

Circulating 25-hydroxyvitamin D status in apparently healthy adolescents and its association with body mass index in Puducherry population

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ABSTRACT

Background: Several earlier studies were focused on Vitamin D status and insulin resistance in diabetes mellitus in adult population. However, very few studies have been done among the healthy adolescents associating Vitamin D status with body mass index (BMI) (anthropometric marker of central obesity). **Objective:** To examine the concentration of 25-hydroxyvitamin D (25[OH]D) in apparently healthy adolescent and its association with BMI in apparently healthy adolescents (12-19 years age group) of Puducherry region. **Methods:** About 60 apparently healthy adolescents were evaluated for anthropometric, physiological, and biochemical (fasting insulin and glucose, lipid profile, and 25[OH]D) parameters. Correlation between Vitamin D levels and BMI was calculated. **Results:** Our study demonstrated a significant low level of 25(OH)D level in overweight (BMI=25-29.9) and obese adolescent (BMI \geq 30) compared to non-obese (BMI<25) group. Pearson's correlation analysis showed strong negative association between BMI and serum 25(OH)D. **Conclusion:** Overweight or obese adolescents are more prone to Vitamin D deficiency. Hence, major efforts should be undertaken to tackle Vitamin D deficiency in adolescents such as food fortification and micronutrient supplementation.

Key words: Adolescents, Anthropometric parameter, Insulin resistance, Vitamin D insufficiency

Vitamin D deficiency or insufficiency prevails in all over the Indian subcontinent with prevalence ranging from 70% to 100% [1]. Vitamin D deficiency is the most under-diagnosed and undertreated global problem affecting majority of individuals irrespective of their age gender, race, and geography [2]. Vitamin D is photosynthesized in the skin form 7-dehydrocholesterol on exposure to sunlight ultraviolet (B) rays. Proper amount of sun exposure is ought to suffice for the prevention of Vitamin D deficiency. In spite of this fact and plentiful of sunlight in countries such as India, it is seen that large population of healthy adolescents has low or borderline low serum 25-hydroxyvitamin (25[OH]D) level. Dietary pattern, fortification of food, limited sun exposure, use of sunscreen, pigmentation of skin, and social taboos facilitate the Vitamin D deficiency in India.

In addition to bone and calcium metabolism, Vitamin D may be having several health protective effects against Type 1 diabetes mellitus, cardiometabolic risk such as hypertension, obesity, and cancer [3]. Several studies in adults have shown

that obese individuals have lower circulating Vitamin D levels as compared to normal weight individuals. Recent studies in adult population suggested that adequate 25(OH)D levels could be associated with increased insulin sensitivity index and consequently weight loss [4]. In one of the previous studies, whole body irradiation produces increased in 25(OH)D level in obese individuals (body mass index [BMI]>30 kg/m²) that was 57% lower as compared to normal weight individual (BMI<25 kg/m²). Several mechanism have been proposed to explain low 25(OH)D levels in obese adults, one of it is sequestration of Vitamin D by adipose tissue [5].

Adolescent obesity is increasing globally and now, it is of utmost concern in India. Obese adolescents with central obesity are more prone to develop the cardiometabolic risk factors such as glucose intolerance, hypertension, and dyslipidemia [6]. The above co-morbidities are associated with the future cardiovascular risk in later life. In above consensus, role of Vitamin D is very much important in insulin sensitivity and glucose tolerance. Adolescent period is more vulnerable

to Vitamin D insufficiency due to increased mineral demands of the skeletal activities. If we know the exact prevalence of Vitamin D deficiency in adolescents, it can be one of the modifiable risk factors in them before the development of obesity and diabetes mellitus.

In South India, data on clinical and subclinical Vitamin D insufficiency status among apparently healthy adolescents are scarce. In the above context, the present cross-sectional study was done with the objective to evaluate Vitamin D status among apparently healthy adolescents (12-19 years age group) from Puducherry population and tried to correlate with BMI and cardiometabolic risk factors among them.

