SHORT COMMUNICATION
EVALUATING THE STATUS OF VITAMIN 25(OH) D LEVELS AMONG FEMALES OF ALL AGE GROUPS IN KARACHI, PAKISTAN

VITAMIN D LEVELS AMONG FEMALES IN KARACHI

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ABSTRACT:
Objective: Vitamin D plays an imperative role in growth, metabolism and reproduction. Low levels of this vitamin D are highly prevalent globally and leads to various disorders besides different skeletal deformities. Women of different reproductive ages are more prone towards developing bone diseases as of low vitamin D levels. The study focuses to evaluate the prevalence of vitamin D deficiencies in females of different ages in Karachi, Pakistan.

Methods: Serum vitamin 25(OH) D levels of 1035 female were measured by electro-chemiluminescence immunoassay. 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, 44.2% (n=458) displayed mild to moderate Vitamin D deficiency while 29.7% (n=307) were having normal levels of serum vitamin D.

Discussion and conclusion: 70.4% of our studied population had lower levels of vitamin D representing that majority of Pakistani women have vitamin D Deficiency. Since hypovitaminosis is a grim public health concern, strong recommendation of health education and vitamin D supplementations for the female population is proposed.

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

INTRODUCTION
Vitamin 25(OH)D is an essential steroid and hormone precursor that is vital for bone metabolism, growth differentiation and its mineralization (Loonis, 1967). Vitamin D containing foods are few, it can be obtained by eating fatty fish flesh which include salmon and tuna, Cod fish oils are also good source of Vitamin D. Beside it Vitamin D can also be taken in diet by eating mushrooms, egg yolk, cheese and beef liver (Ovesen, 2003) It can also be obtained by ultraviolet light emitted by sun which photochemically transforms 7-dehydrocholesterol into precholecalciferol which is provitamin D3 present in the skin; this is further transformed to vitamin D3 or in different products (Loonis, 1967, Holick 1994, Mclaughlin et al., 1974). Vitamins D3 undergoes hydroxylation, initially in liver and then in the kidneys and is changed into the storage form 25-hydroxyvitamin D (25OHD) and then to its active form 1,25dihy-droxyvitamin D respectively (Haddad 1992; Goor1995).

Vitamin 25(OH) D has key role in the absorption of minerals like calcium and phosphorus through intestine, it also modulates the release of parathyroid hormone by inhibiting it and consequently helps in maintaining the bone density (Lee et al., 2014). Recent research shows the relationship of vitamin 25(OH) D deficiency with the increased risk of type I Diabetes, multiple sclerosis, bone diseases, hypertension, heart related diseases and cancers (Lu et al., 2016; Melamed et al, 2008). Many factors which are linked with vitamin 25 (OH) D deficiencies are geographic location, atmospheric conditions, length of time spent in sunlight, type of clothing, poor diet and age. High melanin production in skin also affect the vitamin 25(OH) D absorption (Alagöl et al., 2000). The severe deficiency of Vitamin 25(OH) D can be controlled by dietary and parenteral vitamin 25 (OH) D supplemetations which are effective in improving bone density thereby reduce the morbidity & mortality (Fry and Sanders, 2015). Deficiency and insufficiency of vitamin 25(OH) D is a common problem faced globally not only by children but also observed in adult male and females (Holick, 2007; Nowson et al., 2012). Around 50% of world population is suffering from vitamin 25 (OH) D insufficiencies which is built on vitamin 25(OH) D (25-hydroxy vitamin 25(OH) D) levels below 20 ng/ml. Global estimation shows that around 1 billion people of different ethnicities and age group are having deficiency of vitamin 25(OH) D (Lips et al., 2006; Gordon et al., 2003). In Asian countries, especially Pakistani 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 (Jadoon et al., 2017). Pakistan is located at the subtropical 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 20 ng/ml (Khan et al., 2013). 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 (Marwaha et al., 2011). Because of the significance of vitamin 25(OH) D levels and lack of scientific literature on vitamin 25 (OH) D statuses among women of different age groups in Pakistan, we conducted this study for the determination of the serum vitamin 25(OH) D levels (25-hydroxy-vitamin 25(OH) D) in-between the females of different age groups in Karachi, Pakistan.

