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Research Article

SERUM 25-HYDROXY VITAMIN D, RELATION WITH MILK AND MILK PRODUCTS

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ABSTRACT
It was believed that vitamin D deficiency is rare in northern part of Haryana (India) as per the food habit with milk and milk products, famous in Haryana. The present study is aimed to determine the serum 25-Hydroxy Vitamin D, relation with milk and milk products, Blood samples were collected from employees and students of either sex aged 20-60 years, who were willing to participate, of Maharishi Markandeshwar Institute of Medical Sciences & Research, Mullana, Ambala, in Haryana. 200 apparently healthy subjects were included in the study, out of which 50% males and 50% females were recruited. Institutional ethical committee approval was obtained. After getting informed consent from each subject, a preformed questionnaire containing the details about socioeconomic status, dietary habits was recorded. Venous blood was derived with a standard vain puncture method to estimate the levels of 25-(OH) vitamin D in serum. Among 200 subjects, 57.5% had vitamin D deficiency and 42.5 were found with normal reference range of vitamin D (30-100 ng/ml). Serum vitamin D was not significantly correlated with tea or coffee (p=0.394), pudding (p=0.430). But there was significant correlation with milk (p=0.000), yoghurt (p=0. 001), butter (p=0.000) and cheese (p=0.000). Adequate sun light exposure, supplementation and daily consumption of milk and milk products are the best options to prevent vitamin D deficiency.

Keywords*: Vitamin D, Sun-Exposure, Milk and Milk Products, UV-B*

INTRODUCTION
Vitamin D a fat-soluble vitamin, is dependent on many factors like sun exposure, its duration and time of exposure, skin pigmentation, latitude, air pollution and clothing. The best latitude of the world for abundant sunshine is in between 42°N and 42°S which is good for synthesizing vitamin D subcutaneously from 7-dehydrocholesterol. Sun exposure for half an hour from 10 AM to 2 PM afternoon (maximum ultraviolet-B rays transmitted from the sun) falling on face and arms is adequate for vitamin D deficiency prevention (Holick, 1994).

There are two types of vitamin i.e. vitamin D3 and vitamin D2. Vitamin D3 is derived from the precursor 7-dehydrocholesterol which is synthesized in skin, on the other hand vitamin D2 is obtained from ergosterol found in foods like bread, milk, yeast, mushrooms and cod liver oil. The synthesis of biologically active form from vitamin D3 is a two-step process, first it is hydroxylated to 25(OH)D3 in the liver and followed by hydroxylation in kidney to produce the final active form i.e.1,25$(OH)_2$ D (Norman, 2008). Vitamin D is synthesized by the exposure of sunlight, UV-B photons (wavelength 290-315 nm) can penetrate skin where7-DHC (7-dehydrocholesterol) absorption takes place and UV-B helps to breaking B and makes pre-D3 (pre-cholecalciferol) which isomerizes the double bonds and makes 25(OH)D (Holick, 2003). In the liver, vitamin D is metabolized and circulated to tissues and stored for further activation. Some of these are converted in the kidney as hormone that is 1,25$(OH)_2$D {1, 25(dihydroxy) cholecalciferol} and this form of vitamin D is biologically active. In the kidney, various factors like serum phosphorus and PTH, are responsible for regulating the production of 1, 25 $(OH)_2$ D which in turn, is responsible for regulating calcium metabolism in tissues, bone and intestine (3). VDRs have been reported in almost all the tissues. 1, 25 $(OH)_2$ cholecalciferol helps to maintain cell growth and

maturation, it is also reported that vitamin D plays important role in insulin secretion, inhibition of renin production and regulation of activated T and B lymphocytes and macrophages (Adorini 2002).

The present study was carried out in Biochemistry Department at Maharishi Markandeshwar Institute of Medical Sciences & Research, Mullana, Ambala, in Haryana over a period of 24 months. The study was carried out after the recommendation of Institutional Ethics Committee of Maharishi Markandeshwar University (MMU). The study population was derived from the students and employees of MMU. A total of two hundred "200" healthy volunteers aged 20-60 years of either sex with different socioeconomic background and coming from rural and urban setup were selected for the study. An informed written consent and detailed history of all the subjects was taken. A thorough general and systemic examination was performed and the findings were entered in a pre-designed Performa.

INCLUSION CRITERIA

- Age: 20 years to 60 years, apparently healthy and living in Haryana for about 5 years.