MATERIALS AND METHODS

The participants of this study were sixty apparently healthy adolescents from school located in Puducherry semi-urban population aged 12-19 years male and female (non-pregnant) adolescents from Puducherry population. This cross-sectional study was undertaken in the summer months, i.e., April and May 2013. The parents of each participant were informed about the study protocol and written informed assent was obtained. The study protocol was approved from the Institutional Human Ethical Committee. Adolescents with chronic disease conditions such as tuberculosis, Type 1 or Type 2 diabetes mellitus, on Vitamin D or calcium supplementations or on drug for obesity or doing severe exercise for weight reduction were excluded from this study. General information was collected from parents about adolescents such as age, race, family income, physical activity, food habits, and dietary history.

Anthropometric Measurements

Weight was measured without shoes, using a weighing machine with an accuracy of 0.1 kg. Height was measured without shoes; using a wall stadiometer to the nearest 1 mm. BMI was calculated by weight (in kg)/height (in m²). BMI is recognized as normal (<25), overweight (25-29.9), and obese (≥30) [7].

Biochemical Parameters

Venous blood (3 ml) was drawn from all participants, following overnight fasting of minimum 10 h. From 2 ml aliquot, serum was separated for analyzing lipid profile, insulin, and 25(OH)D levels. About 1 ml was drawn in oxalate fluoride tube and plasma was separated for fasting glucose. The most common and sensitive index for assessing Vitamin D status is 25(OH)D. The serum concentration of 25(OH)D was measured by fully automated Chemi Luminescent Immuno Assay. Vitamin D deficiency was defined as 25(OH)D level less than 20 ng/ml and insufficiency as 25(OH)D level of 20-30 ng/ml [8]. Biochemical parameters such as plasma glucose (glucose oxidase-peroxidase method), serum cholesterol (Enzymatic method), triacylglycerol and high-density lipoprotein (HDL) (by precipitation method),

and serum low-density lipoprotein (LDL) (by indirect method by using Friedwald's equation) estimated in Hitachi 912 fully auto analyzer [9]. Fasting Insulin was estimated on automated electro-chemiluminescence analysis. Reference value of fasting insulin was 6-26 μU/ml [10].

Homeostasis Model Assessment of Insulin Resistance (HOMA-IR)

Insulin resistance was calculated by using HOMA-IR. $HOMA-IR = (\text{Fasting Glucose} \times \text{Insulin}) / 22.5$.

Insulin concentration is reported in μU/ml and glucose in mmol/L. Reference value for HOMA-IR was taken as less than 1.68 [11].

Statistical Analysis

All data were normally distributed. Hence, expressed as the mean±standard deviation (SD) and SPSS 17.0 programs were used for the statistical analysis. Unpaired Student's t-test was used to compare mean±SD (anthropometric and biochemical parameters) in participants and control groups. Pearson correlation coefficient was computed between Vitamin D and insulin resistance and BMI. A significant level of 95% was chosen for all tests ($p < 0.05$).

RESULTS

After appropriate screening the study population, total 70 adolescents were recruited for study; however, 10 adolescents were excluded as their parents did not give consent. Finally, 60 adolescents from semi-urban population were recruited in this study with mean age 14.5±2.3 years including 31 males and 29 females. Participants were divided into two groups. Group 1 consisted of adolescents with no Vitamin D deficiency (serum 25(OH)D>20 ng/ml) and group 2 consisted of Vitamin D deficient adolescents (serum 25(OH)D<20 ng/ml). Vitamin D level was significantly lower in Group 2 (18.09±1.01 ng/ml) as compared to Group 1 (24.14±2.00 ng/ml) ($p < 0.01$).

BMI was significantly lower in Group 1 adolescents (20.96±3.62) as compared to Group 2 (29.39±5.41; $p < 0.01$) as shown in Table 1. Insulin resistance, as calculated by HOMA-IR, was significantly lower in Group 1 (2.31±0.89) as compared to Group 2 (4.29±3.23; $p < 0.05$), as shown in (Table 2). There was no significant difference seen in lipid profile parameters such as total cholesterol, triacylglycerol, and LDL and HDL cholesterol between both the groups (Table 2).

