Study of Vitamin D deficiency and contributing factors in the population of Hyderabad, Pakistan

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Abstract: The study of vitamin D (25-OHD) deficiency was intended to analyse the incidence and some contributing factors amid healthy individuals of different living standards in general population of Hyderabad and its adjacent areas. A total of 1244 healthy individuals (1-84 year of age) (females 725 (58.2%) and males 519 (41.8%)) were selected in this study and the concentration of Serum vitamin D (25-OHD), phosphorus, alkaline phosphatase and calcium was analysed. The prevalence of vitamin D (25-OHD) deficiency was 78.3%. The values <10ng/mL were noted as severe deficiency, 10-20ng/ml moderate and 21-29.9ng/ml as mild deficiency or insufficiency. The degrees of vitamin D (25-OHD) deficiency amongst the healthy individuals were severe (17.2%), mild (18.8%) and moderate (42.3%). Vitamin D (25-OHD) was positively correlated with blood calcium profile whereas alkaline phosphatase and phosphorus correlated negatively. The study showed that vitamin D (25-OHD) deficiency has a high prevalence in healthy subjects.

Keywords: Population, determination, vitamin D, calcium, phosphorus, alkaline phosphatise.

INTRODUCTION

Vitamin D (25-OHD) is a lipid soluble steroid prohormone (Vanchinathan and Lim 2012; Holick, *et al*., 2012). Its endogenous production occurs by the exposure of skin to UVB (280-320nm) which convert the 7-dehydrocholesterol to vitamin D (cholecalciferol). Majority of the people in the world, approximately 90% of vitamin D is synthesized in this way and the remaining 10% may obtain exogenously from nutrients or supplements (Holick, *et al*., 2011; Bikle, 2012). Nutritionally, vitamin D rich foods are fatty fish, eggs, cod liver oil and 25-hydroxyvitamin D fortified supplements (Mazahery and Hurst, 2015). Vitamin D (25-OHD) has an endocrine role in the absorption of calcium by intestine and to be taken up to the blood stream (Bikle, 2011). Generally, two forms of vitamin D (25-OHD) exist which refer to vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol) (Loken-Amsrud, *et al*., 2012). Vitamin D (25-OHD) has a pleiotropic action (Nelson, *et al*., 2012) which is proved by different experimental observation that it is involved in diverse multifactorial conditions (Samuel and Sitrin, 2008; Diaz, *et al*., 2015; Kubis and Piwowar, 2015; Sergeev, 2014). It was reported that its increase role is associated with in a number of health-related activities (Olliver, *et al*., 2013), as in immune function, wound healing, brain homeostasis, neurodevelopment, inflammation reduction, cell proliferation regulation, differentiation, aging, apoptosis and gene regulation (Saad, *et al*., 2015; Cholongitas, *et al*., 2012). Vitamin D (25-OHD) binds with approximately 2700 genes, out of them it alters the expression of about 200 genes (Bischoff, *et al*., 2003; Ramagopalan, *et al*., 2010). Recent research has also revealed that it is also involved in a broad range of disorders, including autoimmunity disorders, multiple cancer syndromes, dementia, autism, cardio-vascular disease, maintaining blood pressure and allograft survival as well as with certain infectious diseases (Kocovska, *et al*., 2012; Zhu, *et al*., 2015). The metabolism of vitamin D (25-OHD) readily occurs in hepatocytes and is closely associated with hepatic function, where it is metabolized to 25-hydroxyvitamin D, an initial step for vitamin D activation (Schiefke, *et al*., 2005; Jones, 2008), removal of vitamin D metabolites through bile to kidney for further hydroxylation to I, 25-dihyroxyvitamin D (calcitriol), biological active form of vitamin D (Young, *et al*., 2010). Vitamin D (25-OHD) has a key role in the maintenance of bone health and homeostasis of calcium (Grober, *et al*., 2013; Lucas, *et al*., 2014; Soto, *et al*., 2008). Occurrence of vitamin D (25-OHD) deficiency is high and is now documented as a worldwide health problem. An estimated calculation shows that about 1billion people worldwide are deficient or having its insufficiency (Holick, 2010). One of the most contributing factors is thought to be due to developmental changes has shift our lifestyle to urbanization (Holick, 2007) and insufficient diet supplementation (Weng, *et al*., 2007). In blood the concentration of 25 (OH) D is commonly used as biomarker for vitamin D profile with a half-life of approximately a few weeks (DeLuca, 2004; Thacher and Clarke, 2011). Making it an ideal marker to measure weather patient is vitamin D deficient, sufficient or intoxicated, normal level of vitamin D (25-OHD) is essential to maintain bone metabolism. The chronic deficiency of vitamin D leads to diminution of bones

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reservoirs of phosphate, calcium and inadequate bone matrix mineralization, which is a risk factor for rickets in children and osteomalasia in adults (Cashman and Kiely, 2011; Reid, 2015). Subsequent investigations proved its role in the protection of the older from osteoporosis (Drake, *et al*., 2015).

