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Vitamin D status of individuals exposed to varying degrees of sunlight due to Occupational Requirements

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Abstract
Vitamin D is a group of fat-soluble secosteroids responsible for enhancing intestinal absorption of calcium, iron, magnesium, phosphate and zinc. 1 billion people worldwide have Vitamin D deficiency or insufficiency. The purpose of present study is to compare serum Vitamin D Levels of individuals exposed to varying degrees of sunlight and correlate with the effect of dietary intake and environmental factors on serum Vitamin D levels, as well as the examine the effect of supplementation for two months on improvement is status of severely deficient subjects. A total of sixty subjects within the age group of 22-60 years were selected for the study. The subjects were selected from different places and occupations based on the degree of exposure to sunlight. Information on Nutritional assessment / dietary intake was collected using a questionnaire. Serum vitamin D levels were assessed by HPLC using a Diode Array Detector**.** The dietary intake of vitamin D rich food is low in all the three groups, and low intake of vitamin D fortified food items as well as vitamin D supplements was observed in all three groups**.** Results with respect to serum vitamin D levels indicated that among group I subjects with zero exposure to sunlight, only 5 could be classified under "deficiency" category and rest of the 15 subjects belonged to the "severe deficiency" category. With respect to group II subjects who were completely exposed to sunlight, 17 out of 20 could be classified under the "deficiency" category and only 3 belonged to the "sufficient" category based on their serum vitamin D levels, inspite of adequate exposure to sunlight, probably due to low intake of vitamin D rich foods. Among group III subjects who were moderately exposed to sunlight, 19 belonged to "sufficient" category and only 1 subject could be classified under "deficient" category.
The results of ANOVA shows significant difference between three group means (p= 1.55E-07). There is significant difference between group I (complete exposure to sunlight) and group II (zero exposure to sunlight) and also between group II (zero exposure to sunlight) and group III (moderate exposure to sunlight). However there is no significant difference between group I (complete exposure to sunlight) and group III (moderate exposure to sunlight), (P<0.01) Significant at 1% level.

Keywords: Cholecalciferol, Secosteroids, HPLC, Nutritional assessment, Fortification, Supplementation.

Introduction
Vitamin D deficiency is pandemic, yet it is the most under-diagnosed and under-treated nutritional deficiency in the world (Holick, 2007) [8, 9] One billion people worldwide have Vitamin D deficiency or insufficiency. It is now recognized that the function of vitamin D extends far beyond that required for calcium homeostasis.
Holick, 2007 [8, 9] reported that vitamin D can play a role in decreasing the risk of many chronic illnesses, including common cancers, autoimmune diseases, infectious diseases, and cardiovascular disease. Women who practice pardah (i.e, the use of clothing and other approaches to screen themselves from men and strangers) and children and adults who avoid all sun exposure or wear sunscreen protection are equally at high risk.
Sensible sun exposure (usually 5–10 min of exposure of the arms and legs or the hands, arms, and face, 2 or 3 times per week) and increased dietary and supplemental vitamin D intakes are reasonable approaches to guarantee vitamin D sufficiency (Holick, 2004) [10].
The present study was therefore designed to assess the vitamin D status of individuals from a free living population exposed to varying degrees of sunlight, examine the results in terms of their dietary habits and evaluate the effects of supplementation for two months on improvement of vitamin D status.

Methodology

A total of sixty subjects within the age group of 22-60 years were selected for the study. The subjects were selected from different places and occupations based on the degree of exposure to sunlight. Subjects were excluded if they were taking medication for vitamin D.

The first group which consisted of 20 subjects who were not exposed to sunlight at all during the day, were selected from a call center at Hi-tech city, Hyderabad. These subjects worked in air conditioned offices and travel by cabs to the office during late evening and return back to their home during early morning before the sun rises. Their shift timings were from 7 p.m. to 4 a.m., and they slept during the day. Their exposure to sunlight was almost at zero level during week days.

The second groups of subjects were those who were completely exposed to sunlight, and these subjects were selected from among the farm field workers at the, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, India. These subjects are completely exposed to sunlight from 7 a.m. to 3.00 p.m., 6 days a week. The blood samples of these subjects were collected during the month of May in order to study the effect of maximum sunlight exposure on the vitamin D status of the individual subjects.

The third group comprised of subjects moderately exposed to sunlight. These subjects were selected from Seed Research and Technology Centre, as well as from the Institute of Biotechnology, Professor Jayashankar Telangana State Agricultural University (PJTSAU), Rajendranagar, Hyderabad. These subjects were exposed to sunlight from 10 a.m. to 12 p.m., with at least 2 hours of exposure to sunlight.