Analysis of the data revealed that serum level of 25(OH)D was having significant negative linear correlation with BMI ($r = -0.768$, $p < 0.001$) (Fig. 1). There was a weak negative correlation between HOMA-IR and Vitamin D status ($r = -0.332$, $p < 0.05$) as shown in Fig. 2.

DISCUSSION

Vitamin D deficiency and insufficiency is increasingly being recognized in India as well as worldwide affecting majority of the individuals irrespective of their age, gender, race, and geography [12]. Majority of the prevalence studies on Vitamin D deficiency are performed on adults, children, and pregnant women in Indian and Western population [13]. However, very few studies have been done among the healthy adolescents in South India associating Vitamin D status with BMI (an anthropometric marker of central obesity). In the present study, we evaluated the concentration of 25(OH)D in apparently healthy adolescents (12-19 years age group), and we have shown its Negative association with BMI in them.

Studies conducted in Delhi urban area population reported Vitamin D deficiency in 90.8 to 100% adolescents

Table 1: Comparative analysis of physiological parameters among two groups of study subjects

Physiological parameters	Mean±SD		p value
	Group 1	Group 2	
Weight (in kg)	56.75±12.18**	82.44±21.90	0.000
Height (in cm)	164.10±7.51	166.33±9.73	0.382
Body mass index (kg/m ²)	20.96±3.62**	29.39±5.41	0.000

*Significant (p<0.05), **Highly significant (p<0.01), SD: Standard deviation

Table 2: Comparative analysis of biochemical parameters among two groups of study subjects

Biochemical parameters	Mean±SD		p value
	Group 1	Group 2	
25(OH) Vitamin D ₃ (ng/ml)	24.14±2.00**	18.09±1.01	0.000
Fasting plasma insulin	11.63±4.50**	21.25±15.27	0.003
Fasting plasma glucose	81.04±8.40	81.66±9.27	0.814
HOMA-IR	2.31±0.89**	4.29±3.23	0.003
Total cholesterol (mg/dl)	159.99±26.29	173.00±27.13	0.110
Triacylglycerides (mg/dl)	83.86±29.42	90.33±44.16	0.549
High-density lipoprotein (mg/dl)	42.21±12.83	40.88±7.24	0.690
Low-density lipoprotein (mg/dl)	106.12±33.028	113.66±21.47	0.348

*Significant (p<0.05), **highly significant (p<0.01), HOMA-IR: Homeostasis model assessment of insulin resistance, SD: Standard deviation

(Table 3) [14-18]. Sahu et al. reported Vitamin D deficiency in 88.6% adolescent female in rural area of North India [19]. In a study from Southern India (Tirupati area), Harinarayan et al. reported more Vitamin D deficiency in urban adolescents (around 81.5%) as compared to rural (72.2%) population [20]. Urban adolescents are more prone to Vitamin D deficiency as compared to their rural counterparts may be because of less exposure to sunlight due to rapid urbanization or less outdoor activities.

In a study conducted in Middle East and North Africa, Vitamin D deficiency varies from 30 to 90% [21]. Healthy Lifestyle in Europe by Nutrition by Adolescence study conducted on 1006 European adolescents showed up to 80% prevalence of Vitamin D deficiency [22]. Another study conducted on Indian postmenopausal women by Agarwal et al. showed significant linear negative correlation between 25(OH)D levels and BMI [23]. Bhatt et al. reported that higher value of abdominal fat were associated with low 25(OH)D