EXCLUSION CRITERIA

- Any problem of gastrointestinal, liver or kidney diseases, lactating and pregnant women, subjects taking minerals and vitamin D supplements, bone tumors, osteomyelitis and the drugs which effect on bone metabolism for example antiepileptic drugs.

Estimation of Serum Vitamin D

Serum vitamin D levels were estimated by direct competitive Chemiluminescence Immunoassay (CLIA) using Monobind Inc. USA kit for CLIA supplied by Lilac Pvt. Ltd (Endres and Rude 1999). In this method, levels <30 ng/ml are considered as deficiency and levels between 30-100 ng/ml are normal.

Statistical analysis

Analysis was done using SPSS version 20. Data were expressed as frequency (percentage). Chi square test was used to find out the significant correlation between the variables. A p-value of <0.05 was considered as significant.

RESULTS

Table given below, showing that 26.5% of all study subjects found with to consume milk daily. 89.5% study subjects were found to consume tea/ coffee regularly. Only 5% of study subjects were found to consume pudding on daily basis. 41.5% of study subjects used to consume yoghurt regularly. 37.5% of respondent used to consume butter regularly while 7.5% of them had never consume butter. Only 0.5% of study subjects used to consume cheese regularly while 29.5% of them were found to consume cheese 1-2 times per week.

Table 1 and figure 1: Distribution of study subjects by vitamin D level with respect to milk

Milk	Vitamin D						χ^2 (df)	p Value
	Normal		Abnormal		Total			
	No.	%	No.	%	No.	%		
Used to daily	46	54.1	7	6.1	53	26.5	68.456 (4)	0.000
1-2 tpw	25	29.4	88	76.5	113	56.5		
1-2tpm	6	7.1	17	14.8	23	11.5		
seldom	2	2.3	1	0.9	3	1.5		
Never	6	7.1	2	1.7	8	4.0		
Total	85	42.5	115	57.5	200	100.0		

tpw=times per week, tpm=times per month

Table 1 showing that association of vitamin D level was found statistically significant (p=0.000) with milk consumption of all study subjects.