It was proved out of several studies that vitamin D (25-OHD) deficiency showed the increased pervasiveness in tropical states such as Turkey, Iran, China, India and Saudi Arabia and have estimated the frequency of vitamin D population between 30 and 93 percent (Azizi, *et al*., 2000; Gowami, *et al*., 2000; Arabi, *et al*., 2010). Although, it is less clear what is the cause of its deficiency in tropical countries that could affect vitamin D status. Furthermore, most of the studies for evolution of determination of the frequency of vitamin D deficiency were limited to a small sample size or were targeted to a specific age group (especially elderly) (Alagol, *et al*., 2000; Du, *et al*., 2001; Dawodu, *et al*., 2003).

MATERIALS AND METHODS

This study was a retrospective, cross-section piloted from March 2015 to February 2016, at the Institute of Biotechnology and Genetic Engineering, University of Sindh, Jamshoro and Isra University Hospital Hyderabad, Pakistan. In this study 1244 healthy individuals (1-84-year age), visited infirmary, filled the inclusion & rejection criteria and were randomly designated. Once scripted consent form referring age, gender, profession and extant reference were recorded. This research was carried out in accordance with protocols of Ethical Committee of “IBGE” at University of Sindh, Jamshoro. This research was carried out in accordance with protocols of ethical committee of “IBGE” at University of Sindh, Jamshoro. About 4ml blood was collected from the individuals in plain vacuatainer and clear supernatant was obtained by centrifugation technique at 3,000gm for 10minutes within 1hour of blood collection. Separated serum was preserved by freezing (at-20^{o}C) until analysed (Zerwekh, 2004). The principle of the test is based on advance technique of chemiluminescence microparticle immunoassay (CMIA). In this method paramagnetic anti-vitamin D coated micro particle binds to vitamin D_2 and vitamin D_3 (25-hydroxyvitamin D) as a capture protein. The chemicals were preserved and used as described by the Abbott Laboratories instructions manual (Cavalier, *et al*., 2012). The alkaline phosphatase, calcium and phosphorus were also analysed in all of these subjects by using manual laboratory techniques for supplementary information about vitamin D status (Hausamen, *et al*., 1967; Tietz, *et al*., 1983; Endres and Rude 2006; Taussky and Schoor 1953). The normal range for vitamin D (25-OHD) was 30-40 ng/ml. The value <20 ng/ml was defined as deficiency while insufficiency was ranged between 20.1-29.9ng/ml (Agarwal, *et al*., 2013). All the results were analysed by SPSS version 21. Continuous variables were calculated as mean and standard deviation (SD). The statistically significant *P*-value was <0.05. Correlation of vitamin D with other parameters and *P*-value <0.01 was taken as statistically acceptable.

RESULTS

The pervasiveness of vitamin D (25-OHD) deficiency was high among general population of Hyderabad, Pakistan. Characteristics of subjects, descriptive statistics and correlation between vitamin D level and their contributing factor variable**s** are given in table-1 and table-2 respectively. Among 1244 subjects ranging age from 1-84 year with mean of 33.48±18.1 year tested for vitamin D, females were 725 (58.2%) while males were 519 (41.8%) of the total sample size. Subjects were mostly residing in apartments (59%), works in offices (14%) and rest of them (27%) uncovered their face while outdoor. In majority of individuals exposure of sunlight was <2hour /24hours (74%). Mostly, the volunteers were colour dressed up (75%). Nine hundred and seventy-four (78.3%) subjects had deficiency and significantly correlated with timing of body exposure to sunlight and the food consumed. The prevalence of vitamin D deficiency was 78.3% (<30ng/ml), the test subjects were placed in four categories according to vitamin D (25-OHD) level in their blood, i.e. with severe deficiency, moderate deficiency, mild deficiency or insufficiency and sufficiency were seen in 17.2% (<10ng/ml) , 42.3% (10-20ng/ml), 18.8% (20.1-29.9ng/ml) and 21.7% (>30ng/ml) respectively.