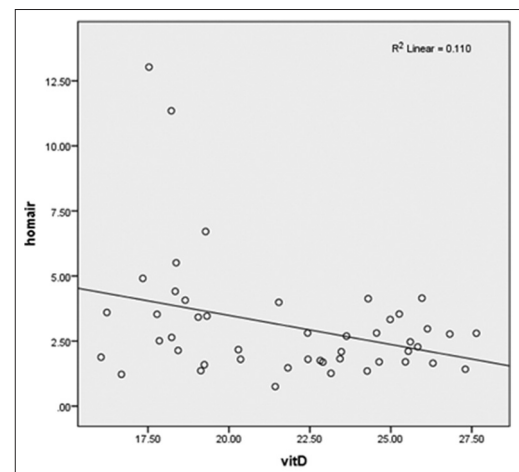


Figure 1: Scatter plot showing Pearson's correlation of Vitamin D with homeostasis model assessment of insulin resistance

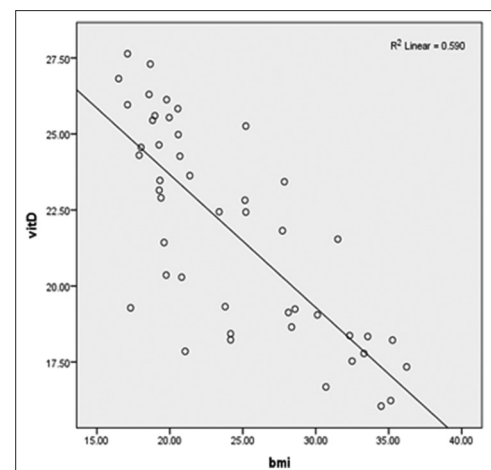


Figure 2: Scatter plot showing Pearson's correlation of Vitamin D with body mass index

Table 3: Comparative analysis of present study with previous on Vitamin D status among Indian adolescents with other countries

Locality	Study subjects (N)	25(OH)D level in ng/ml Mean±SD	Vitamin D status (%)			References	
			Deficient	Insufficient	Sufficient		
Delhi	N=186	Young adult female, age 18.6 years	12.96±9.48	-	-	-	Marwaha <i>et al.</i> 2011 [17]
	N=90	Sport girls from college	21.2±7.57				
	N=96	College girls	5.16±3.08	100	0	0	Puri <i>et al.</i> 2008 [14]
	N=404	Adolescents, female urban, age 12.3 (3.4) years	12.74±6.17	90.8	-	-	
	N=5137	Adolescents, female, urban school age 10-18 years	11.8±7.2	-	-	-	Marwaha <i>et al.</i> 2005 [15]
N=1829	Adolescents, 45% male, 55% female urban school age 13.3 (2.5) years	8.3±5.2	96.9	2.6	0.5	Garg <i>et al.</i> 2013 [16]	
Barabanki, 32 km from Lucknow	N=121	Adolescents, female rural school age 14.3 (2.7) years	13.32±6.4	88.6	-	-	Sahu <i>et al.</i> 2009 [19]
	N=28	Sisters of 34 boys 14.4 (2.7) years, in winter	12.52±5.4	-	-	-	
Tirupati	N=34	Brothers of 28 girls age 14 (3) years, in winter	27±11.6	-	-	-	Harinarayan <i>et al.</i> 2005 [20]
	N=70	Rural children age 13 years Male: 48% Female: 52%	Male: 17±1.3 Female: 19±1.59	Male: 76.5 Female: 72.2	Male: 14.7 Female: 13.9	Male: 8.8 Female: 13.9	
N=69	Urban children age 13 years Male: 43% Female: 57%	Male: 15.57±1.2 Female: 18.5±1.66	Male: 81.5 Female: 62.9	Male: 14.8 Female: 25.7	Male: 3.7 Female: 11.4		
Middle East and North Africa	Adolescent	20 ng/ml	30-90%			Bassil <i>et al.</i> 2013 [21]	
European adolescents, HELENA study	N=1006 (46.8% males)	Adolescents 12.5-17.5 years	Below 20 ng/ml	Up to 80%	-	-	Luis A Moreno <i>et al.</i> 2013 [22]
Puducherry population	N=60	Adolescent (12-19 years age group)	18.09±1.01	-	-	-	Current study

HELENA Study: Healthy Lifestyle in Europe by Nutrition by Adolescence, 25(OH)D: 25-hydroxyvitamin D, SD: Standard deviation

levels in Indian adults without diabetes mellitus [24]. Our study confirms these findings, i.e., low mean level of circulating 25(OH)D in apparently healthy overweight and obese adolescents.

