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Serum 25-Hydroxy Vitamin D, Calcium,Phosphorus and Alkaline Phosphatase Levels In Healthy Adults Above the age of 20 Living in Potheri Village of Kancheepuram District , Tamilnadu

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ABSTRACT

Medical fraternity believed that Vitamin D deficiency is rare in southern part of tropical country like India. This study is aimed to determine the serum vitamin D, calcium, phosphorus and alkaline phosphatase levels in healthy subjects around Potheri, kancheepuram district of Tamilnadu. Total of 81 subjects above the age of 20, out of which 50 males and 31 females were recruited for the study. Institutional ethical committee clearance was obtained. After getting informed consent from each subject, a preformed questionnaire containing the details about socioeconomic status, religion, dietary habits , sun light exposure and skin color were filled. Venous blood was drawn to test vitamin D, calcium, phosphorus and alkaline phosphatase levels in the serum. Among 81 subjects, 73.91% had Vitamin D deficiency. Serum Vitamin D was not significantly correlated with serum phosphorus, serum alkaline phosphatase, skin color and living condition. But there exists positive correlation between serum calcium, sun light exposure with Vitamin D levels whereas socio economic status had a negative correlation. Adequate sun light exposure, fortification of food and oral intake of 800IU-1000IU Vitamin D daily are the best options to combat the Vitamin D deficiency.

INTRODUCTION

Vitamin D is a secosteroid synthesized in the skin when exposed to sun light. Sun light is the major source of this vitamin in the body. Vitamin D is collective term given to Vitamin D3 (Cholecalciferol) and Vitamin D2 (ergocalciferol). Cholecalciferol is synthesized from the skin when exposed to UV radiation. Ergocalciferol is obtained from fungi. Two consecutive hydroxylation reactions, one in liver and another in kidney converts it to active form 1,25dihydroxy Vitamin D (Calcitriol). 25-hydroxy vitamin D is the major circulating form of Vitamin D in blood, its evaluation in the body gives clear indication of vitamin D status in the body (Weaver and Fleet,2004).1,25 dihydroxy vitamin D exerts its action by binding to nuclear receptor called as vitamin D receptor(VDR).VDR is expressed in endocrine glands (pituitary, pancreas, parathyroid, gonads and placenta) (Temmerman, 2011), cardiovascular tissues endothelial cells, vascular smooth muscle cells,cardiomyocytes) (Harel*et al.*, 2011) and also on the immune cells like CD4+ and CD8+ T cells, B cells, neutrophils and antigen presenting cells such as macrophages and dendritic cells (Baeke *et al.*, 2010). In this modern era, Vitamin D has not only remained as a hormone controlling calcium and phosphorus homeostasis, but also evolved its role as an immuno-modulator.

This sunshine vitamin is the topic of debate from the beginning of this century. It was well known that, deficiency of this vitamin causes rickets in children, osteomalacia in adults (McCollum *et al.*, 1922, Wolf, 2004). Researchers are working hard to find out association between deficiency of this versatile vitamin in a variety of cardiovascular disorders like hypertension, obesity, diabetes, high triglyceride levels (Martins *et al.*, 2007), auto immune disorders like rheumatoid arthritis(Kroger*et al.*, 1993), multiple sclerosis (Nieves *et al.*, 1994), cancers like ovarian (Bischoff-Ferrari *et al.*, 2006), breast (Bertone-Johnson *et al.*, 2005), Colorectal (Garland *et al.*, 1991), prostate (Hanchette and Schwartz, 1992) and infections like TB (Nnoaham and Clarke, 2008, Martineau, 2012). It has been documented that more than

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1 billion people around the globe are Vitamin D deficient or insufficient (Holick, 2007).India, being a tropical country, it was believed that Vitamin D deficiency is seldom seen in our country (Harinarayan *et al.*, 1995, Harinarayan, 2005). But, to our surprise, opposite scenario prevails. Physical and sedentary mode of life style enhances Vitamin D deficiency. It is also the consequence of spending most of the time indoors, using the sun screens and protective clothing. Potheri is a suburb of Chennai, belongs to Kancheepuram district having the latitude of12.8255° N with plenty of sunlight throughout the year.

In south India, prevalence studies of Vitamin D status were done only in Tirupathi and Mysore, and such studies were lacking in Tamilnadu. So the study objective is to determine serum vitamin D levels in the study population of Potheri. Other parameters such as serum calcium, phosphorus and alkaline phosphatase levels were also evaluated.

MATERIALS AND METHODS

Healthy adults of both sexes above the age of 20 living in and around Potheri village were included in our study. Total of 81 subjects, out of which 50 males and 31 females were recruited. Institutional ethical committee clearance was obtained prior to the study. Written informed consent was got from every subject before collecting blood.

