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Vitamin D Insufficiency in Adolescents

Mannava Prasanthi Srikanth[1]

[1]Assistant Professor, Department of Biochemistry, Sri Venkateswara Medical College Hospital & Research Centre, Puducherry, India.

Abstract

Background: There is, now increasing evidence that Vitamin D deficiency is widespread across the country and in all age groups. However, we find no studies on prevalence of vitamin d deficiency in healthy adolescent boys and girls and its relationship with overweight and obesity. **Methodology:** This cross-sectional study was carried out in 226 apparently healthy boys and girls aged 12 to 18 years of high schools and junior colleges in the city. A pretested Questionnaire was administered to collect information on socio demographics, sun exposure, medical and nutritional history. Anthropometry and 3 ml of blood sample was collected for Vitamin D. 25 hydoxy-vitamin D was estimated using high-performance liquid chromatography (HPLC). **Results:** 47.3% of the study subjects were boys. The overall Vitamin D deficiency and insufficiency in the study subjects was 79.9% (CI 73.94 to 84.83) and 18.3% respectively. The odds of Vitamin D deficiency was significantly lower 0.37 (CI 0.19,0.73) in boys compared to girls (P =0.006) and higher 2.66 (CI 1.32,5.35) in rural areas (P=0.007). Vitamin D deficiency was not significantly associated with overweight and obesity (P=0.11). **Conclusion:** Our study highlights the high prevalence of Vitamin D deficiency among adolescent girls and boys in and around the city of Hyderabad which is geographically located at 17.3850° N, 78.4867° E in the state, despite abundant sunshine throughout the year. While obesity was not significantly related to vitamin D status, urban residence and being a girl were significantly related; similar to other studies in literature.

Keywords: Vitamin D, Overweight, Obesity.

INTRODUCTION

Hypovitaminosis D is considered to be a worldwide problem in both adults and children.[1] In US data collected from National health and nutrition examination survey (NHANES) 2003-2006 show that approximately 27% adolescents age 12-18 years are vitamin D deficient (5). While in Europe, one study showed that over 90% of teenage girls had 25 (OH)D concentrations<50nmol/l during the winter months.[2] There is a growing awareness that obese adolescents represent a particularly vulnerable group for vitamin D deficiency.[3]

The prevalence of overweight and obesity in adolescents, defined according to the WHO growth reference for school aged children and adolescents (overweight=one standard deviation from body mass index for age and sex, and obese=two standard deviations body mass index for age and sex) is increasing with increasing urbanisation, across the globe.[4] An examination of data collected from 1700 published studies indicates that worldwide prevalence of overweight and obesity rose by 47.1 in children and adolescents in between 1980 and 2013.[5]

Address for correspondence*

Mannava Prasanthi Srikanth,
Assistant Professor, Department of Biochemistry, Sri Venkateswara Medical College Hospital & Research Centre, Puducherry, India.

A recent study conducted among 24,000 school children in south India showed that the proportion of overweight children increased from 4.94% of total students in 2003 to 6.57% in 2005 demonstrating the time trend of this rapidly growing epidemic.[6]

Differing guidelines also exist regarding the proper definitions of vitamin D deficiency in clinical practice. The endocrine society has suggested that 25(OH)D levels of 75 to 250 nmol/l [30-100ng/ml] are sufficient 52-72 nmol/l [21-29 ng/ml] are insufficient and less than 50nmol/l [20ng/l] are deficient.[7] The society for adolescent health and medicine has a definition similar to the endocrine society but considers 25(OH)D levels 75-125 nmol/l [30-50 ng/ml] to be sufficient for the adolescent.[8]

Several factors can influence vitamin D status. Any barrier to the penetration of UVB radiation into the skin epidermis and dermis is inversely correlated with circulating 25(OH)D. These include: decreased solar zenith angle such as occurs during the winter months in temperate climates and at high latitudes year-round; the use of sunscreen or sunblock – even sun protection factor (SPF) as low as 7 can significantly block vitamin D production); high melanin skin pigmentation (which functions as a natural sunscreen);[9] and cultural clothing practices where little/no skin is exposed.[10] Other factors associated with increased risk of vitamin D deficiency or insufficiency include avoidance of milk, fat malabsorption, and excess adiposity.[11]

Analysis of data from 21 European countries and north American adult cohorts predicts that each 1 kg/m2 higher

BMI is associated with 1.15% lower 25(OH) D concentration.[12] This relationship is theorised to be explained by vitamin D"s preferred deposition in body fat compartments making it unavailable for use by other tissues.

