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EVALUATION OF VITAMIN D STATUS IN APPARENTLY HEALTHY WOMEN ABOVE 20 YEARS OF AGE ATTENDING A TERTIARY CARE HOSPITAL

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ABSTRACT

Introduction: There is widespread prevalence of vitamin D deficiency among Indian postmenopausal women. However, there is limited information of the vitamin D status in apparently healthy Indian women of reproductive age group. **Objective:** To estimate the prevalence of Vitamin D deficiency among apparently healthy women aged 20 years and above attending a tertiary care hospital and to study the association of low vitamin D levels with biochemical and socio-demographic parameters. **Methods:** The descriptive study was carried out in 74 apparently healthy women subjects above 20 years of age who were selected randomly from health plan clinic of St John's Medical College and Hospital, Bangalore. These subjects, who were divided in three groups: Premenopausal (21-45years), Menopausal (46-55years) and Postmenopausal (>55 years) age groups, underwent anthropometric measurements, and biochemical evaluation for vitamin D status along with a questionnaire to identify the sociodemographic factors. Descriptive statistics was carried out in the present study. **Results:** Hypovitaminosis D was identified among all the subjects included in the study with a mean serum 25(OH) total vitamin D level (ng/ml) of 13.8 $\pm$ 5.4 S.D (95% confidence interval: 7.0 ng/ml – 26.8 ng/ml). Out of all the subjects, 17.6% were vitamin D insufficient (20.0 –<30.0 ng/ml), 56.8% were vitamin D deficient (<20.0 ng/ml) and 25.7% were moderately vitamin D deficient (<10 ng/ml). The serum 25(OH) D levels showed a negative correlation with serum PTH levels, but was not statistically significant ($p > 0.05$, $r = -0.548$), more than 50% of subjects with severe Vitamin D deficiency had PTH levels within normal range. **Conclusion:** The prevalence of vitamin D deficiency among the study population was 100% across the different age groups, despite the abundant sunshine in India.

To clearly state the enormity of Vitamin D deficiency status in India continued research and epidemiological investigations are required.

KEYWORDS: 25(OH) D = 25-hydroxyvitamin D; PTH = Parathyroid hormone.

INTRODUCTION

Vitamin D is a fat soluble vitamin required for bone mineralization and musculoskeletal health. There is emerging evidence on the wide spread extra skeletal effects of Vitamin D other than its musculoskeletal effects. Adequate levels of Vitamin D are maintained through cutaneous synthesis on sun exposure to ultraviolet B rays (290 – 315nm) and through limited dietary sources (fatty fishes such as salmon, mackerel and sardine, cod liver oil, egg yolk and milk). A number of factors such as duration and time of sun exposure, latitude, season, atmospheric pollution, clothing style, use of sun blocks, skin pigmentation as well as obesity and the presence of several chronic diseases also influence the biosynthesis and bioavailability of Vitamin D and contribute to the risk of impaired Vitamin D status of the body.[1,2]

Vitamin D inadequacy is a widespread disorder across all the age groups among men and women in developing countries like India regardless of their geographical location. A circulating level of 25(OH) Total Vitamin D of > 30ng/ml or 75nmol/L is essential to provide the physiological functions. Vitamin D deficiency causes rickets in children, and will precipitate osteopenia, muscle weakness, myalgia, osteoporosis and fracture risk in adults.[3, 4]

The most recent reports on Vitamin D status from Indian population demonstrated a prevalence of more than 70% despite of its abundant sunshine.[2] The aim of this paper is to provide a glimpse of idea about the gravity of Vitamin D deficiency among apparently healthy women above 20 years of age, and so that we might know which age groups are most affected and Vitamin D is a preventable disorder with adequate supplementation. This study aimed to evaluate the association of low Vitamin D levels with biochemical parameters such as calcium, inorganic phosphorus and parathyroid hormone and sociodemographic factors such as locale, duration of sun exposure, use of sun screens, socioeconomic status and diet pattern.