Demographic data such as height, weight, BMI & other details like diet, sun light exposure, socioeconomic status, skin color were collected using a preformed questionnaire.3 ml of the venous blood was drawn from each subjectand serum levels of Vitamin D, calcium, phosphorus and alkaline phosphatase were estimated.

Inclusion Criteria

1. Healthy male and female subjects above the age of 20.
2. Subjects working in SRM University, Potheri.

Exclusion Criteria

Subjects having diseases like TB, HIV, asthma, hypertension and diabetes mellitus.

Estimation of Serum Vitamin D

Method used was chemiluminescence micro particle assay. Architect 25- OH Vitamin D kit is loaded in Architect i system to estimate serum Vitamin D levels. In this method, levels below 20 ng/ml is considered as deficiency, 20-30 ng/ml is insufficiency and levels between 30-80ng/ml is optimum.

Estimation of Serum Calcium

This was done by using modified O-cresolphthalein Complexone method using Erba Mannheim Calcium estimation kit. Optical density of 10µl of test serum was read against blank and Standard at a wavelength of 578nm using UV spectro-photometer. Normal values of calcium using this kit will be 8.4-10.4mg/dl.

Estimation of Serum Phosphorus

This was done by molybdate UV method using Liquimax Phosphorus–SLR kit purchased from Avecon Healthcare Pvt Ltd. Optical density of 10 µl of test serum was read against blank and standard at a wavelength of 340nm using UV spectrophotometer. In this method, normal values of serum Phosphorus is in a range of 2.4-5.0mg/dl.

Estimation of serum alkaline phosphatase

This was done quantitatively by DEA buffer/DGKC method using Liquimax alkaline phosphatase purchased from Avecon Healthcare Pvt Ltd. Change in the optical density of 20µl of test serum was read against distilled water at 405nm. Normal range of alkaline phosphatase ranges from 80-315.

Statistical analysis

Analysis was performed using SPSS version 16. Continuous variables were expressed as mean ± SD. Discrete variables were expressed as frequency (percentage). Pearson's correlation test was used to find out the significant correlation between the variables. A p value of <0.01 or <0.05 was considered as significant. Significant difference between gender was analysed using independent t test and results were considered statistically significant if the p value is < 0.05.

RESULTS

Serum Vitamin D status was expressed in Table1.Study revealed, 73.91% had deficiency of Vitamin D, 17.39% had insufficiency and only 8.69% had optimum levels. Deficiency was more in females (87%) when compared to males (76%). Insufficiency was more in males (20%) compared to females (6%). Optimum levels of Vitamin D was seen in 4% and 6% of male and female subjects. 91.3% had less than optimum level of vitamin D.

Descriptive statistics and laboratory biochemical parameters are shown in Table 2. Subjects ranged in age from 20-72 years with a mean of 41.93±13.02. Mean sun light exposure daily was found to be 23.58±42.59 (minutes) in the study population. Mean serum Vitamin D, Calcium, phosphorus and alkaline phosphatase levels were 15.49±7.58, 8.95±2.03, 4.70±0.87 & 126.54±64.25 respectively. When we compared the genders serum calcium & serum phosphorus mean values were found to be high in females.

Table 3 compared serum Vitamin D levels with study variables. Serum Vitamin D was not significantly correlated with serum phosphorus, serum alkaline phosphatase, consumption of egg, nonvegetarian food & milk, skin color . There was significant correlation between serum Vitamin D and serum calcium, socio economic status, living condition and sunlight exposure. Vitamin D significantly correlated positively with serum calcium with correlation coefficient of 0.257 and sunlight exposure with correlation coefficient of 0.187 and living condition with a correlation coefficient of 0.307 whereas negatively correlated with

socioeconomic status with a correlation coefficient of -0.339. Also, there exists significant negative correlation between socio economic status and sunlight exposure with a P value of 0.001. **(**Correlation coefficient= -0.356)

Table 1: Basic characteristics.