There is paucity of data on prevalence of vitamin D deficiency in adolescents in India. Studies have been mostly restricted to western countries / developed countries and or from other parts of India. So, the present study is taken up to assess prevalence of vitamin D deficiency in adolescent group.

METHODS

This study was a cross sectional study carried out in Junior college and high school in around the city. Subjects includes boys and girls aged between 12 to 18 years.

Sampling: systemic random sampling was adopted for each class of high school/college

Inclusion Criteria: Boys and girls aged between 12 to18 years after taking assent from them and also written informed consent from their parents and guardians.

Exclusion Criteria: Boys and girls who have taken vitamin D supplements in last 3 months. Completely clothed individuals (according to customs in some religions hinders the development of Vitamin D in skin) were excluded from the study.

Before the start of the study, Institutional ethics committee clearance was taken before the start of the study.

Study parameters included

a. A pretested questionnaire was administered to collect socio demographics, physical activity, medical and nutritional history.
b. Anthropometry including Height to the nearest 0.1 cm, weight (to nearest 100 grams, Hip circumference (to the nearest 0.1 cm) and waist circumference (to the nearest 0.1 cm) were measured using standard equipment.
c. 5 ml of blood sample was collected for estimation of Vitamin D, PTH. Vitamin D was estimated using HPLC method (given below)

Vitamin D estimation (HPLC method)

Sampling: National committee for laboratory standards (NCCLS) Guidelines were followed for sample collection, handling and processing.

Blood sample collection: 3ml venous blood specimen was collected from all the subjects. Median cubital vein in ante-cubital fossa was selected for venepuncture. The area around the intended puncture site was cleaned with a gauze pad saturated with 70% isopropanol in circular motion and from the site outward. A tourniquet was applied six inches above the intended puncture site and venepuncture was done with evacuated or vacuum gel tubes using a sterile needle. The blood was drawn gradually and within one minute of the application of torniquet. After collecting the desired amount of blood the needle was withdrawn, separated from the needle holder and discarded appropriately.

Blood sample handling: The blood samples were allowed to stand for complete clot formation at room temperature and subsequently centrifuged for 10 minutes at approximately 15000 X g ensuring no particles or traces of fibrin.

Extraction of vitamin D from serum: To 500µl of serum sample 100µl of Titrated 25(OH) D3 (1500cpm) was added and mixed for 30 seconds, incubate for 10 min at room temperature. 500µl of Methanol & Isopropanol (90:10) were added and vortex mixed for 15 seconds incubated in ice cold for 10 min to precipitate the proteins, then 1.0 ml of n-Hexane was added and vortex mixed for 1 min and centrifuged at 3000rpm for 10 min. The upper Hexane layer was dispensed into another tube and the residue was re-extracted with another 0.5 ml of n-Hexane and centrifuged. The upper Hexane layer was pooled with the previous aliquot in the tube. The contents of the tube were evaporated to dryness under Nitrogen and reconstituted in 100µl of Methanol. An aliquot of 25.0µl was loaded onto a reverse phase (C18) HPLC system and 25(OH) D3 and 25(OH) D2 were eluted by Methanol & Water (85:15 V/V) 2.0 ml/min without guard column or 2.3 ml/min with guard column.

Preparation of standard 25(OH) D3 and 25(OH) D2: standard concentration at265 nm in a Spectrophotometer was checked and stock was prepared and working standards of 1.0 ng and 50 ng tubes respectively. Internal standard (H3) 25(OH) D was taken in 5 µl of label into 10 ml of Ethanol and vertex and label counts of 100 µl in β Counter was taken.