MATERIALS AND METHODS

Study Subjects

A descriptive study was conducted in 74 apparently healthy women above 20 years of age attended the health plan clinic and occupational and staff clinic of St John's Medical College and Hospital, Bangalore during the period of one year (July 2013 to June 2014). None of them had been treated with calcium or vitamin D supplements, hormone therapy, oral contraceptive pills, thiazides, steroid preparations, anticonvulsants or other medications that might affect vitamin D metabolism. Women with thyroid and parathyroid diseases, chronic liver and kidney diseases, overt bone and mineral metabolic diseases, mal absorption syndromes, obesity, metabolic syndrome and who had undergone bowel surgery were excluded. The study was approved by the Institutional Ethics Committee and all subjects gave written informed consent.

Method of Sample Collection

Serum 25-hydroxy total Vitamin D (25 –OH total vitamin D), Parathyroid hormone (iPTH), Calcium (Ca) and inorganic phosphorus (P) were measured along with a questionnaire to identify the socio-demographic factors like Body Mass Index (BMI), type of work, socioeconomic status, locale, regular use of sunscreens, duration of sun exposure and diet pattern. Random venous blood samples were collected in serum separator tubes observing all safety precautions, and the biochemical estimation was done within 3 – 4 hours of sample collection after checking for sample integrity. The serum 25(OH) D is the most reliable marker of Vitamin D status.

Biochemical estimations were carried out using automated analyser (Dimension Clinical Chemistry System RxL Max, Siemens Diagnostics) and commercial kits (Siemens). The biological reference interval for serum total calcium is 8.5-10.1 mg/dl and inorganic phosphorus is 2.5-4.9 mg/dl. The serum concentrations of 25(OH) D (biological reference interval: >30 ng/ml) and PTH (biological reference interval: 11.1- 79.5 pg/ml) were measured by CLIA (Advia Centaur XP system from Siemens Diagnostics). Serum 25(OH) D level of 20 –<30 ng/ml was classified as Vitamin D insufficiency, and levels <20 ng/ml were classified as Vitamin D deficiency and levels <10 ng/ml as severe Vitamin D deficiency. Secondary hyperparathyroidism was defined by serum PTH level of >65pg/ml.

Statistical Analysis

Statistical analysis was carried out using SPSS.18. Descriptive statistics was carried out. Data were presented as mean ± SD or number (%) for categorical variables. Independent t test was done to compare Vitamin D levels with sociodemographic factors. Analysis of Variance (ANOVA) was used to compare Vitamin D levels with Calcium, Inorganic Phosphorus and PTH. Pearson Correlation was used to assess simple association between Vitamin D and PTH levels. A p value less than 5% was considered to be statistically significant.

RESULTS

The characteristics of the subjects are shown in Table 1. The mean (± SD) serum 25(OH) D concentration for all 74 subjects was 13.8±5.4ng/ml (95% confidence interval: 7.0 ng/ml – 26.8ng/ml). The prevalence of Vitamin D deficiency among the study population was 100%. Out of the 74 apparently healthy women above 20 years of age participated in the study, 13 (17.6%) were Vitamin D insufficient, 42(56.8%) were Vitamin D deficient and 17 (25.7%) were severely Vitamin D deficient. Among the subjects with Vitamin D insufficiency and deficiency, the mean serum 25(OH) D levels (ng/ml) were 21.8 ± 2.3 S.D, 14.3 ±2.9 S.D. The mean serum 25(OH) D levels (ng/ml) among premenopausal, menopausal and postmenopausal age groups are 13.6 ±5.8 S.D, 14.5 ± 5.2 and 13.3 ± 5.3 respectively. The estimates were based upon the parameters with a Gaussian distribution of values. Women in menopausal age group showed a better vitamin D status when compared to premenopausal and postmenopausal age groups. The distribution of Vitamin D deficiency was equal among premenopausal, menopausal and postmenopausal age groups (p = 0.718). However, there was an increase in moderate Vitamin D deficiency (< 10 ng/ml) among premenopausal age group (n=8) especially among professionals, when compared to menopausal (n=5) and postmenopausal age groups (n=6).