Variables		Total (n=81)	Male (n=50)	Female(n=31)
			N (%)	n(%)
Serum vitamin D	Deficiency	73.91	38(76)	27(87)
	Insufficiency	17.39	10(20)	27(87)
	Optimum	8.69	2(4)	2(6)
Diet	Non veg	74	46(92)	28(90.3)
	Veg	6(7.4)	3(6)	3(9.7)
	Veg & egg	1(1.2)	12(24)	0
Egg consumption/ wk	1	19(23.5)	12(24)	7(22.6)
	2	39(48.1)	21(42)	18(58.1)
	3	17(21)	14(28)	3(9.7)
	No egg	6(7.4)	3(6)	3(9.7)
Non veg consumption/ wk	1	29(58.8)	15(30)	14(45.2)
	2	32(39.5)	21(42)	11(35.5)
	3	6(7.4)	3(6)	3(9.7)
	4	4(4.9)	4(8)	0
	7	1(1.2)	1(2)	0
	Vegetarian	7(8.6)	4(8)	3(9.7)
	Twice per month	1(1.2)	2(4)	0
No. of cups of milk consumption/ day	0.5	1(1.2)	0	1(3.2)
	1	42(51.9)	27(54)	15(48.4)
	2	29(35.8)	18(36)	11(35.5)
	3	7(8.6)	4(8)	3(9.7)
	4	2(2.5)	1(2)	1(3.2)
Skin color	Dark	26(32.1)	18(36)	8(25.8)
	Fair	15(18.5)	9(18)	6(19.4)
	Wheatish	40(49.4)	23(46)	17(54.8)
BMI Status	Normal	57(70.4)	37(74)	20(64.5)
	Overweight	19(23.5)	10(20)	9(29)
	Underweight	5(6.2)	3(6)	2(6.5)
Living condition	Concrete	60(74.1)	33(66)	27(87.1)
	Hut	1(1.2)	1(2)	0
	sheet	20.(24.7)	16(32)	4(12.9)

Table 2: Descriptive statistics and laboratory biochemical parameters.

Variable	mean±SD for total (n=81)	mean±SD for male (n=50)	mean±SD for female (n=31)
Age	41.93±13.0	44.72±13.53	37.42±10.91
Sunlight Exposure in min.	23.58±42.59	31.90±52.37	10.16±7.47
Serum vitamin D level	15.49±7.58	16.17±7.40	14.39±7.86
Serum calcium	8.95±2.03	8.81±2.09	9.18±1.95
Serum phosphorus	4.70±0.87	4.56±0.75	4.93±1.01
Serum alkaline phosphatase	126.54±65.54	130.96±67.35	119.41±59.29

Independent t-test was used to find the significant difference between the male and female subjects as depicted in Table 4. Significant difference exists between male and female in sun light exposure at 0.05 level. Mean sun light exposure was more in males (31.90±52.37) when compared to females (10.16±7.47) which makes p value significant.

Table 5 shows the correlation of Vitamin D with study variables with respect to genders. Among males, serum Vitamin D was significantly positively correlated with serum calcium, sun light exposure and living condition whereas it was negatively correlated with serum phosphorus and socioeconomic status. P value was significant for serum calcium, serum phosphorus, sun light exposure, socioeconomic status and living condition. Correlation was significant at 0.05 level. Among females, serum vitamin D was positively correlated with serum calcium and negatively correlated with socioeconomic status and milk consumption.P value was significant for all these 3 parameters at 0.05 level.

Table 3: Correlation of Vitamin D with study variables.

variables	Pearson correlation	P value
Serum calcium	0.257	0.021
Serum phosphorus	-0.207	0.064
Serum alkaline phosphatase	-0171	0.128
Diet	-0.101	0.37
Egg condumption/wk	0.021	0.858
Milk consumption/day	-0.211	0.059
Non veg consumption/wk	-0.152	0.182
Skin color	-0.002	0.982
Living condition	-0.307	0.005
Socioecon omic status	-0.339**	0.002
Sunlight exposure in min	0.187*	0.034

**correalion is significant at 0.01 level
*correlation is significant at 0.05 lavel.

Table 4: Male and female difference.

variables	Independent t test	P value
Vitamin D	-1.139	0.258
Serum calcium	0.76	0.449
Serum phosphorus	1.649	0.103
Serum alkaline phosphatase	-0.713	0.478
Socio economic status	1.723	0.089
Sunlight exposure in min	-3.187	0.002*

*Correlation is significant at 0.05 level.

Table 5: Correlation of Vitamin D with study variables with respect to gender.

Variables	Male		female	
Vitamin D	Pearson correlation	P value	Pearson correlation	P value
Serum calcium	330*	0.019	0.466*	0.008
Serum phosphorus	-0.319*	0.024	-0.03	0.872
Serum alkaline phosphatase	-0.181	0.208	-0.187	0.314
Socio economic status	-0.325	0.021	-0.411	0.022
Sunlight exposure in min	0.327*	0.02	-0.005	0.978
Skin color	0.138	0.339	-0.194	0.296
Living condition	0.283*	0.046	0.327	0.073
Diet	-0.017	0.907	-0.302	0.098
Egg consumption/wk	0.016	0.911	0.021	0.916
Milk consumption/day	-0.208	0.148	-0.390	0.03
Non veg consumption/wk	-0.168	0.254	-0.195	0.294

*Correlation is significant at 0.05 level.

