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Evaluating the status of vitamin 25(OH) D levels among females of all age groups in Karachi, Pakistan.

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Abstract:

Introduction: Vitamin D plays an important role in growth, metabolism and reproduction. Deficiency of this vitamin is highly prevalent globally and leads to various disorders besides different skeletal deformities. Women of different reproductive ages are at higher risk of developing bone diseases because of low vitamin D levels.

Objective: This study aimed to assess the prevalence of vitamin D deficiencies in females of different ages in Karachi, Pakistan

Methodology: A retrospective investigation of vitamin D levels during a six month period at the Clinical Lab, Al-Khidmat Diagnostic Center, Karachi; Pakistan was performed. Serum vitamin 25(OH) D levels of 1035 female were measured by Electrochemiluminescence (ECL) assay. The data was analyzed through SPSS version 16.

Results: Among 1035 participants of different reproductive ages, 26.1% (n= 270) showed severe vitamin D deficiency, 44.3% (n=458) displayed mild to moderate Vitamin D deficiency while 29.7% (n=307) were having normal levels of serum vitamin D.

Results: The data of 1035 subjects was examined and the mean serum vitamin 25 (OH) D levels of the studied subjects were found as 21.04±14.13 with the minimum and maximum range of 4.97ng/ml to 70.71ng/ml

Conclusion: 70.4 % of our studied population had lower levels of vitamin D representing that the majority of Pakistani womenfolk have vitamin D Deficiency.

Keywords: Vitamin 25 (OH) D levels, Female population, different age, Karachi

Introduction:

Vitamin 25(OH) D is an essential steroid and hormone precursor that is vital for bone metabolism, growth differentiation and its mineralization.¹ Vitamin 25(OH) D can be obtained from diet by using fatty fish and from dairy products fortified with vitamin 25(OH) D.² It can also be obtained by ultraviolet light emitted by sun which photochemically transforms 7-dehydrocholesterol a provitamin D₃ in to pre-cholecalciferol which is pre-vitamin D₃ in the skin, which is further converted to vitamin D₃ or in different products.^{1,3,4} Vitamins D₃ undergoes hydroxylation, initially in liver and then in the kidneys and is converted into the storage form 25-hydroxyvitamin D (25OHD) and then to its active form 1,25dihydroxyvitamin D respectively.^{5,6}

Vitamin 25(OH) D plays important role in the absorption of minerals like calcium and phosphorus through intestine and also modulates the release of parathyroid hormone by inhib-

iting it, consequently helps in maintaining the bone density.⁷ Recent research shows the association of vitamin 25(OH) D deficiency with the increased risk of type 1 Diabetes, multiple sclerosis, rheumatoid arthritis, hypertension, cardiovascular diseases and cancers.^{8,9} Many factors which are associated with vitamin 25 (OH) D deficiencies are geographic location, atmospheric conditions, length of time spent in sunlight, type of clothing, and high melanin production in skin also affect the vitamin 25(OH) D absorption, poor diet and age.¹⁰ The severe deficiency of Vitamin 25(OH) D can be controlled by dietary and parenteral vitamin 25(OH) D supplementations which are effective in improving bone density thereby reduce the morbidity & mortality.¹¹

Deficiency or insufficiency of vitamin 25(OH) D is a common problem faced globally not only by children but also observed in adult male and females.^{12,13} Around 50% of world population is suffering from vitamin 25 (OH) D insufficiencies which

is based on vitamin 25(OH) D (25-hydroxy vitamin 25(OH) D) levels below 20 ng/ml. It has been estimated that globally around 1 billion people of different ethnicities and age group are having deficiency of vitamin 25(OH) D.^{14,15} In Asian countries, especially Pakistan I population facing vitamin 25(OH) D deficiency and around 53.5% of its population is vitamin 25(OH) D deficient including individuals at extremes of age.¹⁶ Pakistan is located at the sub-tropical region and despite having sufficient sunny climate its population is becoming severely vitamin 25(OH) D deficient especially women of all ages from reproductive to post-menopausal age. A study reports that 90% of pre-menopausal females have serum concentrations of vitamin 25(OH) D below 20ng/ml.¹⁷ Normal vitamin 25(OH) D levels are essential for women during reproductive age because osteoporosis is commonly seen in females who have repeated pregnancies and nurse their babies. Maternal Vitamin 25(OH) D deficiency affects fetal development that would culminate in deficiency states in their next generation.¹⁸

Objective:

Because of the importance of vitamin 25(OH) D levels and lack of scientific literature on vitamin 25(OH) D status in women of different age groups in Pakistan, we conducted this study to determine the serum vitamin 25(OH) D levels (25-hydroxyvitamin 25(OH) D) among females of different age groups in Karachi, Pakistan.