Circulating 25(OH)D levels have strong negative correlation with BMI and central adiposity. Moy and Bulgiba reported that Vitamin D insufficiency is independently associated with abdominal obesity in Malaysian subjects [25]. Cheng et al. reported that lower 25(OH)D were strongly associated with greater regional adiposity in the Framingham Heart Study [26].

They further showed that correlation of 25(OH)D and visceral fat was more than that of subcutaneous abdominal fat with 25(OH)D. Study by Dong et al. in the United States suggested that there are positive correlation between Vitamin D level with healthy lifestyle factors including adiposity, physical activity, and cardiovascular fitness independent of gender, height, and sexual maturation [27].

Adolescents with central adiposity are at greater risk for Vitamin D deficiency as Vitamin D is thought to be sequestered by adipose tissue and its bioavailability decreases.

Association of obesity with low level of 25(OH)D may be attributed to decreased exposure to sunlight because of limited mobility of obese people and negative feedback from 1, 25(OH)D and parathyroid hormone (PTH) on the synthesis of 25(OH)D in liver [28]. Abnormal calcium metabolism has been associated with weight gain and high micronutrient intake is believed to decrease obesity indirectly linked with Vitamin D deficiency.

In the present study, significant difference was seen in insulin resistance (calculated by HOMA-IR) in Vitamin D sufficient versus deficient group with negative association between insulin resistance and 25(OH)D levels. This is an agreement with a 10-year prospective study conducted by Forouhi et al., which showed that baseline 25(OH)D level is associated with fasting glucose, insulin fasting, and HOMA-IR in [29]. They also showed that 25(OH)D levels were negatively associated with first and second phase insulin response during hyperglycemic clamp study. Thus, hypovitaminosis D not only impairs β -cell functions but also alters glucose tolerance and insulin sensitivity index.

Study by Pittas et al. has shown that the deficiencies in 25(OH)D levels influence insulin secretion and increases peripheral insulin resistance via its effect on intracellular calcium in T2DM [30]. Overweight or adolescents with central adiposity are more prone to Vitamin D insufficiency or deficiency and have higher risk of glucose intolerance. Ekbom et al. reported low Vitamin D levels in obese Swedish adolescents were strongly associated with impaired fasting glucose [31]. Study on 443 Chinese adolescents and children by Liu et al. reported significant association between Vitamin D and insulin resistance [32].

Although cardiovascular events occur most frequently during or after the fifth decade of life, pathologic evidence suggests that precursors of cardiovascular disease originate in childhood. Poor Vitamin D status is thought to be associated with a higher prevalence of BMI, insulin resistance, and this risk is same in adults as well as in adolescents. Overall observation of low circulating Vitamin D level with abdominal obesity are important for South Indian adolescents because both contribute to insulin resistance and may also increase cardiovascular risk. Based on this study, we can suggest that Vitamin D measurement is of paramount importance and should be included in the armamentarium of biochemical tests related to adolescent obesity and insulin resistance.

This study has some limitations. The first is that being cross-sectional nature of the study, no cause or effect of 25(OH) D levels could be studied. The seasonal variation and sunlight exposure and nutritional status need to be considered in future studies. The other limitations were small sample size; more subjects should be studied from rural and urban population and non-availability of PTH measurement.

CONCLUSION

Overweight or obese adolescents are more prone to Vitamin D deficiency. Hence, major efforts should be undertaken to tackle Vitamin D deficiency in adolescents such as food fortification and micronutrient supplementation in school mid-day meal programme.

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