Table I – Subject Characteristics.

Subject Characteristics	Frequency (n)	Percent (%)
Age distribution		
Premenopausal	25	33.8
Menopausal	25	33.8
Postmenopausal	24	32.4
Socioeconomic status		
APL	57	77
BPL	17	23
Locale		
Rural	15	20.3
Urban	59	79.7

Duration of Sun exposure (per day) < 15minutes 15 – 30 minutes >30 minutes	 57 16 1	 77 21.6 1.4
Use of Sun Screens Yes No	 26 48	 35.1 64.9
Diet Pattern Veg Non Veg	 38 36	 51.4 48.6
Body Mass Index Normal Under weight	 60 14	 81.1 18.9

Table II – Vitamin D status among the study population.

Vitamin D Status	Frequency (n)	Percent (%)
Normal	0	0
Insufficiency	13	17.5
Deficiency	61	82.5
TOTAL	74	100

*****No missing cases.

The serum 25(OH) D levels showed a negative correlation with serum PTH levels, but was not statistically significant ($p > 0.05$, $r = -0.548$). There was no statistically significant correlation of low vitamin D levels with total calcium and inorganic phosphorus. However, there was a significant difference in calcium ($p=0.004$) and inorganic phosphorus ($p = 0.00$) levels between the women of different age groups. The calcium and phosphorus were low in postmenopausal women when compared to women of other age groups. The mean serum total calcium (mg/dl), serum inorganic phosphorus (mg/dl) and serum PTH (pg/ml) among the study population was 7.6 $\pm$0.6 mg/dL, 3.1 $\pm$ 0.4 mg/dL and 53.7 $\pm$ 19.01 pg/ml respectively.

Table III – Comparison of Vitamin D levels with Biochemical Parameters.

	Vitamin D	Calcium	Phosphorus	PTH
n	74	74	74	74
Pearson Correlation				
Vitamin D	1	0.606	0.441	-0.548
Calcium	0.606	1	0.442	0.486
Phosphorus	0.441	0.442	1	0.300
PTH	0.548	0.486	0.300	1

**Tested using Pearson Correlation bivariate analysis (p value < 0.01).*

Table IV – Comparison of Biochemical Parameters between the study groups.

BETWEEN GROUPS	Sum of Squares	dF	Mean Squares	F	P value
Vitamin D	19.599	2	9.800	0.332	0.718
Calcium	3.584	2	1.792	5.880	0.004
Phosphorus	2.333	2	1.167	9.119	0.000
PTH	1628.124	2	814.062	2.335	0.104

**Tested using Analysis of Variance (ANOVA).*

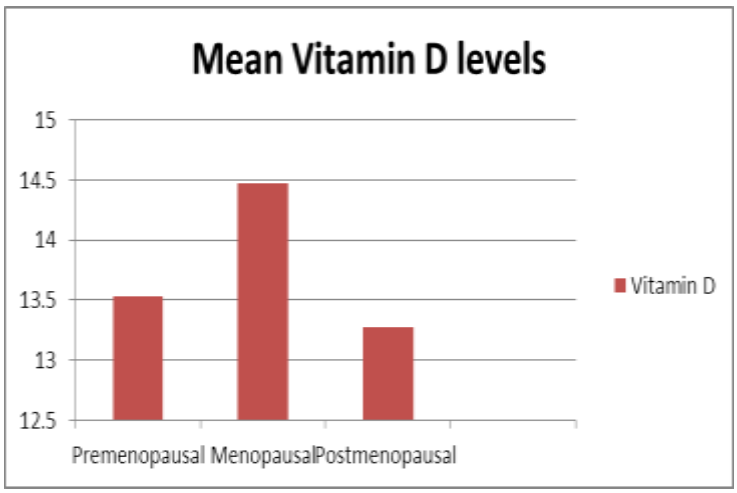

Figure 1 – Mean Serum 25(OH) D levels in women of different age groups.

