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VIT D STATUS AND DETERMINANTS IN INDIAN CHILDREN AND ADOLESCENTS: A MULTI-CENTRE STUDY

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Abstract: Research on vitamin D status in Indian children has been confined to studies conducted on small samples from certain regions and using non-standard techniques to quantify 25(OH)D3. Vitamin D insufficiency was observed in this multicenter research that used a standardized technique to evaluate 25(OH)D3 concentrations from dried blood spots (DBS) in children and adolescents from 5 to 18 years old in India. This school-based cross-sectional observational research used a multi-stage stratified random sampling technique. We picked a city and its surrounding hamlet from six states in India, which span a lot of ground. Data was gathered on demographics, anthropometry, body composition, food intakes, and DBS samples. Liquid chromatography with tandem-mass spectrometry was used to evaluate 25(OH)D3 in DBS. A total of 2450 youngsters had their vitamin D level checked, with a subset (n = 672) having extra data obtained for the purpose of evaluating predictors. In the study, 36.8% of participants had adequate vitamin-D levels (> 50 nmol/L), with greater concentrations seen in rural respondents and boys ($p < 0.05$). The mean vitamin-D concentration was 45.8 ± 23.9 nmol/L.

Keywords: Vitamin-D, Children, Adolescents, Indian, Anthropometry, Liquid Chromatography, Tandem-Mass Spectrometry.

1. INTRODUCTION

An insufficient amount of vitamin D affects more than one billion individuals throughout the world and has lately emerged as a significant problem in the field of public health. A wide variety of nonskeletal disorders, such as type 1 diabetes mellitus, cardiovascular disease, infectious disease, multiple sclerosis, autoimmune disease, depression, schizophrenia,

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 03, 2024

obesity, chronic obstructive pulmonary disease, and infectious disease, are also linked to low serum 25-hydroxyvitamin D levels. [1,2] A number of musculoskeletal disorders, such as osteoporosis, rheumatoid arthritis, fractures, and bone metabolism, are also linked to low serum 25-hydroxyvitamin D levels. The specific nature of the association between vitamin D and a few of these ailments, on the other hand, is not entirely understood. [3,4]

The question of whether or not these ailments are the cause of vitamin D deficiency or if vitamin D deficiency is a cause of these conditions is not yet established. [5] There is a growing body of research suggesting that a lack of vitamin D is linked to a variety of health issues; hence, it is essential to maintain sufficient levels of vitamin D in order to achieve maximum health. [6,7]

Vitamin D is essential to a wide variety of functions that are essential to the body. Its conventional tasks include the regulation of mineral homeostasis and the absorption of calcium. In addition to this, the body cannot absorb minerals such as iron, zinc, magnesium, and phosphorus without vitamin D. [8,9] Vitamin D is essential for the body to absorb these elements. A significant contribution to the regulation of parathyroid hormone (PTH) secretion is made by the active form of vitamin D. Bone loss and fragility, which are both induced by excessive levels of parathyroid hormone (PTH), are linked with an increased risk of osteoporosis.[10]

Also, recent studies suggest that vitamin D may be able to assist in the regulation of glucose levels and the release of insulin. [11] As a result of its contribution to the generation of antimicrobial peptides, vitamin D promotes cellular immunity and the body's defenses against infections. Recent research has shown that vitamin D controls both innate and adaptive immunity, showing that it has the potential to act as an immunomodulator for the treatment of autoimmune illnesses and cancer. [12] Psoriasis, autoimmune thyroid issues, thyroid cancer, and other autoimmune diseases have all been associated to vitamin D deficits, according to a number of studies.[13,14]

. vitamin D deficiencies have also been connected to thyroid cancer. In addition to this, vitamin D has the ability to control the development of cells and also influences the differentiation and proliferation of cells. [15] The presence of vitamin D receptors in a wide variety of organs is evidence that vitamin D is used in a wide variety of therapeutic and physiological applications. In view of the many functions that vitamin D plays in human health, it is abundantly evident that vitamin D is an essential component for ensuring that one is in the best possible condition.Vitamin D deficiency is a global issue that is becoming more urgent yet is often ignored. [16]

This is a problem that affects people all over the globe. There are still significant gaps in our information regarding how frequent vitamin D deficiency is, despite the fact that findings from research indicate that it is getting increasingly prevalent all over the world. [17] As an example, when the 25(OH)D $<$ 20 ng/ml threshold is taken into consideration, it is estimated that 24 percent of the American population, 37 percent of the Canadian population, and forty percent of the European population do not have sufficient quantities of vitamin D. On the

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 03, 2024

other hand, it seems that certain regions, such as Asia, the Middle East, and Africa, have a substantially greater frequency of vitamin D deficiency than others. [18,19]