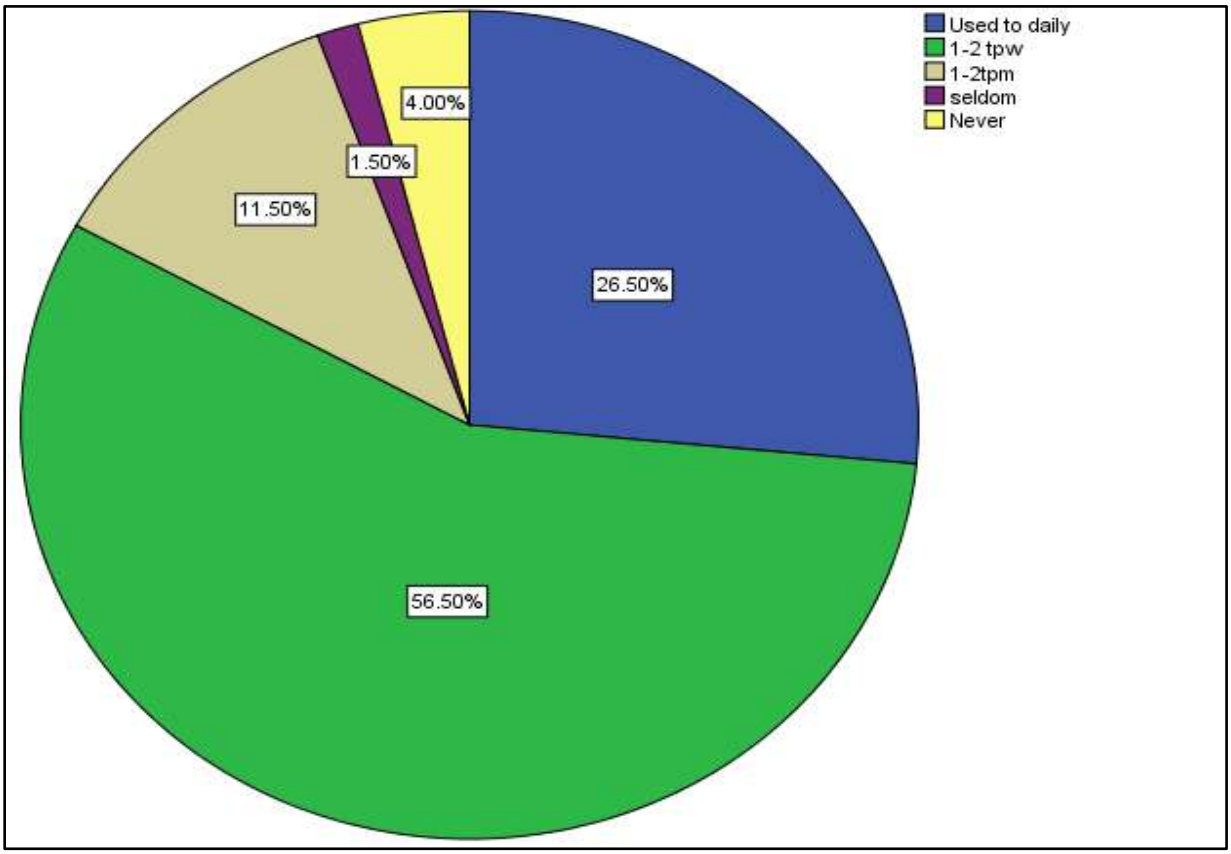

Figure 1

Figure 1 showing that the consumption habit of milk in all the study subjects in percentage. Maximum 56.5 % of subjects used to milk 1-2 times per week and least 1.5 % of subjects were found with the habit to consume milk seldom.

Table 2 and figure 2: Distribution of study subjects by vitamin D level with respect to tea or coffee

Tea or Coffee	Vitamin D						χ^2 (df)	p Value
	Normal		Abnormal		Total			
	No.	%	No.	%	No.	%		
Used to daily	75	88.2	104	90.5	179	89.5	4.090 (4)	0.394
1-2 tpw	2	2.4	2	1.7	4	2.0		
1-2tpm	3	3.5	2	1.7	5	2.5		
Seldom	2	2.4	0	--	2	1.0		
Never	3	3.5	7	6.1	10	5.0		
Total	85	42.5	115	57.5	200	100.0		

tpw=times per week, tpm=times per month

Table 2 showing that association of vitamin D level was found statistically not significant (p=0.394) with the consumption of tea / coffee

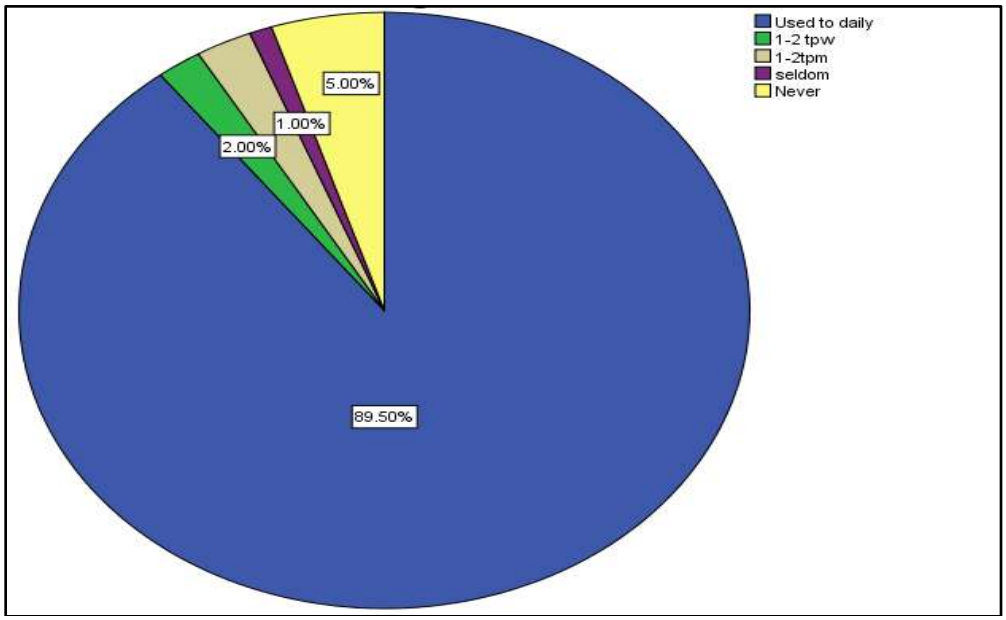

Figure 2
Figure 2 showing that the maximum 89 % of subjects were found used to tea & coffee daily.