Formation of ethical committee

The ethical committee was formed which included the doctor, a nurse, the Chairman of the advisory committee, and the analyst. The committee's approval was obtained before drawing the blood of the subjects.

Vitamin D status was assessed using two different methods

a. Information on Nutritional assessment / dietary intake was collected using a questionnaire which was designed to measure the dietary intake of all vitamin D rich food sources, quantity of intake, as well as to measure exposure of sunlight and gain access to information about the general health status and the regular intake of any vitamin D supplements**.**
b. Serum vitamin D levels were assessed by HPLC using a Diode Array Detector**.** Vitamin D status in 60 subjects from three groups of individuals to varying degrees of sunlight, was assessed by measuring the serum concentration of 25-hydroxy vitamin D_3 [$25(OH)D_3$] by the method described by Turpeinen *et al*., 2003 [30].

The method was described to be easy to use, sensitive, and rapid with simple sample preparation technique. Separation and quantification of $25(OH)D_3$ from $25(OH)D_2$were achieved with isocratic elution and a mobile phase composition consisting of 760mL/L methanol in water, at a flow rate was 1mL/min. Detection was at 265nm, and the injected volume was 50µl. Stock standard vitamin $D_3$was prepared by dissolving 1 mg of vitamin D_3 in 2.5 ml of ethanol to give a concentration of 0.4mg/ml. Working standards were prepared to give standard concentrations of 4, 8 and 16 ng/µl.

Samples were prepared by mixing 0.5 mL of serum with 350 µL of methanol–2- propanol (80:20 by volume) and mixed in a Multitube vortex mixer for 30 s. $25(OH)D_3$ was extracted by mixing three times (60 s each time) with 2 mL of hexane. The phases were separated by centrifugation, and the upper organic phase was transferred to a conical tube and dried under nitrogen. The residue was dissolved in 100µl of mobile phase consisting of 760mL/L methanol in water, for injection. For chromatographic separation, Agilent 1200 HPLC system with a 1260 quaternary pump and 1260 Diode Array Detector was used. The mobile phase was. Standards and samples were injected in triplicates and regression coefficient was derived for standards to establish linearity. Column used was Microsorb 100-5, C18 Octadecylsilane (ODS) 250 mm length X 4.6 mm diameter.

The percentage of methanol in the mobile phase is critical for separation of these analytes. Extraction of the serum samples with hexane before HPLC analysis was simple and fast (10 min), and it was adequate for reproducible recoveries of $25(OH)D_3$.

HPLC method with Diode array detection enables reliable quantification of $25(OH)D_3$ and, if present, $25(OH)D_2$. The short and relatively simple sample preparation and ease of use make it useful for routine determinations. Quantification of $25(OH)D_3$ was done using the following formula: PEAK AREA * PEAK FACTOR/ 250* 10. Peak factor was calculated from the regression coefficient of the standard.

Results and Discussion

The data collected from the subjects through questionnaire method was analyzed and the results are presented in Table no I. The results showed low consumption of milk, yoghurt, milk puddings, butter, cheese, breads, bread rolls, wraps, fortified vitamin D products and dietary supplements in all three groups of individuals.

The serum vitamin D levels were assessed using HPLC method described by Turpeinen *et al*., 2003 [30], and were classified according to classification given by vitamin D status National Nutrition Council (Meyer 2006) in Table II.

From the 60 subjects selected 15 were found to be severely deficient and all these 15 subjects belonged to Group I, that is those completely not exposed to sunlight these subjects were administered supplemented with vitamin D_2 at a dose of 60,000 IU/ week. After 8 weeks of supplementation, the serum vitamin D levels were assessed and the results are presented in Table III.

Time of day during sun exposure, season, latitude, and degree of skin pigmentation dictate how much vitamin D_3 is produced during sun exposure. Exposure of the arms and legs (abdomen and back when possible) to sunlight 2 to 3 times a week for approximately 25% to 50% of the time it would take to develop a mild sunburn (minimal erythemal dose), will cause the skin to produce enough vitamin D.

For a white person, if 30 minutes of June noon-time sun would cause mild sunburn, then 10 to 15 minutes of exposure followed by good sun protection should be sufficient to produce adequate vitamin D (Holicks, 2007) [8, 9]. There is no need to ever expose the face because although it is the most sun exposed of all the body areas, it provides little vitamin D_3.