MATERIAL AND METHODS

This study is retrospective, 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 Clinical Lab, Al-khidmat Diagnostic Center, Karachi, Pakistan within a period of 6 months from September 2014 to February 2015. The females within the age range of 13 to 75, having no systemic disease and self-reported healthy were included in the study whereas, females having age group less than 13 and above 75 and who were not willing to participate in the study were excluded from the study. Serum levels of vitamin 25(OH) D were estimated by electro-chemiluminescence immuno assay (ECLIA) method on E-170 Modular immuno analysis analyzer (Roche, Germany) having detection limits of 3.00-70.00ng/mL. QC was determined by running normal and high level standards.

On the basis of serum concentration of Vit. D study subjects were categorized into 3 groups: (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 SPSS ver. 16.0. The results were expressed in means, standard deviation, percentages and standard error. To evaluate the significance of mean differences chi-square test was applied and p-value ≤0.05 was taken 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. Mean serum vitamin 25(OH) D levels in the studied subjects were found as 21.04±14.13 with the minimum and maximum range of 4.97 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 1: Status of serum vitamin 25(OH) D3 levels among studies population (n=1035)

<table>
<thead>
<tr>
<th>Vitamin 25(OH) D Levels (ng/ml)</th>
<th>No of participants</th>
<th>Percentages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe Deficiency (&lt;10)</td>
<td>270</td>
<td>26.1</td>
</tr>
<tr>
<td>Mild to Moderate Deficiency (10-25)</td>
<td>458</td>
<td>44.2</td>
</tr>
<tr>
<td>Normal (&gt;25-80)</td>
<td>307</td>
<td>29.7</td>
</tr>
</tbody>
</table>

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 2: Age-wise evaluation and distribution of 25-hydroxy vitamin 25(OH) D levels among study participants

<table>
<thead>
<tr>
<th>Age Groups (years)</th>
<th>n=1035</th>
<th>Normal Vitamin 25 (OH) D levels (n=307)</th>
<th>Mild to Moderate Deficiency (n=458)</th>
<th>Severe Deficiency (n=270)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>Average (Range min-max)</td>
<td>Average (Range min-max)</td>
<td></td>
</tr>
<tr>
<td>Less than 20</td>
<td>76 (7.3)</td>
<td>19 (25)</td>
<td>38.7 (25.1+62.1)</td>
<td>29 (38.15)</td>
<td>8.177</td>
</tr>
<tr>
<td>21-30</td>
<td>306 (29.6)</td>
<td>65 (21.24)</td>
<td>39.81 (25.4+70.71)</td>
<td>139 (45.42)</td>
<td>5.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

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.
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 41-50 years, 19.19% (n=43) were severely deficient, 50% (n=112) were mild to moderately deficient while 30% (n=69) were having 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) D 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 was detected in-between 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 bone deformities in children, while in adults autoimmune disorders and osteoporosis are common. Vitamin 25 (OH) D deficiency in females is most prevalent in different reproductive ages. To date there have been several studies which have signified the part of vitamin 25(OH) D in women’s health. The data suggest the relationship of vitamin 25(OH) D with several factors including fertility and reproductive health. Although vitamin 25(OH) D status can also be inclined by environmental factors including to sun exposure time and dietary habits, different reproduction related 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 was 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 (10-25 ng/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 linked with women of fertile age (21-30 years) i.e. 37.8%. On the other hand, women of age group with least fertile health of above 70 years did not display severe vitamin 25 (OH) D deficiencies (0.4%).

Women lying in most fertile age group i.e. the age range of 21 to 30 years, showed 37.8% of females were 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% were mild to moderately vitamin 25(OH) D deficient. Difference in vitamin D levels in various age groups (p value <0.05) was found significant among all the groups.

Our findings of severe deficiency of vitamin 25(OH) D levels are also supported by literature, a study conducted in Karachi reported that 84% of
their study subjects had serum 25(OH) D concentrations < 75 nmol/L (Sheikhet al., 2012). A similar study conducted in 2015 Lahore, Pakistan report that 73% of their women of child bearing age were vitamin 25(OH) D deficient whereas 43% showed profound Vitamin 25(OH) D deficiency (Junaid et al, 2015). Different studies conducted in Asia reveals the increased rates of vitamin 25 (OH) D deficiencies among women (Islam et al, 2006; Gammna et al., 2014; Alsuwadia et al., 2013). In India, a study reveals the levels of vitamin 25(OH) D were estimated in reproductive age women which showed that 88% of women among their study group were suffering from vitamin 25 (OH) D deficiencies (Sofi et al., 2017).

There are multiple limitations associated with our study. 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 (Voulgaris et al., 2017).

This study concluded that deficiency of vitamin 25(OH) D is prevalent in 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 environmental temperature remains low especially in the coming winter season.

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Disclaimer: The authors hereby declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

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