



RESEARCH PAPER

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Vitamin D level and its correlation with age in female population of district Peshawar, Pakistan

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Abstract

Vitamin D is one of the fat soluble vitamins which increase the intestinal absorption and metabolism of calcium and phosphorous. Vitamin D is necessary for maintenance, growth, and development of healthy skeleton from birth to death. The present cross sectional study was carried out in which 260 females, randomly selected from the Diabetes and Endocrinology, Medical and Orthopedics outpatient setting (OPDs) of Hayatabad Medical Complex Hospital Peshawar who were enrolled in March 2012 to February 2013. Serum concentrations of 25-hydroxyvitamin D [25(OH) D] were measured by CMIA immunoassay. Information on demographic and lifestyle factors was derived from interview-administered questionnaires. The data was analyzed by SPSS version 16. The mean serum 25(OH) D concentration was 14.45ng/mL. Only 5% of the population had 25(OH) D level was normal (40-100ng/mL) while 95% of the population had vitamin deficiency (<40 ng/mL), but the prevalence of vitamin D severe deficiency (<10 ng/mL) was 46%, 34% had moderate deficiency levels (10-20 nmol/mL), while 13% had hypovitaminosis (20-40ng/mL). Those female who were at risk had peak vitamin D deficiency included young female (10.83ng/mL), aged (12.53ng/mL), unmarried (13.13ng/mL), pregnant women (13.70ng/mL), high level of education (12.77 ng/mL), work in office 12.10ng/mL, middle socio economic background (14.04ng/mL), poor nutrition (13.17ng/mL), use of sunscreen (11.06ng/mL), not exposure to sunlight (4.37ng/mL), closed houses (14.53ng/mL), white skin colour (12.92ng/mL), musculoskeletal pain (14.43ng/mL), early fatigue (13.99ng/mL), obesity (13.61ng/mL), Diabetes mellitus (13.55ng/mL), Cardiovascular disease (12.87ng/mL) and Asthma (11.41ng/mL).

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Introduction

Vitamin D is also known as the sunlight vitamin. It is one of the fat soluble vitamins which increase the intestinal absorption and metabolism of calcium and phosphorous (Mealy *et al.*, 2011). Vitamin D is necessary for maintenance, growth, and development of healthy skeleton from birth to death. Evolutionary history of vitamin D reveals that it first appeared in the phytoplankton and zooplankton life, which have been present in oceans for more than 750 million years ago synthesis vitamin D when exposed to sunlight (Holick, 2003).

The sunlight exposure is the chief source of vitamin D for human (Chung *et al.*, 2009). The human beings obtain twenty percent vitamin D from diet and derived eighty percent from sunlight exposure (ultraviolet – B rays) on conversion of 7-dehydrocholesterol in the skin (Jan *et al.*, 2011). The naturally occurring common food sources are egg yolk, beef liver and fatty fishes, butter, mushroom, milk and cheese (Chung *et al.*, 2009). There are little vitamin D dietary sources in Pakistan that show the relationship of ingestion of these foods and deficiency of vitamin D (Karim *et al.*, 2011).

The 7-dehydrocholesterol present in the skin epidermis and dermis converts to previtamin D₃, when the wavelength 290-315nm of solar ultraviolet B radiation (UVB) enters the skin after exposure to sunlight. The previtamin D₃ after that convert to vitamin D₃. The “D” represents D₂ or D₃. The Vitamin D₂ formation occurs through UVB radiation of ergosterol from yeast. The Vitamin D₃ formation can occur through the UVB radiation of 7-dehydrocholesterol from lanolin (Holick, 2007).

Vitamin D metabolization from the diet and skin occur in the liver to 25-hydroxy vitamin D [25(OH)D]. The 25(OH)D in the kidney is metabolized by the enzyme 25 hydroxy vitamin D -1- α hydroxylase(CYP27B1) to its active form 1, 25 dihydroxy vitamin D (Holick 2007). The 25-hydroxy vitamin D is the chief form of vitamin D that circulates in the blood stream but it has low biological

activity. Generally serum 25(OH)D level show dietary status. The plasma parathyroid hormone concentrations, serum calcium and phosphorous levels control the renal formation of 1, 25 dihydroxy vitamin D (Holick, 2007). Other tissues which contain the enzyme that catalyses the conversion of 25(OH)D to 1, 25(OH)₂D, 25-hydroxyvitamin D₃-1- α -hydroxylase, include keratinocytes, macrophages, prostate, osteoblasts, mammary gland, antigen-presenting cells, and colon (Chung *et al.*, 2009). The Vitamin D binding protein (DBP) attaches vitamin D and its metabolites then carried it to the bloodstream (Lips, 2001).