Detection identification and quantification of 25(OH) D3: All the solvents were filtered through 0.45 µm membrane filter, prior to analysis; the analytical column was thoroughly washed with Methanol. The column temperature was maintained at 40oC in column oven throughout the analysis period. The isocratic solvent system consisting of Methanol & Water in the ratio of 85: 15 V/V was used as polar mobile phase. The mobile phase was mixed thoroughly and degassed before use. The flow rate of the mobile phase was maintained at 2.3ml/min with guard column or 2.0 ml/min without guard column throughout the assay. The detector wavelength was set at 265 nm. An aliquot of 25 µl of the sample extract which was re-constituted in 100 µl Methanol after evaporation to dryness under Nitrogen is loaded on to HPLC. Elution of vitamin D was accomplished with mobile phase and stationary phase interaction. The standard was used for peak identification and quantification. The concentration of standards ranged from 1.0 ng to 8.0ng concentration on HPLC. Peak identification of 25(OH) D3, 25(OH) D2 in the sample based on retention times and comparison with standards. Further confirmation was achieved by spiking the sample with pure standards and known concentration and co-migration of sample peaks. Retention times were confirmed by re – chromatography. The quantification of vitamin D in the sample was accomplished by comparing the sample peak area with that of a known concentration of standards. The results were expressed in ng/ml of serum sample.

Data Analysis

The data was entered into CSPro software (version 6.1). R Programming software (Version 3.1) was used for data analysis. After data cleaning and validation it was statistically analysed using various methods appropriately as needed (eg;

for continuous variables Mean and SD, for categorical variables, frequencies and crosstabs will be calculated. For prevalence, binomial test was used to calculate the confidence intervals (p values). Logistic regression was carried out to assess the risk factors for vitamin D deficiency with and without adjustment for age. P<0.05 was considered to be statistically significant.

RESULTS

Data on 226 adolescent boys and girls were available for analysis. Among them, 47.3% of them were boys. Median age of the study subjects was 15 years (Range 12 to 18 years). Mean age and SD of boys and girls are given below. Girls were slightly older than boys and the difference was statistically significant (P <0.001).

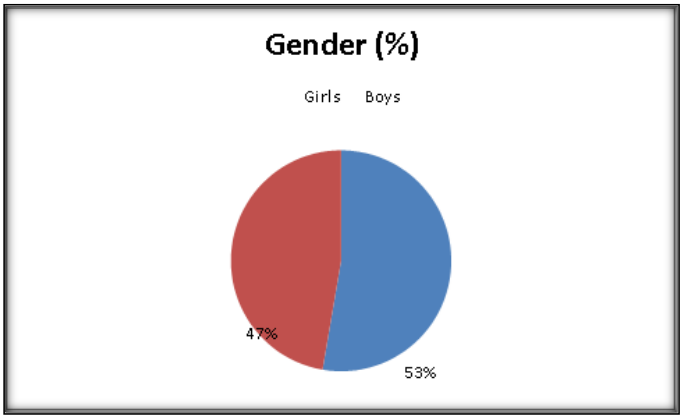

Figure 1: Distribution (%) of boys and girls in the study

Table 1 Mean age and SD (in parenthesis) of boys and girls in the Study.

Sun exposure variables: The median duration of sun exposure among study subjects was 60 minutes (Range 0 to 180 minutes). The duration of sun exposure was significantly higher in boys than girls (P < 0.001). Only 5.4% had dark colour skin, and the rest were either intermediate or fair skin. Girls reported to have significantly fair skin compared to boys. 8.5% of the study subjects reported to use sun screen lotions. The usage of sun screen lotions was also significantly higher in girls (13.6%) compared to boys (2.9%) (P = 0.009).

Anthropometry: Mean (SD) weight of the study subjects was 55.8 (12.3) kg. Mean height was significantly higher for girls compared to boys (P <0.001). Mean (SD) height of the study subjects was 152.2 (11.6) cm. Mean height was also significantly higher for girls compared to boys (P = 0.002). Mean (SD) Waist and hip circumference of the study subjects was 70.6 (10.0) cm and 84.9 (11.3) cm respectively. Mean waist and hip circumference was significantly higher in girls compared to boys.

Obesity: Mean (SD) Body mass index for Age Z (BMIZ) score was -0.65 (1.33). There was no significant differences in BMIZ scores in boys and girls (P=0.658). 12.6% of study subjects were obese, while 35.1% were overweight. Obesity was higher among girls (16.4%) compared to boys (8.5%). Overweight was similar in girls (34.5%) and boys (35.8%).