In the current study, the comparison of low Vitamin D levels with various sociodemographic factors such as locale, duration of sun exposure, regular use of sun screens, socioeconomic status, diet patterns and BMI did not show any statistically significant association ($p > 0.05$). Majority of the participants in this study, with apparently deficient Vitamin D status were symptomatic; however back pain and muscle pain were the most common symptoms amongst 6.7% (n = 5, not statistically significant) belonging to severely deficient group.

Table V – Comparison of Vitamin D levels with the Sociodemographic Factors.

Socio demographic Factors		N	Mean (ng/ml)	SD	P value
Diet pattern	Veg	38	13.213	5.645	0.366
	Non Veg	36	14.351	5.101	0.365
Sun exposure	< 15 minute	57	13.372	5.567	0.200
	15-30 minute	16	15.343	4.630	0.162
Sunscreen use	Yes	26	13.273	6.140	0.565
	No	48	14.034	4.971	0.590
Type of work	Indoor	72	13.284	5.538	0.657
	Outdoor	2	13.567	5.104	0.542
Socioeconomic status	APL	57	13.707	5.663	0.862
	BPL	17	13.967	4.447	0.844

Locale	Rural	15	13.475	5.016	0.816
	Urban	59	13.841	5.508	0.807
Body mass index	Normal	60	13.891	5.659	0.622
	Underweight	14	13.233	4.105	0.683

**Tested using Independent t test. P value less than 0.05 is considered to be statistically significant.*

DISCUSSION

Widespread prevalence of Vitamin D deficiency across Indian population is undeniable. Several risk factors have been identified, which include female gender, extremes of age, covered clothing style, low socioeconomic background and winter season. Varying prevalence of Vitamin D deficiency among women has been reported globally. The most recent studies demonstrated that more than 70% of Indian population suffers from Vitamin D insufficiency (< 30ng/ml).[2] The present study extended the assessment of vitamin D status in apparently healthy women above 20 years of age and showed that the 100% of the subjects had Vitamin D inadequacy across all the age groups. The mean 25(OH) D level observed by us in this study population was 13.8±5.4 ng/ml. This is in good agreement with a large systematic review including fifty studies on Vitamin D status of ostensibly healthy Indians from different parts of the country done by Ritu and Ajay et al.[2]

In the present study, the mean serum 25(OH) D levels (ng/ml) among premenopausal, menopausal and postmenopausal age groups are 13.6 ±5.8 S.D, 14.5 ± 5.2 and 13.3 ± 5.3 respectively. The distribution of Vitamin D levels was equal among premenopausal, menopausal and postmenopausal age groups. However there was a significant increase in severe vitamin D deficiency among young women which could be attributable to their restricted indoor activities, regular use of sun blocks (SPF>8) and inadequate intake of Vitamin D rich foods as a part of weight reduction. Among elderly postmenopausal women, multiple factors can contribute to Vitamin D inadequacy including dietary deficiency, undetected fat mal absorption and reduced cutaneous biosynthesis. The serum calcium and inorganic phosphorus levels were low in postmenopausal women. Low calcium and high phytate (brans) and diluted milk in Indian diet can be the major risk factors for calcium inadequacy. Without enough Vitamin D level in blood stream, dietary calcium will not be absorbed.[6,7]

Many studies have shown significant negative correlation between Vitamin D and PTH, but our study even though showed a negative correlation (r = -0.548) between the variables, it is not statistically significant. We found similar findings in a large observational study by Lappe et al[8], which showed no significant correlation between Vitamin D and PTH (p = 0.54). PTH levels are often normal when 25(OH) D levels fall below 20ng/ml. No universal guideline exists on exact serum 25(OH) D levels at which PTH rises. This is very evident in our study wherein Vitamin D insufficiency did not correlate with PTH levels (r = -0.548, p>0.05).