There are many factors which effects the cutaneous productions of vitamin D₃ which includes season, latitude, skin pigmentation, time of day, aging, Sunscreen, malabsorption (Suibhne *et al.*, 2012). Dark skinned, veiled, pregnant women have greatest frank deficiency (80%) and also great range present in infant mothers treated for rickets (Brot *et al.*, 1999). Others essential factors are smoking, BMI (body mass index), dietary intake of vitamin D, physical activity, cultural factors and 25(OH)D fortified food, Obesity, air pollution (Mannion *et al.*, 2006, Holick, 2007, Barake *et al.*, 2010). The wearing of full burqa in Muslim regions women reduced the exposure of sunlight and thus vitamin D status (Lowe *et al.*, 2006, 2011). Dietary deficiency of vitamin D is the common reason, in the absence of any malabsorption or disorder of kidney, livers, or vitamin D metabolism (Jan *et al.*, 2011).

Females experience globally from vitamin D deficiency although they would be mothers and will have pressure of nursing and pregnancy. The significant of this problem will be realized only, when we understand that the growth period of females are short, so they do not have sufficient time to balance healthy skeleton (Shakiba *et al.*, 2009).

The Studies were carried out in Greece and Iran, they found that a more incidence of vitamin D insufficiency among Turkish females and their infants and among pregnant females and their newborns. A

widespread vitamin D deficiency among expecting females and correspondence of maternal and cord blood levels of vitamin D was reported in India (Zuberi *et al.*, 2008).

Atiq *et al.* (1998) reported minimum vitamin D levels in 48% of healthy Pakistani nursing mothers. The mothers belonging to the upper socioeconomic group showed higher prevalence of low vitamin D level. The females belonging to lower socioeconomic background, nursing mothers were low vitamin D concentration (Zuberi *et al.*, 2008).

In Pakistan, a high frequency of vitamin D deficiency has been examined from the upper socioeconomic group among breastfed infants and nursing mothers although the problem has not been disclosed for major group of females and their newborns that belong to lower earnings status (Karim *et al.*, 2011). In Pakistan the urban newborns and their mothers had greatest Vitamin D deficiency (Karim *et al.*, 2011).

Vitamin D insufficiency has been linked with different chronic diseases and clinical disorders, include decreased autoimmune disorders such as multiple sclerosis and diabetes types I and II, muscle strength, impaired balance, mood and cognitive dysfunction, myocardial infarction, tuberculosis, chronic obstructive pulmonary disease and schizophrenia (Holick *et al.*, 2008., Evatt *et al.*, 2011). This was the first study conducted in this area for the determination of vitamin D level in the women. The present study was conducted with the aim and objective to determine the vitamin D level in the human population of the district Peshawar and to aware people about the negative effects of vitamin-D deficiency.

Materials and methods

Study design

In this cross sectional study enrolled 260 females out of 300, randomly selected from the Diabetes and Endocrinology, Medical and Orthopedics outpatient setting(OPDs) of Hayatabad Medical Complex

Hospital Peshawar from January 2012 to February 2013. The patients were participating, after filling of a pre-evaluation pro forma.

Sampling and data collection

All patients give printed informed proforma after being fully aware of the study aims and objectives. Then face to face interview were filled out by questionnaires. The questionnaires in the proforma about their Name, Address, Date, ages, type of their residential house(open or close), use of sunscreen, exposure to sunlight, symptoms such as muscle pain, weakness, fatigue, presence of other diseases, obesity, skin colour, socioeconomic background, feeding habits (poor diet, good diet) etc were recorded in a designed proforma. Then after taking permission from all contestants, 5ml of eight hours night fasting blood samples were collected from every patient in an aliquote tubes for measuring 25-OH-D₃. After separation of serums quickly they were freeze under 4C° centigrade.

The patients were divided into three analytical groups based on their serum 25 vitamin D levels for the intention of this study. Those with vitamin D levels < 9 ng/ml were group to severe deficiency, levels between 10 - 20 ng/ml as moderate deficiency and levels of 20 - 40ng/ml as hypovitaminosis. The protocol of research was accepted by the institutional review board and local medical ethics committee.

Measurement of 25-OH-D₃ was carried out by automatic Architect instrument of Abbot Company by the process of Chemiluminescent Microparticle Immunoassay (CMIA).

Statistical analyses

The statistical analysis was accomplished by using SPSS version 16. Descriptive analysis was done for clinical and demographic variables.