Mean (SD) Vitamin D of the study subjects was 13.7 (7.4) mcg/dl. Mean Vitamin D was significantly higher in boys compared to girls (P <0.001). The overall Vitamin D deficiency and insufficiency in the study subjects was 79.9% and 18.3% respectively. The prevalence and confidence intervals for Vitamin D deficiency was 79.9 % (CI 73.94 to 84.83). Vitamin D deficiency was significantly higher in girls (87.3%) compared to boys (71.7%).

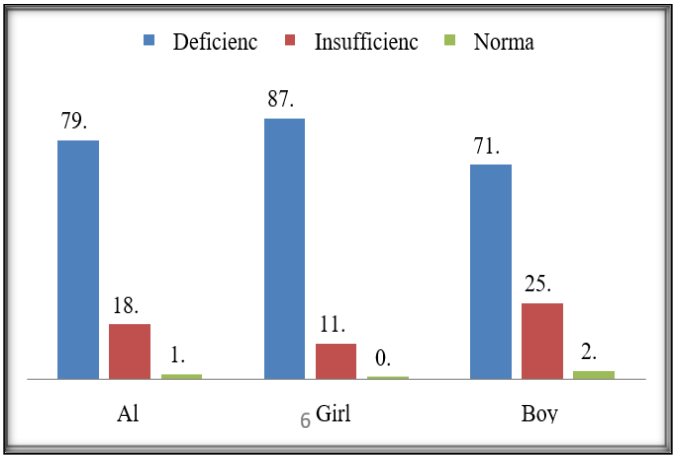

Figure 2: Vitamin D deficiency and insufficiency (%) in study subjects

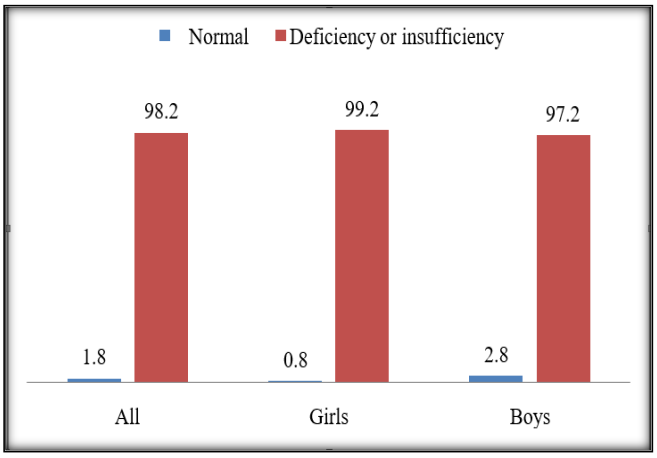

Figure 3: Vitamin D deficiency or insufficiency (%) in study subjects

Vitamin D deficiency and associated factors: Binomial logistic regression was carried out with Vitamin D deficiency as dependent variable (Deficiency = Yes, Insufficiency or normal= No) and risk factors of the study taken as independent variable. Age was taken as confounding variable

Vitamin D deficiency in relation to gender: The odds of Vitamin D deficiency was significantly lower (P =0.006) in boys compared to girls.

Vitamin D deficiency in relation to place of residence: The odds of Vitamin D deficiency was significantly higher (P =0.007) in urban compared to rural area.

Table 1: Consumption of Milk per day among study subjects

	All	Girls	Boys	P value
Milk consumption in ml (mean (sd))	100.86 (105.02)	48.68 (73.88)	155.59 (105.25)	<0.001

Table 2: Sun exposure duration per day among study subjects

	All	Girls	Boys	P value
Sun exposure in minutes (mean (sd))	46.18 (36.87)	29.65 (33.38)	65.15 (31.21)	<0.001

Table 3: Mean Weight in kg and Height in cm of study subjects

	All	Girls	Boys	P value
Weight (mean (sd))	52.47 (11.62)	55.76 (12.35)	48.87 (9.60)	<0.001
Height (mean (sd))	152.25 (9.59)	154.13 (7.06)	150.19 (11.43)	0.002