Table 3: Distribution of study subjects by vitamin D level with respect to pudding

Pudding	Vitamin D						χ^2 (df)	p Value
	Normal		Abnormal		Total			
	No.	%	No.	%	No.	%		
Used to daily	--	--	--	--	--	--	3.826 (4)	0.430
1-2 tpw	6	7.1	4	3.5	10	5.0		
1-2tpm	31	36.5	51	44.3	82	41.0		
Seldom	24	28.2	27	23.5	51	25.5		
Never	24	28.2	33	28.7	57	28.5		
Total	85	42.5	115	57.5	200	100.0		

tpw=times per week, tpm=times per month

Table 3 showing that association of vitamin D level was found statistically not significant (p=0.430) with the consumption of pudding.

Table 4: Distribution of study subjects by vitamin D level with respect to yoghurt

Yoghurt	Vitamin D						χ^2 (df)	p Value
	Normal		Abnormal		Total			
	No.	%	No.	%	No.	%		
Used to daily	32	37.6	51	44.3	83	41.5	19.616 (4)	0.001
1-2 tpw	18	21.2	44	38.3	62	31.0		
1-2tpm	31	36.5	14	12.2	45	22.5		
Seldom	3	3.5	2	1.7	5	2.5		
Never	1	1.2	4	3.5	5	2.5		
Total	85	42.5	115	57.5	200	100.0		

tpw=times per week, tpm=times per month

Table 4 showing that association of vitamin D level was found statistically significant (p=0. 001) with consumption of yoghurt.

Table 5: Distribution of study subjects by vitamin D level with respect to butter

Butter	Vitamin D						χ^2 (df)	p Value
	Normal		Abnormal		Total			
	No.	%	No.	%	No.	%		
Used to daily	53	62.4	22	19.1	75	37.5	42.797 (4)	0.000
1-2 tpw	9	10.6	46	40.0	55	27.5		
1-2tpm	10	11.8	23	20	33	16.5		
Seldom	7	8.2	15	13.1	22	11.0		
Never	6	7.0	9	7.8	15	7.5		
Total	85	42.5	115	57.5	200	100.0		

tpw=times per week, tpm=times per month

Table 5 showing that association of vitamin D level was found statistically significant (p=0.000) with consumption of butter.

Table 6: Distribution of study subjects by vitamin D level with respect to cheese

Cheese	Vitamin D						χ^2 (df)	p Value
	Normal		Abnormal		Total			
	No.	%	No.	%	No.	%		
Used to daily	1	1.2	--	--	1	0.5	28.448 (4)	0.000
1-2 tpw	14	16.4	45	39.1	59	29.5		
1-2tpm	52	61.2	36	31.3	88	44.0		
Seldom	8	9.4	28	24.4	36	18.0		
Never	10	11.8	6	5.2	16	8.0		
Total	85	42.5	115	57.5	200	100.0		

tpw=times per week, tpm=times per month

Table 6 showing that association of vitamin D level was found statistically significant (p=0.000) with consumption of cheese.

DISCUSSION

Its worldwide deficiency has becomes a health issue, even after all medical advancement of the century and it is still epidemic. More than a billion populations, all over the world are vitamin D deficient. Still there is not any government or international health organization body has declared this vitamin D deficiency a public health emergency to aware the population for the requirement to achieve the sufficient amount of vitamin D levels in blood. About fifty to ninety percent of 25(OH) D is synthesized by ultraviolet-B rays in the skin and the remaining obtains from diet or supplements (Holick 2007). Studies from different parts of India had reported the prevalence of vitamin D deficiency varying from 30% to 100 % (Angurana, Angurana, Mahajan, Kumar, Mahajan, 2014).

Deficiency of vitamin D generally does not occur as it can be synthesized in the body but if it occurs in children called rickets and in adults called osteomalacia. The normal reference range of vitamin D taken in this study is 30-100 nano-grams per milliliter. 25(OH)D deficiencies may lead to defects in insulin secretion, impaired glucose tolerance, impaired immunity and carcinogenesis etc. Hypervitaminosis D can

cause hyper-calcemia, hyper-phosphatemia, anorexia, nausea, diarrhea and vomiting, besides bone pain and metastatic calcification of the soft tissues including the myocardium, the kidney, the pancreas and the uterus (Lal and Pandey 2016).

