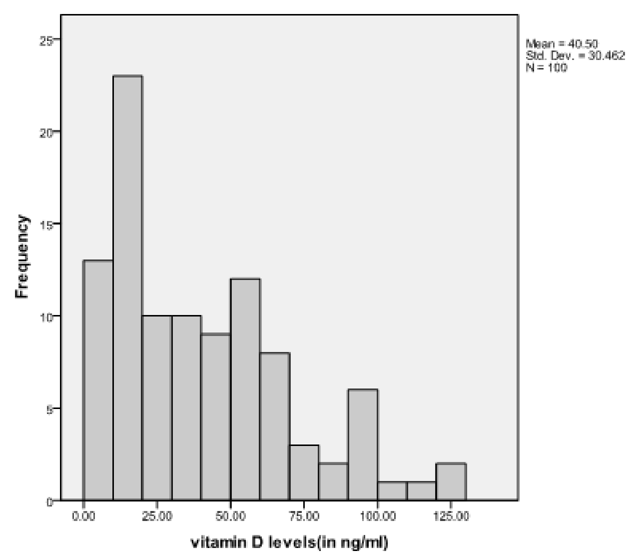

Fig-1. Frequency distribution of vitamin D

The mean MBI among males was 25.29 with sd 2.93 while females had mean BMI of 25.27 with sd 3.19. The relationship of high BMI and vitamin deficiency was statistically nonsignificant. (p-value = .282), Fig 2.

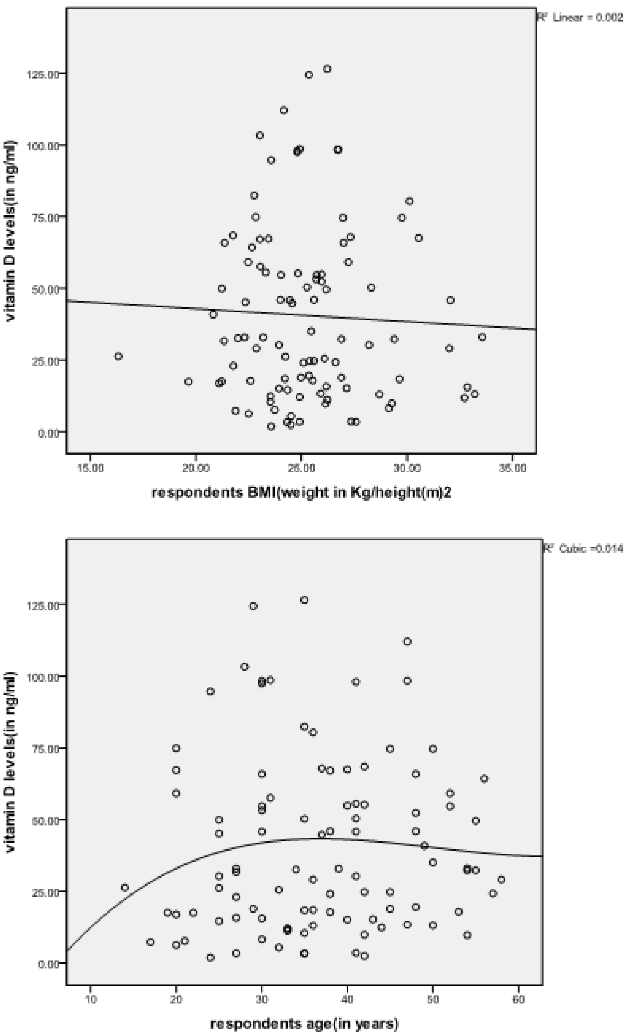

Fig-2. Scatter plot between vitamin D and BMI

DISCUSSIONS

Vitamin D deficiency is a worldwide problem; it is present in healthy individuals of all age groups, and in both males and females13,15. Subclinical vitamin D deficiency is extensive in the adult ambulatory care patients[24,25,26].

Over study reveal low levels of vitamin D (< 30 ng/ml) in 46% of the population studied. This finding is very significant considering the fact that our study sample comprise of asymptomatic healthy adults in the community. It is also common even in the populations of sunniest countries[20].

In Pakistan a prevalence of 84.3% (sample size 300) of low vitamin D has been reported recently among healthy population from Karachi[25].

A high prevalence of 92% (sample size 119) and 90% (sample size 123) was reported by Zuberi et al and Mansoor et al respectively. Both these studies were conducted at Karachi on apparently ambulatory patients. Our study reveal low incidence (46% with sample size of 100) of vitamin D deficiency as compared to above studies. The above reported variation in prevalence can partially be explained by the difference in study settings. Both the above mentioned studies were conducted in a tertiary care hospital setting, while our selection of patients involved multiple small clinics and healthy volunteers. Differences in dietary habits or exposure to sun light may be responsible for the differences.

Similarly a study conducted only on female patients with fibromyalgia shows a prevalence of 80% (sample size 40)3. Again the incidence of vitamin D deficiency in our female patients is 48.22 % (sample size 56). In our study the selection of the female patients was not confined to fibromyalgia symptoms only. This could explain the difference in the observed deficiency levels of vitamin D.

In Pakistan all the above mentioned studies were conducted in Karachi. Could differences in consumption of amount of dairy products in Karachi and Sargodha or exposure to sun light be responsible for this difference?

In our study 36% of the individuals had vitamin D deficiency (< 20 mg/ml). Again this figure is lower than the 79.6%, 78.3% and 57.7% prevalence of vitamin D deficiency reported in studies on general asymptomatic population in Karachi, Tehran and India respectively[21,22,25]. The difference in sample size, exposure to sun light, dietary habits, the time of the year when blood samples were collected and, possibly, the different laboratory methodologies used.

The result from these studies is evident that there is definite wide spread vitamin D deficiency, although the proportion of population affected varies.

The result of our study, that 46% of general population has insufficient vitamin D levels, is a great concern since it points toward the future skeletal health of these individuals. The 25(OH) concentration is an independent determinant of peak bone mass and that adequate vitamin D levels can prevent osteoporosis related hip fractures.

Obesity is considered as one of the risk factors for low vitamin D levels. Sequestration of vitamin D into fat has been postulated to be a possible mechanism. Obesity has been associated with low vitamin D levels in Saudia, Europe and the USA27,28,29,30. Our study results do not directly correlates the BMI and the obesity. The relation of low vitamin D and obesity was not statistically significant (p-value = .282) and Figure 2 and table II. vitamin D levels(in ng/ml)

CONCLUSIONS

Our study reveals that 46% of the asymptomatic general population is having low level of vitamin D. Due to potentially serious and long term nature of health sequelae associated with low levels of vitamin D, an urgent action must be undertaken to implement the rules and regulations of food fortification. Further both health professionals and public needs to be educated in regards to benefits of vitamin D supplementation.

Limitations of the study

1. Small sample size, financial constraints were the main reason. Increasing sample size with inclusion of more pockets of population would help in generalize ability.
2. A single sample of blood has been used for analysis, and the study period extends from winter to summer. A longitudinally designed study with serial blood sampling performed in both winter and summer months is recommended

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