Methodology:

In this retrospective study, 1035 females of different age groups ranged from 13 to 90 were randomly included, who were referred by physicians for investigation of serum vitamin 25(OH) D levels to the Clinical Lab, Al-khidmat Diagnostic Center Karachi, Pakistan over a period of 6 months from September 2014 to February 2015. Serum levels of vitamin 25(OH) D were measured by Electrochemiluminescence immunoassay (ECLIA) method on E-170 Modular immunology analyzer (Roche, Germany) having detection limits of 3.00 - 70.00 ng/mL. QC was determined by running normal and high level standards. Study subjects were categorized into 3 groups based on serum concentration of vit D: (1) severe vitamin 25(OH) D deficient [25(OH) vit D < 10 ng/mL], (2) mild to moderate vitamin 25(OH) D deficient [25(OH) vit D ≥ 10-24.9 ng/mL] and (3) normal serum vit D [25(OH) vit D ≥ 25-70 ng/mL]. Subjects were also segregated into different age groups viz; <20, 21-30, 31-40, 41-50, 51-60, 61-70, >70 years.

Data was analyzed by using the Statistical Package for the Social Sciences software (SPSS), version 16.0 (IBM Corporation, Armonk, New York, USA). The results were expressed in means standard deviation and percentages. The significance of difference in means was tested by chi-square test and p-value ≤0.05 was considered as significant.

Results:

The data of 1035 subjects was examined for serum vitamin 25(OH) D levels. The mean age of the participants was 38.15±13.50 with 76% (n=789) belonging to 21-50 years age group. The mean serum vitamin 25(OH) D levels of the studied subjects were found as 21.04±14.13 with the minimum and maximum range of 4.97ng/ml to 70.71ng/ml. According to the set laboratory cutoff values, 26.1% (n= 270) of studied population exhibited severe vitamin 25(OH) D deficiency; 44.3% (n=485) presented mild to

moderate vitamin 25(OH) D deficiency and only 29.7% (n=307) out of 1035 participants were having normal vitamin 25(OH) D levels (Table-1).

Table No 1: Vitamin D level

Vitamin 25(OH) D Levels (ng/ml)	No of Participants	%
Severe Deficiency (<10)	270	26.1
Mild to Moderate Deficiency (10-25)	458	44.3
Normal (>25-80)	307	29.7

For age wise evaluation and distribution of serum vitamin 25(OH) D levels, our examined subjects were further stratified into different age groups. The comparison of vitamin levels in different age groups is represented in Table-2.

Table No 2: Vitamin D level in different age groups.

Age Group In Years	n (%)	Normal Vitamin 25(OH) D levels (n=307)	Mild to Moderate Deficiency (n=458)	Severe Deficiency (n=270)	p-value
Less than 20	76 (7.3)	19 (25)	29 (38.15)	28 (36.84)	<0.001 *
21-30	306 (29.6)	65 (21.24)	139 (45.42)	102 (33.77)	
31-40	259 (25)	71 (27.41)	115 (44.40)	73 (28.18)	
41-50	224 (21.6)	69 (30.80)	112 (50)	43 (19.19)	
51-60	111 (10.7)	56 (50.45)	37 (33.33)	18 (16.21)	
61-70	50 (4.8)	24 (48)	21 (42)	5 (10)	
Above 70	9 (0.9)	3 (33.33)	5 (55.5)	1 (11.1)	

In a group of female with <20 years of age The severe vitamin 25(OH) D deficiencies were prevailed in 21 to 30 years age group comprising of 306 individuals. 33.7% (n=102) subjects of this age group exhibited severe deficiency whereas 45.42% (n=139) were observed with mild to moderate deficiency of vitamin 25(OH) D. Sixty five (21.2%) females belonging to the same age group were having normal vitamin 25(OH) D levels. Similarly among the 259 females aged 31-40, severe deficiency was observed in 28% (n=73), mild to moderate deficiency in 44.4% (n=115) whereas normal vitamin 25(OH) D status was observed in 27.41% (n=71). In the next age group ranging from 41 to 50 years, 19.19% (n=43) were severely deficient, 50% (n=112) were mild to moderately deficient while 30% (n=69) were reported to have normal serum levels of vitamin 25(OH) D. Among 111 participants of age group 51-60, 16.21% (n=18), 33.3% (n=37) and 50.45% (n=56) were reflected severe deficiency, mild to moderate deficiency and normal levels of vitamin 25(OH) D3 respectively.