It was observed that in male and female there was a little difference regarding vitamin D level as shown in table-3. The age and gender distribution of the under-study subjects are presented in table-4. Vitamin D (25-OHD) was correlated positively with duration of body exposure to sunlight and colour of clothes, whereas there were significantly no correlation of its level with age, gender and locality. Subsequently, vitamin D (25-OHD) was significantly correlated positively with serum calcium whereas serum phosphorus and alkaline phosphatase correlated negatively**.**

DISCUSSIONS

There are many contributing factors towards the prevalence of hypo-vitaminosis among general population worldwide (Mithal, *et al*., 2009). In residual population limitation and avoidance of sunlight exposure due to fear of over tanning of the skin or traditionally, veiling of the entire body, veil (Saraiva, *et al*., 2005; Erkal, *et al*., 2006) and air pollution were the main contributing factors (Hashemipour, *et al*., 2004; Meddeb, *et al*., 2005). These factors hamper the ultraviolet β rays penetration thereby decreasing level of vitamin D (low synthesis). It was

Table 1: Basic characteristics features of under study subjects (n=1244)

Gender	Male/Female	519(41.7%)/ 725(58.3%)
Vitamin D in diet	Low / Adequate	1050(84%)/ 194(16%)
Living Standards	Office workers/ Beggars House wives/ Students Labour class/ Others	175(14%)/189(15%) 461(37%)/ 69(5.5%) 67(5.3%)/ 283(22.7%)
Area of skin exposed	Face Exposed/ Face & hands exposed Face, hands & forearms exposed/ Whole body Covered	405(32.5%)/ 527(42.3%) 258(20.5%)/ 54(4.3%)
Duration of sun exposure	<1 hour /d/ 1-2 hours /d >2 hours /d	636(51.1%)/ 352(28.2%) 256(20.5%)
Colour of clothes	Black/ White Others	405(32.5%)/ 78(6.3%) 761(61.1%)

Table 2: Descriptive statistics (n=1244)

	Minimum	Maximum	Mean ± S.D	P
Age of Patients (Years)	1	84	33.48±18.1	.662
Serum Vitamin D (ng/ml)	0.7	160	22.1±18.2	.206
Serum Calcium (mg/dl)	5.1	12.1	9.29±0.96	.047
Serum Phosphorus (mg/dl)	2	4.8	3.7±0.43	.229
Serum Alkaline phosphatase (IU/L)	32	351	86.8±31.1	.801

Table 3: Vitamin D Status in the under-study population expressed in terms of age groups, gender and degree of severity.

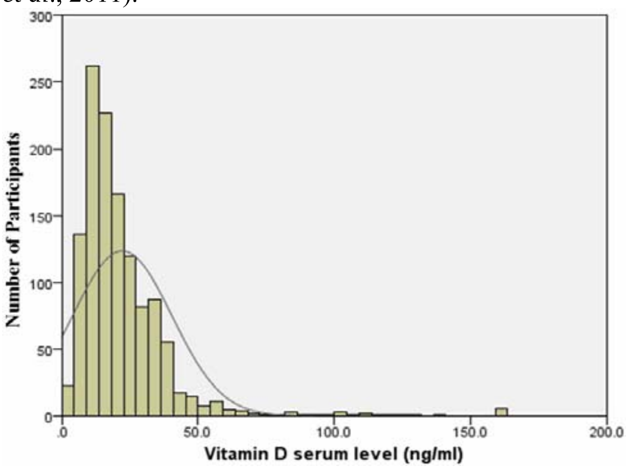
Age Group	Number of subjects in each Vitamin D status category expressed as 25(OH)D level				The actual Mean SD serum 25 (OH)D level	
	<10	10-20	20.1-29.9	>30	Male	Female
1-20Year	46/13.3%	161/43.5%	73/21.2%	73/22.0%	23.10±10.3	20.9±14.3
21-40Year	59/15.5%	148/38.9%	82/21.7%	91/23.9%	25.6 ± 16.3	22.2±14.8
> 40 Year	108/20.7%	215/41.3%	91/19.5%	106/18.5%	19.2±15.9	17.8±15.2
All cases	213/17.2%	524/42.3%	237/18.8%	270/21.7%	22.4±18.04	21.0±18.3

Vitamin D status expressed in terms of the subjects numbers each vitamin D status category (<10,10-20, 20.1-29.9 and >30ng/ml) per three age groups (1-20,21-40 and >40year) and also expressed in terms of mean±SD of circulating vitamin D in males and females separately as per same age groups.

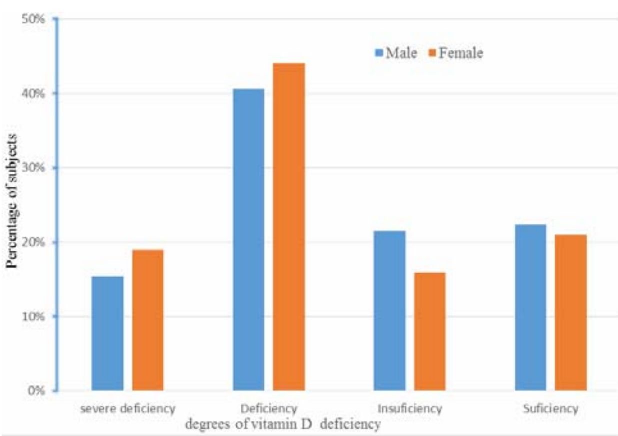
Table 4: Different age and gender groups distribution of Participants