Table 4: Mean Waist and Hip circumference in cm of study subjects

	All	Girls	Boys	P value
Waist circumference (mean (sd))	70.56 (10.02)	73.93 (11.15)	66.88 (7.01)	<0.001
Hip circumference (mean (sd))	84.86 (11.31)	89.87 (11.38)	79.37 (8.33)	<0.001

Table 5: Mean BMIZ scores of study subjects

	All	Girls	Boys	P value
BMIZ Score (mean (sd))	0.65 (1.33)	0.69 (1.44)	0.61 (1.20)	0.658

Table 7: Mean Vitamin D of study subjects

	All	Girls	Boys	P value
Vitamin D (mean (sd))	13.70 (7.42)	11.98 (6.61)	15.63 (7.82)	<0.001

DISCUSSION

In our study the Odds of Vitamin D deficiency was significantly higher in girls compared to boys. After adjustment for age, boys were having lower odds, 0.38 (0.19,0.77) of developing Vitamin D deficiency. This is similar to a recent study in malaysia, where the prevalence of vitamin D deficiency was more severe in girls than boys (160). In that study, girls had higher odds (OR=8.98; 95% CI 6.48 to 12.45) of having vitamin D deficiency compared with boys. Our findings confirm previous studies which have shown again and again to be much more prevalent in females of this age range (aged 9–18 years) then it is among boys of the same age range.[13] Habibesadat et al also reported similar results of vitamin D deficiency among adolescents aged 7–18 years old in North Khorasan, Iran where the age-adjusted odds of serum 25(OH)D <30 nmol/L was 21.12 higher in girls compared with boys.[14] Postulations as to why girls are more susceptible to vitamin D deficiency include the more modest dress codes observed by many girls in this region, as well as the habit of avoiding the sun for cosmetic reasons.

Religion and Community: In our study, the odds of Vitamin D deficiency was not significantly different (P =0.452) in relation to religion. However, there were significant differences with respect to type of community. The odds of Vitamin D deficiency was lowest in Scheduled Tribe (ST) community compared to OBC (P<0.001). Schedule Tribes had the lowest odds 0.13 (0.04, 0.36) of developing vitamin D deficiency. This is similar to another study where low socio-economic group had significantly lower mean 25(OH)D concentrations than did the Upper socio economic group of adolescents in North India.[15] In an another study in Brazil, that compared low and high socioeconomic status groups, the study did not find any significance difference in mean vitamin D concentration between the two groups.[16] The likely reason that lower socio economic group have higher Vitamin D levels is linked to the lifestyle factors such as increased physical activity and sun exposure duration.

High dietary consumption of phytates and low dietary intake of animal proteins is one of the suggested hypothesis for vitamin D deficiency.[17] While, vegetarians had 1.85 (0.72,4.75) odds of higher Vitamin D, it was not statistically significant (P =0.183). There was also no significant relationship with Milk and Fish intakes with Vitamin D deficiency in our study. Contrary to other studies which shown that Milk intake was inversely related to Vitamin D deficiencies,[18] although one study result showed limited statistical significance due to the small-number statistics in their study.[19] It is known that low dietary calcium converts the 25OHD to polar metabolites in the liver and leads to secondary 25 OHD deficiency. Also, low calcium intake increases parathyroid hormone (PTH) which increases conversion of 25OHD to 1,25-dihydroxyvitamin D. In addition, 1,25- dihydroxyvitamin D induces its own destruction by increasing 24-hydroxylase. This probably explains the low 25OHD concentrations in persons on a high-

phytate or a low-calcium diet underlining the importance of dietary calcium for not only maintaining good bone health but in interpretation of 25OHD levels and subsequent therapy.

CONCLUSION

Our study highlights the high prevalence of Vitamin D deficiency among adolescent girls and boys in and around the city of Hyderabad which is geographically located at 17.3850° N, 78.4867° E in the state despite abundant sunshine throughout the year. Our findings are similar to other studies in this age group in northern and western India. The risk factors that significantly affected the vitamin D status were urban residence, female gender and type of community. We found no significant association with weight, body mass index and BMIZ scores. Our findings are not entirely different from the literature as few studies have highlighted no relationship between vitamin D in adolescent age group as well as adults in south India.

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