The comparison of low Vitamin D levels with various sociodemographic parameters such as locale, time and duration of sun exposure, regular use of sun screens, socioeconomic status, diet pattern and BMI did not show any statistically significant association (p>0.05). Recent epidemiological data documents the higher prevalence of Vitamin D inadequacy irrespective of the region, latitude, season, age, gender or ethnicity. A study from Kashmir [34.3^0N] by Zargar et al[10], showed a prevalence of 83% among 92 healthy adults (mean age 28.15±4.9 years) and with a mean serum 25(OH) D levels of 11.26±4.42 ng/ml. Harinarayanan et al[11], from Tirupathi [13.4^0N] also reported a similar picture of Vitamin D levels 12.1±3.32 ng/ml. From this study it can be inferred that the change in latitude did not affect Vitamin D levels. A population based study by Islam et al[12] from Dhaka [23.7^0N], n = 96 showed a similar vitamin D status among Veiled women (12.12±9.04 ng/ml) and unveiled women (12.24±4.4 ng/ml). In a study of normal healthy individuals including doctors, nurses and soldiers in New Delhi by Goswami et al[13], serum 25(OH) D levels were suboptimal (<30 ng/ml) in all the groups irrespective of age, gender, BMI, region and duration of sunlight exposure.

The major factors attributing for the high prevalence of Vitamin D deficiency in India, despite plentiful sunshine could be many including cultural taboos, nutritional factors and inadequate sun exposure.[2] Most widely consumed dietary sources of Vitamin D such as dairy products have very low Vitamin D content, and they are rarely fortified. Cooking practices such as deep frying, pressure cooking, repeated boiling of milk and adulteration of dairy products destroys available Vitamin D content of foods. Indian socio religious and cultural practices restrict adequate exposure to sunlight.[9] Indian skin tone with high melanin pigment (natural sunscreen) reduces cutaneous synthesis of Vitamin D. The extreme heat of summer sun, cosmetic concerns, urbanisation, atmospheric pollution and high population density limits the sun exposure and endogenous Vitamin D synthesis.[14,15] Publications indicating the wide prevalence of Vitamin D deficiency in healthy Indians have studied

subjects mostly from middle and upper classes. Similarly in our study also subjects from below poverty line were not represented well. The under nourished socioeconomically backward people constitute a larger percent of Indian population.

The sources of Vitamin D for Indian population are adequate sun exposure (15 to 45 minutes, 3 to 4 times a week between 10 am to 2 pm), vitamin D supplements (400 – 800IU Vitamin D3) and limited amount of dietary sources. 40IU Vitamin D3 per day increases the circulating 25(OH) D by 0.4 – 1.6 ng/ml.[2] Screen those who are at risk of deficiency based on diet, sun exposure, obesity indices, age and co-morbid diseases. Though some researchers in India have tried to establish the cut-off limits for Vitamin D deficiency, it is still largely based on the reference interval provided by the manufacturer's supplying the kit or the international standards. India is unique with its population diversity and genetic polymorphisms which can affect the Vitamin D levels in many ways. It is the need of the hour for each medical testing laboratory estimating and reporting Vitamin D levels to establish a population-based biological reference interval and thereby preventing the bias for over reported Vitamin D deficiency in India. The need for nutritional public health awareness campaigns and implementation of national health policies which care takes national level food fortification is important in India with a high prevalence of Vitamin D deficiency.

We recognise some limitations to our study. Although efforts were made to recruit subjects that would be typical of healthy women of different age groups and sample size was appropriate, it was relatively small. The blood samples were collected only once during the months of July 2013 through December 2013 and it would have been better if the subjects were evaluated at different time of the year to determine the effects of season on Vitamin D.

CONCLUSION

Despite the abundant sunlight in India, the prevalence of Vitamin D deficiency among women is 100% across the age groups. The aetiology for the high prevalence of Vitamin D deficiency is multifactorial, where lack of sun exposure and inadequate dietary intake are the most important factors. The evidence we have for the role of Vitamin D in multiple health outcomes is controversial, however we have to make sure that appropriate measures are taken to improve the nutritional status needed for good health.

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