Majority of the senior females aged > 61 showed normal levels

of vitamin 25(OH) D. A statistically significant difference (< 0.01%) of vitamin 25(OH) D levels were detected among these different age groups.

Discussion:

According to health care professionals vitamin 25 (OH) D deficiencies (VDD) is still the biggest challenge faced globally. Pakistan being an underdeveloped and malnourished country, deficiency of micro and macro nutrients is leading to severe health concerns. vitamin 25 (OH) D deficiencies is also reported to be associated with different diseases like stunted growth and rickets in children, autoimmune disorders and osteoporosis in adults. Vitamin 25 (OH) D deficiencies in females is most prevalent in different reproductive ages. To date there have been several studies which have signified the role of vitamin 25(OH) D in women's health. The data suggest the association of vitamin 25(OH) D with several factors including fertility and reproductive health. Although vitamin 25(OH) D status may be influenced by environmental factors such as sun exposure and diet, different reproduction associated factors have also been associated with varied levels of vitamin 25(OH) D. According to WHO, the reproductive age group is in the range of 15-50 years, however, whether circulating vitamin 25(OH) D levels are affected by different reproductive age group is somewhat unclear. Studies with several factors of reproduction including pregnancy, menstrual cycle, PCOs and infertility have revealed varied effects. Severe vitamin 25(OH) D deficiency in younger females prone to develop higher risk in advanced age of reproductive cycle.

Our current study is concluded from a retrospective data over a period of six months carried out in Clinical Laboratory Al-Khidmat Diagnostic Centre, Karachi. It includes females of all age groups. We classified our females from puberty to menopause and after menopause with most of the women falling in the range of moderate Vitamin 25(OH) D deficiency (10-25ng/.mL) i.e 44.3 % as compared to 26.1 % of the population being severely deficient (<10 ng/.mL) and 29.7% in normal range (>25-80 ng/.mL). We found that severe vitamin 25(OH) D deficiency was significantly associated with women of fertile age (21-30 years) i.e 37.8%. On the other hand, women of age group with least fertile health (above 70 years) do not display severe vitamin 25(OH) D deficiency i.e. 0.4%. Women lying in most fertile age group i.e. the age range of 21 to 30 years, shown 37.8% of our females are having severe low levels of vitamin 25 (OH) D whereas 30.3% showed mild to moderate deficiency of vitamin 25(OH) D. By the age of 31 to 40 years, the fertility levels are decreased and at this age, 27% of our females are severely vitamin 25(OH) D deficient and 25.1% are mild to moderately vitamin 25(OH) D deficient. Significant difference in vitamin D levels with respect to age groups (p value < 0.05) was found among all the groups.

Our findings of severe deficiency of vitamin 25(OH) D levels are also supported by other investigators, a study conducted in Karachi reported that 84% of their study participants had serum 25 (OH) D concentrations < 75 nmol/L.¹⁹ A similar study conducted in 2015 Lahore, Pakistan report that 73% of their women of child bearing age were vitamin 25(OH) D deficient while 43% showed profound Vitamin 25(OH) D deficiency.²⁰ Different studies conducted in Asia reveals the high rates of vitamin 25 (OH) D deficiencies among women.²¹⁻²³ In a study conducted in India,

the levels of vitamin 25(OH) D were estimated in women of reproductive age and showed that 88% of women among their study group were suffering from vitamin 25 (OH) D deficiencies.²⁴

Our study had several limitations. From this retrospective data, we could not evaluated correlation with other relevant parameters like health status including their BMI or any other fertility associated factors such as regular menstrual cycle, pregnancy and PCOs.²⁵

Conclusion:

This study concluded that deficiency of vitamin 25(OH) D is prevalent among the women of reproductive age in Karachi, Pakistan. The results of this study would provide a valuable data for dissemination of awareness in our society where women of fertile age fall among high risk individuals with impending osteoporosis, auto immune disorders or pregnancy related complications. To overcome this problem, an intake of vitamin 25(OH) D rich diet and supplements are highly suggested. However, as our country is exposed to sunlight almost round the year, we could benefit from this natural resource during the morning and evening time periods when the exterior temperature remains low especially in the coming winter season.

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