DISCUSSION

Vitamin D deficiency among Indians is on the raise. In our study, 73.91% of total subjects had deficiency which was in close association with the study conducted in urban adults of Lucknow (Arya *et al.,* 2004), females of reproductive age group in Tirupathi (Harinarayan *et al.,* 2011), rural pregnant women of Barabanki (Sahu *et al.,* 2009) and healthy adults of Karachi, Pakistan (Mahmood *et al.,* 2009) where it was found to be 78.3 %, 76.3 %, 74 % & 76.2 % respectively. Subjects included in this study were working in SRM University from 8am -4pm. Sun light exposure in these subjects was very limited. Mostly people were leading sedentary indoor life style and not taking Vitamin D supplements or Vitamin rich diet which might be the reason for higher deficiency levels in this area. Darker complexion might be another contributing factor for higher deficiency levels since high

level of melanin reduces cutaneous synthesis of vitamin D (Matsuoka *et al.,* 1991). Serum calcium was positively correlated with serum Vitamin D in our study indicating that Vitamin D is involved in the absorption of calcium from the gut (Heaney *et al.,* 2003, Heaney, 2003). Serum phosphorus and alkaline phosphatase levels were within the normal limits but not correlated with Vitamin D status in our study, was similar to the study conducted in Karachi (Shaheen *et al.,* 2012). Vitamin D increases the efficiency of intestinal absorption of calcium to 30-40% and phosphorus absorption approximately by 80% (Holick, 2007). Vitamin D deficiency state leads to secondary hyperparathyroidism which results in loss of phosphorus in the urine and decreases intestinal absorption of phosphorus. This cause low or low normal phosphorus concentration. Low normal calcium and low normal phosphorus both will cause insufficient calcium phosphorus product which is important for bone mineralization process. Defective mineralization causes rickets in children and osteomalacia in adults (Holick, 2002, Holick, 2003,Holick, 2004). During deficiency of Vitamin D, there should be low levels of calcium and phosphorus but alkaline phosphatase levels should raise. But in our study, calcium was reduced but phosphorus and alkaline phosphatase levels were within normal limits indicating that bone mineralization was not yet affected.

We found positive correlation between sun light exposure and Vitamin D status. It is already well known fact that major source of Vitamin D is the sun light. Our study was supported by a study conducted in Saudi where exposure to sun light resulted in 2½ fold increase in 25-OH Vitamin D3 levels (Sedrani *et al.,* 1983).

Socioeconomic status was having negative correlation with Vitamin D. And also, there was a significant negative correlation between socioeconomic status and sun light exposure.It is assumed that people belonging to higher socioeconomic status had limited sun light exposure which made them prone for vitamin D deficiency. Present study was supported by a cross sectional hospital based study conducted in children at Chennai where similar findings were observed (Vasudevan *et al.,* 2014).

It was proven from study conducted in Belgium that skin pigmentation had negative influence on Vitamin D synthesis (Libon *et al.,* 2013), but our study fails to give correlation between D vitamin and skin color. Our study supports that Vitamin D deficiency is common irrespective of skin color (Cavalier *et al.,* 2009).

Diet such as milk, egg or non-vegetarian food consumption also had no influence on Vitamin D levels in this study. Dietary sources are very low in Vitamin D content. Indians are not usually eating salmon, sardines, tuna, mackerel which are rich in D content. Egg yolk is also having only 20IU of Vitamin D. Dietary source of Vitamin D for vegetarians is unfortified milk which contain only 2IU of Vitamin D /100ml. Dilution and adulteration of milk will not be able to provide sufficient D vitamin. This might be the reason for negative correlation of Vitamin D and milk in this study. In India Vitamin D fortified food products are not commonly sold as in case of Western countries which made study subjects vulnerable to Vitamin D deficiency. In this study, subjects living in concrete houses had a positive correlation with Vitamin D may be because concrete is a good reflector of sun light which makes it as a source of this sun shine vitamin.

CONCLUSION

Study concludes that Vitamin D deficiency is common in Potheri, suburban part of Chennai, Tamilnadu. Even though sun light is adequate in this part of country, sun light exposure is not sufficient to synthesize Vitamin D in the body. Food products like milk, yogurt, butter, ghee, infant formulas, orange juice , mango juice, wheat flour, maida, rice & rice flour should be fortified to ensure sufficient Vitamin D. It is advisable to take oral intake of 800IU-1000IU Vitamin D daily. Sun light exposure daily for 10 minutes on face and arms is very much needed to raise the sun shine vitamin. Indian government should lay proper guidelines for attaining adequate Vitamin D levels in this subcontinent .

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