Age (Years)	Total Number	Female	Male
1-20	344(100%)	199(57.8%)	145(42.2%)
21-40	380(100%)	222(58.5%)	158(41.5%)
>40	520(100%)	309(59.4%)	211(40.6%)
Total	1244(100%)	725(58.2%)	519(41.8%)

observed from survey that males also seen scared of sunlight exposure because of insufficient education and relative knowledge regarding the source of vitamin D (25-OHD)**.** The main source for obtaining of vitamin D (25-OHD) is sunlight. It was revealed that the area of skin exposed and the duration of sunlight exposure strongly correlated with vitamin D levels (as per this study) as also shown in other studies (Hashemipour, *et al*., 2004; Alagol, *et al*., 2000; Moussavi, *et al*., 2005). Vitamin D (25-OHD) deficiency is an innovative worldwide emerging problem among all age groups (Saraiva, *et al*., 2005; Moussavi, *et al*., 2005; Islam *et al*., 2006). The vitamin D level is used as biomarker for bone metabolism. When the level of vitamin D (25-OHD) decreases (<10ng/ml), the physicians prescribe for taking sufficient doses constituting 50,000 IU of vitamin D weekly up to 2-3 months (Kennel, *et al*., 2010). Vitamin D (25-OHD) enhanced profiles are necessary for muscle strength. Recent studies confirm the hypovitaminosis D to cause as cardiovascular illness, diabetes mellitus, malignancy, particularly of the intestine and endocrine (Bjelakovic, *et al*., 2011; Cranney, *et al*., 2007). Adequate vitamin D is necessary for the expression of about 200 genes (Bischoff, *et al*., 2003). The present study has revealed that majority of individuals are vitamin D deficient in Hyderabad region. Present results are in agreement with the national data reported from Faisalabad, 77.5% of the sample presented vitamin D deficiency in Punjab whilst 18% showed the insufficiency of vitamin D (Masood, *et al*., 2010). Another study had shown about 90.1% vitamin D deficient subjects covering 305 premenopause stage (females of Karachi) (Khan, *et al*., 2012). It has been observed that <20ng/ml serum level of vitamin D is the cut out need of 97.5% of world population (Veugelers, *et al*., 2014). The value of vitamin D less than this is considered as deficiency whilst about 30ng/ml level is considered as vitamin D insufficiency and vitamin D sufficiency is defined as >30ng/ml (Holick, *et al*., 2011).

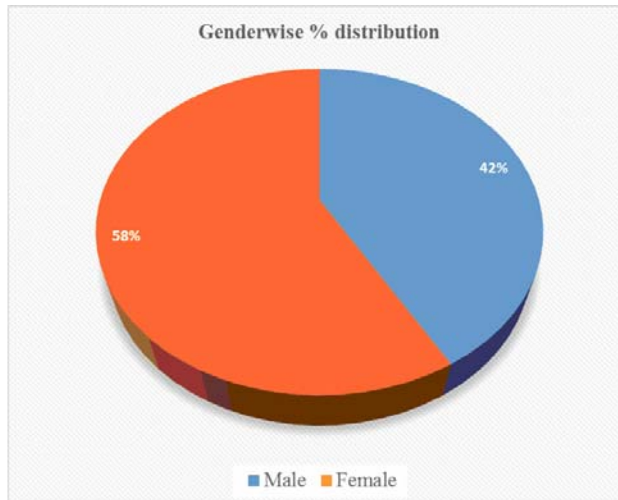


Graph 1: Histogram represents percentage distribution of vitamin D deficiency level in the under-study subjects/volunteers.



Graph 2: Distribution of participants according to the degrees of vitamin D deficiency in varied gender groups

The prevalence of vitamin D deficiency was shown to be high in office workers and housewives as such they are involved indoor or in office work. The exposure of sunlight was meagre because of traditional, religious and social standards hence they cover the entire body except hands and face. The prevalence of vitamin D deficiency raises the question as why the healthy individuals do not intake or maintain adequate level of vitamin D. Actually, these anticipated values have been well-known based on international/global population. As normal values may fluctuate according to gender, age and diet, it is necessary to set up our specific normal value by analysing a considerable number of normal individuals within Hyderabad domain and signifying what results are achieved to be standarized for them. There was lack of consciousness concerning amply stable nutrient and the overcooking practice were contributory elements to the current vitamin D needs.



Graph 3: gender wise percentage distribution of participants

CONCLUSIONS

The current study revealed that the office workers and housewives are more likely to have decreased vitamin D levels due to their life style, insufficient diet and less exposure to sunlight. This is due to the lack of knowledge among common people, in this regard health committees and concerned authorities should take immediate measures and arrange open public seminars and using media for awareness on vitamin D dietary supplements and problems due to deficiencies.

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