Results

Severity of Vitamin D deficiency

It was observed that out of 260 subjects, 13(5%) were normal, 247(95%) were vitamin D deficient. it is evident from the table that female of age group 1-15

years and above 61 years have high vitamin D deficiency (100%) in table 3.1.

Out of 247 the incidence and percentage of, 35(13.46), 90 (34.61%) and 122(46.92) were hypovitaminosis, mild vitamin D deficiency and severe vitamin D deficiency respectively.

Different categories of vitamin D deficiency levels

Table 1. Vitamin D deficiency in female population of Peshawar region.

Age group (Year)	Total Sample N (%)	Normal vitamin D (40-100 ng/ml) N (%)	Vitamin D deficiency (<40ng/ml) N (%)
1-15	11(4.23)	0	11(100)
16-30	65(25)	1(1.53)	64(98.46)
31-45	81(31.15)	6(7.40)	75(92.59)
46-60	65(25)	6(9.23)	59(90.76)
61-75	35(13)	0	35(100)
76-90	3(1.15)	0	3(100)
Total	260	13(5)	247(95)

Age wise vitamin D deficiency in female population

The mean vitamin D level of the study population was calculated by using SPSS software version 16. The mean vitamin D level was 14.45ng/ml).The study population was subdivided into age groups and analyzed (Table 3.3). The maximum numbers of subjects were between 31 and 45 years of age. The

mean vitamin D levels range between 12.53-16.42 ng/ml in all the age groups studied. The minimum mean level(12.53ng/ml) of vitamin D were seen in subjects between 76 and 90 years of age, while maximum mean level(16.42ng/ml) of vitamin D as observed in subjects between 31 and 45 years of age.

Table 2. Different graded of vitamin D level in female population of District Peshawar.

Age group (Years)	Vitamin D deficiency (<40) N (%)	Hypovitaminosis (20-40ng) N (%)	Mild /Borderline vitamin D (10-20ng/ml) N (%)	Severe vitamin D deficiency (<10ng/ml) N (%)
1-15	11(100)	2(18.18)	3(27.27)	6(54.54)
16-30	64(98.46)	3(4.61)	22(33.84)	39(60)
31-45	75(92.59)	13(16.04)	33(40.74)	29(35.80)
46-60	59(90.76)	9(15.25)	18(30.50)	32(54.23)
61-75	35(100)	7(20)	14(40)	14(40)
76-90	3(100)	1(33.33)	0	2(66.66)
Total	247(95)	35(13.46)	90(34.61)	122(46.92)

Risk factors related with deficiency of vitamin D

A total of 260 female were investigated in which 50 were unmarried and 250 were married. It was evident that maximum amount of vitamin D (14.76ng/ml) was recorded in married as compared to unmarried female population (Table 3.5).

It was recorded that maximum amount of vitamin D (14.49ng/ml) was recorded in non pregnant as compared to pregnant female population. The different categories of education were studied in which high vitamin D (15.02ng/ml) was present in

middle educated female while low vitamin D (13.66ng/ml) in high educated categories. The various activity levels of female population were studied in which high vitamin D level was recorded in the female who did office work.

In order to tests the significant among various categories of socioeconomic status about vitamin D level in female population. It was observed that high vitamin D level (15.39ng/ml) is present in female who belong to poor economic status, then middle one and high group were low vitamin D level.

Table 3. Distribution of female patients according to age and vitamin D levels in peshawar region.

Age groups (years)	Sample (N)	Mean Vitamin D level (ng/ml)	Standard Deviation
1-15	11	13.74	9.28
16-30	65	10.95	7.26
31-45	81	16.42	14.15
46-60	65	16.11	14.94
61-75	35	13.69	7.59
76-90	3	12.53	6.69
Total	260	14.45	12.10

It was evident that vitamin D (16.69) deficiency more prevalent in female of poor diet than in good diet individuals. It was manifested that maximum amount of vitamin D (14.61ng/ml) was recorded in female not use sunscreens as compared to use of sunscreen in female population.

In order to tests the significant differences among various categories or levels of exposure to sunlight about vitamin D level in female population. The females who exposed to sunlight for three hours were high vitamin D level 20.48ng/ml, and low vitamin D (4.37ng/ml) in female who were not exposed to sunlight.