An adult in a bathing suit exposed to 1 minimal erythemal dose (slight pinkness to the skin 24 hours after exposure) is the equivalent to taking approximately 20,000 IU (500 mg) of vitamin D_2 orally (Holick, 2007 and Holick, 2012) [8, 9, 13, 14, 15, 16, 17, 18]. Thus, exposure of arms and legs to 0.5 minimal erythemal doses is equivalent to ingesting approximately 3000 IU of vitamin D_3 (Holick, 2007 and Holick, 2011) [8, 9, 11, 12].

The subjects in the first group of the present study were not

exposed to sunlight due to their work timings and they can be compared to people in living in climatic conditions having minimum sunlight or cold climatic condition where exposure to sunlight is minimal due to poor climatic conditions resulting in vitamin D deficiency. In addition, these subjects did not consume significant amount of vitamin D rich foods to compensate for low exposure to sunlight.

The second group of individuals, who were completely exposed all day, also did not consume significant amounts of vitamin D rich foods. However, it is evident from the results presented in Table III that this group had highest serum vitamin D levels in comparison with the low exposure and moderately exposed groups.

Table 1: Information on Dietary Intake of Three groups of subjects

Type of Foods Consumed	Group I Zero Exposure to Sunlight	Group II Complete Exposure to Sunlight	Group III Moderate Exposure To Sunlight
	(% of Subjects)		
Milk	40% Consume	40% Consume	30% Consume
Kind Of Milk	80% Consume Whole Milk, 20% Consume Semi Skimmed Milk.	40% Consume Whole Milk	80% Consume Whole Milk, 20% Consume Semi Skimmed Milk
Milk In Tea Or Coffee	60% Consume	40% Consume	80% Consume
Breakfast Cereal With Milk	90% Do Not Consume	100% Do Not Consume	80% Do Not Consume
Milk Pudding, Rice Pudding Or Custard	20% Consume	30% Consume	10% Consume
Yoghurt	20% Consume	50% Consume	20% Consume
Butter/Spread	10% Consume.	100% Do Not Consume	100% Do Not Consume
Cheese	10% Consume.	100% Do Not Consume	100% Do Not Consume
Cheese (All Kinds) On Pizza, On Toast, On Lasagne Or With Any Other Kinds Of Food	10% Consume.	100% Do Not Consume	100% Do Not Consume
Bread, Wraps, And/Or Rolls	15% Consume	100% Do Not Consume	80% Consume
Kind Of Toast/Bread/Rolls	From 15% 5%White Bread, 5%Brown Bread. 5% Both	-	80% White Bread, 20% Brown Bread.
Fish	60% Consume	90% Consume	80% Consume
Meat	90% Consume	100% Consume	80% Consume
Eggs	95% Consume	100% Consume	90% Consume
Cakes, Chocolate And/Or Biscuits	40% Consume	10% Consume	90% Consume
Alcohol	100% Do Not Consume	20% Consume	10% Consume
Carbonated Drinks	100% Consume	100% Consume	85% Consume
Beverages Fortified With Vitamin D And/Or Calcium	10% Consume	100% Do Not Consume	100% Do Not Consume
Dietary Supplements	10% Consume	100% Do Not Consume	100% Do Not Consume
Use Sunscreen	15% Use	30% Use	15% Use

In the three groups, the first group of subjects who were not at all exposed to sunlight were economically stable and could afford the food items compared to group II subjects who were completely exposed to sunlight. These subjects belonged to the lower income category and they could not afford the expensive food items like milk and yogurt. However, consumption of certain food items was found to be equal for milk (40%) and consumption of yoghurt was more among the subjects completely exposed to sunlight than those not exposed to sunlight, the percentage of consumption being 50% and 20 % respectively. Among the third group of subjects who were moderately exposed to sunlight, their income levels were are also higher, but consumption of milk was only 20% and consumption of yoghurt was equal when compared to subjects not exposed to sunlight (20%).

Intake of caffeine from tea and coffee is very high in India. Studies have reported association of high caffeine intake with increased risk of low bone mineral density, osteoporosis, and osteoporotic fractures in middle-aged women. This situation is exacerbated in women with low calcium intake, especially in lean subjects (Beaudoin, 2011) [2]. 60 % of the subjects from group I who were not exposed to sunlight consume tea, 40 % of the completely exposed group consume tea and 80 % of the moderately exposed group consume tea.

Vitamin D_3 is also found in animal food sources e.g., fatty fish (e.g., salmon, mackerel and tuna) cod liver oil, milk, *etc*. Vitamin D_2 is found in vegetable sources like sun-exposed yeast and mushrooms.