Despite the fact that India receives a significant amount of sunlight, several studies conducted on a smaller scale have shown that the country has a widespread problem of vitamin D deficiency. Estimates of vitamin D deficiency in India range from 34% to an incredible 94%, depending on the kind of demography being considered. It is important to keep in mind that these studies only worked with small samples, and as a consequence, the findings may not be applicable nationally. [20,21]

2. REVIEW OF LITERATURE

Mele (2020) [22]Vitamin D may be useful as an immunomodulator for cancer and autoimmune diseases, according to recent studies that found it to have a function in both types of immunity. Several studies have linked autoimmune illnesses such as psoriasis, thyroid cancer, and autoimmune thyroid disorders to insufficient vitamin D levels.

Amrein (2020) [23]Unfortunately, vitamin D deficiency is still a growing problem throughout the world that doesn't get nearly enough attention. Vitamin D deficiency is on the rise around the globe, according to studies, but there are still some unknowns about how common it is. Approximately 24% of Americans, 37% of Canadians, and 40% of Europeans are believed to have inadequate levels of vitamin D when taking into account the cutoff of 25(OH)D < 20 ng/ml.

Harinarayan (2019) [24]Ensuring sufficient vitamin D levels is crucial for general health and wellness throughout adolescence, a time of significant physical and mental growth. Vitamin D deficiency is common among Indian teenagers, according to earlier research; however, no study has reached a large-scale, representative scope. Furthermore, no one has investigated the anthropometric and biochemical variables linked to vitamin D insufficiency in this group in any detail.

National Institute of Health (2022) [25]There are many important roles that vitamin D plays in the body. Its traditional function is to keep mineral levels stable and to control calcium absorption. The absorption of other vital minerals, including zinc, iron, magnesium, and phosphorus, also depends on vitamin D.

3. Significance of the study

This work is noteworthy because it uses the LCMS/MS technology, which is a new methodology in multicenter research, to comprehensively analyze the vitamin D level among adolescents and children in India. There is an immediate need for health promotion efforts due to the high frequency of vitamin D insufficiency and deficiency, as well as regional and ethnic disparities. In addition, by showing its importance in many communities, this work adds to the ongoing worldwide conversation on vitamin D insufficiency. In order to enhance

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 03, 2024

the wellness of Indian youth, this study aims to shed light on the geographical differences and identify risk factors.

4. Statement of the Problem

Vitamin D insufficiency and inadequacy is a common concern among kids and teens in India, according to this study's problem statement. Although there is some evidence of high prevalence rates in different countries, there is a dearth of large-scale investigations that use LCMS/MS or other advanced evaluation approaches. The demographic and interstate differences in vitamin D levels are also poorly understood. To fill these gaps, this research wasexamining the absence of vitamin D in Indian youths and what factors contribute to it. The results werehelp develop solutions that are more likely to be effective in improving adolescent health.

5. RESEARCH METHODOLOGY

Data was collected from July 2022 to October 2023 as part of a multicenter, cross-sectional, observational research that took place in schools. A multistage stratified random sample approach, as reported earlier11,12, was used for the sampling. In a nutshell, six Indian states—Madhya Pradesh, Rajasthan, Uttar Pradesh, Punjab, Tamil Nadu, and Assam—were chosen at random, and within each of those states, a city and a neighboring hamlet were randomly picked. Dobara, Haripura, Khanpuriya, Laxmipura, chennai, and Borkui were chosen as nearby villages from Rajasthan, Uttar Pradesh, Madhya Pradesh, Assam, Tamil Nadu, and Punjab, respectively, while Bhopal (18.5° N), Kota (20.7° N), Lucknow (21.2° N), Amritsar (25.8° N), Hyderabad (14.0° N), and Dispur (30.7° N) were chosen as cities. The research concluded with the creation of a list of schools from chosen centers. Out of a hundred schools that were contacted, forty schools consented to participate. The procedures followed the guidelines laid forth in the Declaration of Helsinki for the Protection of Human Subjects in Medical Research. An institutional ethics committee's stamp of approval was finally secured. All participants were required to provide written informed permission, which was acquired from all children (older than 7 years old) and from health authorities, schools, and parents or legal guardians. A pediatrician checked over each child's medical history and rejected those with chronic conditions or those who were expected to have problems with calcium and vitamin D metabolism, such as malabsorption, or who were already taking supplements of these nutrients.