It was evident that greater amount of vitamin D (15.53ng/ml) was recorded in closed house as compared to open house. It was evident that maximum amount of vitamin D (15.24ng/ml) was recorded in non musculoskeletal pain as compared to musculoskeletal pain. It was marked that maximum amount of vitamin D (15.70ng/ml) was recorded in late fatigue as compared to early fatigue. It was observed that maximum amount of vitamin D (14.77ng/ml) was recorded in non obesity female as compared to obesity ones.

It was apparent that maximum amount of vitamin D (14.74ng/ml) was recorded in non Diabetics as compared to Diabetics female. It was marked that maximum amount of vitamin D (14.51ng/ml) was recorded in non cardiovascular as compared to cardiovascular disease female. It was obvious that maximum amount of vitamin D (14.51) was recorded in non asthmatics as compared to asthmatics female.

Discussion

This was the first research conducted on vitamin D level in female population of all ages (1 to 90 years) in district Peshawar.

In the present study 260 female of all ages were investigated. It was found that only 5 % have normal vitamin D level (40- 100 ng/ml) while 95% (< 40ng/ml) were vitamin D deficiency. This may be due to social setup of the region because mostly female are confined to homes, customs of Purdah and they may have less exposure to Sunlight, nutritional deficiency, metabolic disorders (diseases) and air pollution in district Peshawar.

In the present study 46.92% (<10ng/mL), 34.61% (10-20 ng/mL), 13.46 % (20-40 ng/mL) were severe vitamin D deficiency, mild deficiency,

hypovitaminosis respectively. The present study was consistent to Lips classification. According to the Lips taxonomy (Lips, 2001), hypovitaminosis D was showed in 92.6% of the lower socioeconomic status group (severe: 11.2%; moderate: 39.5%; and mild: 42.1%) and in 84.9% of the upper socioeconomic status group (severe: 4.9%; moderate: 25.5%; and mild: 57.6%), (Marwaha *et al.*, 2005).

A study on female students in Yazd city presented that 60% of the girls have vitamin D deficiency with the serum level of 25-OH vitamin D < 20ng/ml and out of them 20% experienced from severe deficiency. Moussavi *et al.* (2005) in Isfahan also study (2004), in which the total frequency of vitamin D in both categories of males and females has been 46% and 14.5% of girl suffered from severe deficiency.

In the present study, 260 females of all age groups (1-90 years), who suffer with 'aches and pains' were investigated for vitamin D deficiency using 25-OH-D₃. All subjects belong to Pakistan and with similar cultural practices, and religion. The mean vitamin D level of the study population (14.44 ± 12.10ng/ml) was recognized to be sound within the reference range (9-36 ng/mL). Investigation based on age subgroups presented low level (10.83ng/ml) of vitamin D in female between 16 and 30 years of age. The female of this age group are mostly student and confined to rooms for the purpose of study. The female of this age group also fully cover their selves. These also showed with the period of greatest fecundity in Pakistan, and females of this age group are mostly house-bound. Generally breastfeeding is experienced and may also effects vitamin D level. Greatest vitamin D levels (16.42ng/ml) were found among women of 31-45 years, of middle age group, frequently married women with comparatively more outside activities. While female >75 year of age vitamin D level was (12.53ng/ml), this is due to immobility confined to rooms and not expose to sunlight.

The present multivariate study also found that vitamin D insufficiency improved gradually with age and higher in women in all age groups. The women

aged >75 years, vitamin D deficiency mean (12.53ng/l) was over four-fold greater than women aged 31-45 years (16.42ng/ml).

Conclusion

It was concluded from the present study that 95% of the female population of Peshawar had vitamin D deficiency (<40 ng/mL). Those female who are at risk had peak vitamin D deficiency included children (13.74ng/mL), young female (10.83ng/mL), old (12.53ng/mL), obese (13.61ng/mL), poor nutrition (13.17ng/mL), highly educated female (12.77ng/mL) and those who work in offices (12.10ng/mL), upper socio economic background (14.04ng/mL) and inhabitant in closed houses (14.53ng/mL) and those not expose to sunlight (4.37ng/mL). While it was heartening that 5% of the population had a normal or optimal level of vitamin D (> 40 ng/mL). Out of 95 % Vitamin D deficiency, the severe deficiency (<10ng/mL), mild deficiency (10-20 ng/mL), Hypovitaminosis (20-40 ng/mL) were 46.92%, 34.61%, 13.46% respectively. The existing data have associated with suboptimal vitamin D level with many chronic diseases. The responsible factors for vitamin D deficiency in the present study were social setup of the region because mostly female are confined to homes, customs of Purdah and they may have less exposure to Sunlight, nutritional deficiency, metabolic disorders (diseases) and air pollution in district Peshawar.

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