Very few foods naturally contain vitamin D; examples of foods with ample vitamin D stores include wild-caught salmon and UV-exposed mushrooms (Holick, 2007) [8, 9]. Frequent fish consumption is believed to help maintain adequate concentrations of serum $25(OH)D_3$ in elderly Japanese women during the winter (Kazutoshi, 2000) [23]. 60% of the subjects not exposed to sunlight consume fish, 90 % of the subjects completely exposed consume fish and 80% of the subjects belonging to the moderately exposed group consume fish.

It was found that low habitual dietary vitamin D intake did not affect vitamin D status in summer, and fish was reported to be the major contributor to vitamin D intake independent of sex, age, vitamin D status, BMI, and the income of subjects (Jungert, 2014) [21].

Vitamin D (relatively) rich dietary sources are unaffordable and mostly limited, especially for vegetarians. Vitamin D supplements are unaffordable and not feasible as a population based approach. Fortification of widely consumed staple foods with vitamin D is the only viable solution towards attaining vitamin D deficiency in India. Unlike supplementation strategies, fortification of food with vitamin D poses a negligible risk of toxicity. Though the subjects who were completely exposed to sunlight and those moderately exposed to sunlight could afford either supplements or vitamin D rich foods, as they are economically stable, due to lack of knowledge, ignorance and lack of interest they were found to consume very amounts of these foods. Whereas, the subjects who were completely exposed to sunlight belonged to the low income group and with low purchasing power they could not afford to purchase either vitamin D rich foods or supplements.

Despite a predominantly non-vegetarian dietary pattern, approximately 60% of the intake of vitamin D from food comes from fortified foods in USA (Fulgoni, 2011) [6] and Canada (Langlois, 2010) [25].

Alcohol consumption, especially if long-term and heavy, increases the risk of hip fracture (Felson, 1988) [5]. 20 % of the subjects who were completely exposed to sunlight, being from the low income category, consumed alcohol, 10 % of subjects moderately exposed to sunlight consumed alcohol and 100 % of subjects not exposed to sunlight did not consume alcohol at all, and the reason for this could be that most of these subjects were females.

Intake of sugar-sweetened beverages including colas, other carbonated beverages and sweet fruit drinks was assessed using a validated food frequency questionnaire among 741 premenopausal women. Plasma concentrations of $25(OH)D_3$ were quantified by radioimmunoassay. The association between intake of sugar-sweetened beverages and $25(OH)D_3$ concentrations was evaluated using multivariate generalized linear models and Spearman correlations. Higher intake of colas was associated with lower mean $25(OH)D_3$ levels. No association was observed between intake of sweet fruit drinks and $25(OH)D_3$ concentrations. These results suggest that high intake of colas may decrease $25(OH)D_3$ levels in premenopausal women (Caroline, 2014) [3].

In the present study, all the subjects in the first two groups, that is, those who were completely exposed to sunlight and those who were not at all exposed to sunlight consumed carbonated beverages whereas 85 % of subjects from the moderately exposed group consumed carbonated beverages.

The consumption of at least 0.5 egg/day among children could prevent health problems because of their high amount of vitamin D (Rodriguez-Rodriguez 2013) [28]. 95 % of subjects not exposed to sunlight consumed eggs, 90 % of subjects moderately exposed to sunlight group consumed eggs and 100 % of completely exposed to sunlight group consumed eggs.

Dietary consumption of whole egg can attenuate hyperglycemia, stabilize weight gain, and maintain normal circulating $25D_3$ concentrations in Type 2 Diabetes in rats (Jones, 2015) [20]. It was observed in the present study from the data collected that when any of the subjects consumed egg, they consume one full egg, but very rarely. Hence, the consumption of eggs by subjects from any of the three groups could not significantly contribute to improved vitamin D status.

Cooking practices in India like baking is done mostly above 175°C but the temperature in the food does not reach such high temperatures, therefore stability of vitamin D during baking is well within acceptable range (Natri, 2006) [27]. Shallow and deep frying of foods is very popular in India. When foods are fried, vitamin D in the food comes out into the cooking medium and is thermally degraded (Lu, 2007) [26].

On the other hand, the high salt content of Indian diet is likely to increase urinary calcium excretion. A direct relation between high sodium intake and lower bone mass has been reported (Caudarella, 2009) [4].

In the scenario of inadequate calcium intake, vitamin D insufficiency and high phytate content in diet, environmental pollutants such as fluoride add insult to injury. Toxins like fluoride affect bone metabolism severely in the conjunction with inadequate calcium intake, especially in children (Harinarayan, 2006 and Khandare, 2005) [7, 24].