In the present study a total of two hundred (N=200) healthy volunteers aged 20-60 years of either sex with different socioeconomic background and coming from rural and urban setup were found with elevated prevalence of 25(OH) D deficiencies. The gender distribution among males & females in the study were equally distributed. Among the total 200 study subjects, 115 (57.5%) were found with 25(OH) D levels < 30 ng/ml which were considered, vitamin D deficient. Similar findings of higher prevalence were also observed by Bachhel *et al.,* 2015 (90%), Kiran *et al.,*2014 (73.91%), Arya *et al.,* 2004 (66.3%). Also, 85 (42.5%) subject had normal (30-100 ng/ml) levels of vitamin D. This is despite the fact that this region gets a good sunshine throughout the year and people are well aware and can afford good nourishment. Recently, another study was conducted by Misra *et al.,* 2017, among adults, aged 20–60 years, residing in a rural community of rural Ballabgarh (Haryana) and found the prevalence of vitamin D deficiency 90.8%.

In the present study, the food habit of all the subjects was analyzed and the probability of significant was taken ($p<0.05$). The association between vitamin D level & milk consumption was ($p=0.000$), similar finding were observed by Fentaw *et al.,* 2017, that the significant association was exist between deficiency of vitamin D and intake of milk and milk made items , consumption of yoghurt ($p=0.001$), consumption of butter ($p=0.000$) and consumption of cheese ($p=0.000$) of all study subjects as per table number 1,4,5 and 6. Furthermore, the association of 25-hydroxy-vitamin D levels were found statistically non significant ($p>0.05$) with consumption of tea / coffee ($p=0.394$), consumption of pudding ($p=0.430$) of all study subjects as per table number 2 and 3, with of all study subjects. The observation regarding food habit comes out by Kiran *et al.,* 2014, that is not corroborate with our finding that we had observed the same, In the study furnished by Gaafar and Badr, 2013 it was found that the association between consumption of milk ($p=0.0001$) & vitamin D was statistically highly significant and there were a significant association between vitamin D & consumption of cheese ($p=0.02$), thus the study conducted by Gaafar and Badr, 2013, corroborates the present study. Our study is contrast with the study done by Gordon *et al.,*2004, in which negative correlation (r) between the 25(OH) D deficiency & consumption of milk and no any significant correlation was exist between 25(OH) D deficiency and consumption of yogurt and cheese, Cod & other fish liver oils may be better source of vitamin D if peoples used to non-vegetarian contents in their diet but for vegetarian peoples milk and milk products are best sources (with less amount of vitamin D as compare to non vegetarian diet). Vitamin D content in milk generally depends upon the diet of cow, on their exposure to sunlight. Human milk had lower contents of vitamin D as compare to cow's milk. Plants food and vegetables oil had low amount of this nutrient, so we can say that 25-hydroxy-vitamin D in the diet is only obtained from animal sources.

SUMMARY AND CONCLUSION

Vitamin D deficiency is still pandemic as seen in the previous literature that it observed in almost all the countries in the world. So there may be many causes due to lack of awareness about vitamin D deficiency is an important public health issue. Cod liver oil, oily fish, cow's milk and mushrooms exposed to ultraviolet-B radiation are the only natural source. Requirement of fortifying foods with 25(OH) D may be long-lasting. Daily lifestyle changes that decrease outdoor exposure of sun for all age groups including children. The tradition of deficiency of vitamin D rise the risk of major chronic illnesses like heart disease, diabetes, stroke, cancer, asthma, infectious diseases and autoimmune diseases etc in whole population of the world. The possible causes of vitamin D deficiency in Indians, of its wide spread might be due to low dietary intake and high fiber diet intake that decreases 25(OH) D levels in blood.

The Council of Medical Research in India (ICMR) not has given any particular approval regarding daily requirement of 25-hydroxy-D excluding the particular recommendation on medical ground *i.e.* only 400

IU of vitamin D per day. Indian Council of Medical Research recommends only outdoor activities during sun-shine for normal healthy children and adults so-that they could receive proper amount of vitamin D.
It is clear from the above facts that most of the natural foods including those derived from animals are low in vitamin D content so-that peoples should therefore try to obtain as much as sunlight as possible to adopt vitamin D and increase in their diet in proper amount to meet the optimum level.

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