Table 2: Vitamin D status as per the classification of the National Nutrition Council (Meyer 2006).

25(OH)D in serum or plasma	Description
>50 nmol/l*	Sufficient
25-50 nmol/l	Suboptimal
12.5-25 nmol/l	Deficiency
<12.5 nmol/l	severe deficiency

The following Table presents data from serum vitamin D levels of the three groups of subjects exposed to varying degrees of sunlight.

Table 3: Serum Vitamin D levels of three groups of subjects exposed to varying degrees of sunlight.

S. No.	Group I Zero Exposure To Sunlight	Group II Complete Exposure To Sunlight	Group III Moderate Exposure To Sunlight
		Vitamin D_3 ng/ml	
1	5.7	24.0	15.1
2	16.2	23.2	14.3
3	11.0	25.2	27.7
4	12.0	20.0	70.0
5	14.0	16.0	20.8
6	12.0	14.6	24.7
7	8.0	19.0	27.4
8	5.4	14.0	17.2
9	4.6	33.3	25.7
10	8.8	18.4	20.3
11	7.7	31.0	14.7
12	8.1	18.8	24.6
13	8.0	24.1	29.8
14	8.1	24.0	22.2
15	11.0	32.0	16.0
16	5.4	28.3	12.5
17	11.0	24.1	23.1
18	7.49	18.2	21.2
19	5.53	26.0	17.5
20	2.6	14.0	18.7
Mean	8.6	22.4	23.1
Standard Deviation	3.4	5.9	12.9

It is evident from the above table that among group II subjects completely exposed to sunlight, 17 out of 20 were in "deficiency" category and only 3 belonged to "sufficient" category, whereas in in group I subjects who had zero exposure to sunlight, only 5 belonged to "deficiency' "category and remaining 15 were classified as having severe deficiency, and in group III subjects who had moderate exposure to sunlight, 19 were found to belong to "sufficient" category and only 1 could be classified as "deficient" based on serum vitamin D_3 levels.

Inspite of low consumption of vitamin D rich food items, serum vitamin D levels of group II subjects completely exposed to sunlight group and subjects having moderate exposure to sunlight were more than that of group I subjects who had zero exposure to sunlight. Though among group II subjects, only 3 members were found to have sufficient vitamin D_3 levels, which accounts for only 15%, indicating that adequate exposure to sunlight alone is not sufficient, and it is required that along with vitamin D supplementation is needed in some form or the other, that is either through diet or fortified foods or supplements, in order to maintain adequate serum vitamin D levels..

Sensible sun exposure (usually 5–10 min of exposure of the arms and legs or the hands, arms, and face, 2 or 3 times per week) and increased dietary and supplemental vitamin D intakes are reasonable approaches to guarantee vitamin D sufficiency (Holick, 2004) [10].

Table 4: Anova: Single Factor of three groups of subjects exposed to varying degrees of sunlight.

Anova: Single Factor						
Summary						
Groups	***Count***	***Sum***	***Average***	***Variance***		
Column 1	20	448.2	22.41	34.67989		
Column 2	20	172.62	8.631	11.62725		
Column 3	20	463.5	23.175	145.9714		
Anova						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	2679.827	2	1339.913	20.90581	1.55E-07	3.158843
Within Groups	3653.293	57	64.09286			
Total	6333.12	59				

There was found to be significant difference between three group means (p=1.55E-07). There was significant difference between group I and group II and also between group II and group III. However there was no significant difference between group I and group III.

Table 5: Vitamin d_3 levels before and after supplementation.

Subject No.	Vitamin d Leves before Supplementation (Severely deficient)	Vitamin d Leves after Supplementation Deficient
1	7.75	15
2	7.7	10
3	8.11	14
4	5.4	17.5
5	8.02	11.2
6	5.4	7.8
7	5.5	17
8	2	11
9	8.14	9
10	7.5	10
11	5.7	13
12	8.8	15
13	8.1	12
14	5.5	19
15	9.7	21

Table 5 shows the vitamin D_3 levels before and after supplementation for two months. It is shown that before supplementation 15 out of 60 were severely deficient and after supplementation for 2 months their serum vitamin D levels were increased and they were found to have insufficient Vitamin D_3 levels. More 2 months supplementation is recommended for increasing serum levels to sufficient category.

The results of the study were statistically analyzed using a paired T–test for assessing improvement in vitamin D_3 status after supplementation. There was found to be a significant difference between the serum levels before and after supplementation (p<0.01).

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