- **Sample size**

Using the formula [z2*P(1 − P)/d2),

Where z = 1.96 and d = 5%,

The sample size per area was determined to be "n = 370 children/region," in accordance with other research that assumed an average prevalence of vitamin D insufficiency (VDD) of around 60%. Therefore, in order to evaluate VDD in 6 areas of India, a minimum of 2200 individuals were needed. Using G power (software version 3.1.9.2) for post hoc power analysis, we found that a subset of the sample (n = 672) had enough validity to meet the study's power requirements (1 − beta error probability) = 0.83 at the 0.05 level of alpha. We

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 03, 2024

used a two-tailed logistic regression model (Z test family) and found that age/other factors were significant predictors of VDD with an odds ratio (OR) of 0.754 (0.671-0.841).

- **Demographic data**

A pre-validated questionnaire was used to gather information on the participants' date of birth, socio-demographic details, sun exposure, sunscreen use, and medical history. This data was then cross-verified with school records.

- **Anthropometry**

We used the established methods to measure the subjects' heigh and body weight. In order to determine body mass index (BMI), the following formula was used: Weight in kilograms divided by height in meters is the body mass index (BMI).2. The non-dominant hand was used to measure TSFT according to conventional methods using a Harpenden calliper, to the nearest millimeter. Afterwards, Z scores were generated from the height, weight, TSFT, and BMI using reference data from India.

- **Body composition**

After a minimum of three hours of fasting and voiding before measurements were taken while standing, the subjects' fat mass, fat-free mass, and total body water were measured using a Bioelectrical Impedance Analyzer (BIA), specifically a Tanita Model BC420MA. Z scores for body fat and muscle mass were computed using data from India as a reference.

- **Dietary intakes**

Expert nutritionists documented food consumption over the course of two days that were not consecutive, excluding a holiday or Sunday, using the 24-hour dietary recall technique. Using the multiple-pass approach and conventional measuring implements, interviewees were asked to estimate their food consumption in Hindi, English, or the local language. The nutritive values of both raw and cooked foods were used in the C-Diet program to estimate nutrient intakes. Consumption of calcium in milligrams per hundred calories was used to calculate calcium density.

- **Dried blood spot collection and vitamin D estimation**

Professional phlebotomists collected blood samples on untreated Whatman filter paper no. 903. Before the operation, we made sure each youngster was comfortable by making them sit down and explaining the technique. After the selected finger was cleaned with a spirit swab (70% ethyl alcohol), a sterile, single-use safety lancet was used to puncture the skin. Additional drips were seen on pre-labeled filter paper after the first drop was discarded. Each filter paper was air-dried after detecting three or four drips, and then sealed in plastic bags with a 1 gram desiccant bag inside. To prevent the bags from being exposed to sunlight, they were each placed in a black, sealed plastic bag with a label. Until further investigation, each bag was kept in a freezer set to -80 °C. Using hexane as a solvent, the DBS samples were extracted using the liquid-liquid extraction technique. Vitamin D (25(OH)D3) content was evaluated using the LC-MS/MS system Shimadzu 8045 triple quadrupole mass spectrometer

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 03, 2024

(MS) [Shimadzu Corporation, 2019, Japan] with the electrospray ionization (ESI) source in positive ion polarity mode coupled with Nexera X2 LC-30A UHPLC system, as previously described10. The samples were separated using ultra-high-performance liquid chromatography (UHPLC). The same equipment and trained lab staff were used to test all of the samples. The coefficient of variance within the test was 7% and between the assays was 8%.

- **Data entry and warehousing**

All of the data was input twice into MySQL, and range checks were used to catch any mistakes. Next, we used distributions, ranges, and measures of central tendency to check whether the dataset was consistent. For categorical variables, frequency tables were made to spot anomalies and discrepancies in the data.

- **Statistical analysis**

Version 26.0 of the IBM Statistics Data Editor (SPSS) for Windows was published in 2018 by IBM Corp. Visual Studio 2017 (Windows), IBM SPSS Statistics 26.0. Armonk, NY: IBM Corp.) was used for statistical analysis, whereas StataIC12 was employed for proportional testing. Mean ± SD was used to describe normally distributed data, whereas non-normally distributed variables were given as median (IQR). The variables were then examined for normality. For normally distributed data provided as mean ± SD, suitable procedures such independent sample t-tests were used to evaluate the disparities between children residing in urban and rural areas in relation to anthropometry, 25(OH)D3 concentrations, nutritional intakes, and body composition characteristics. For the purpose of analyzing differences in parameters provided as median (IQR) but not normally distributed, the Mann Whitney-U test was used. The differences across the states were examined using ANOVA for both boys and girls independently. Using data from each state's 2021 census, we additionally provide survey-weighted parameters that are divided up according to urban/rural settings and states. Using the SPSS software21, we obtained a percentage factor appropriate to the sample data for the survey weighting technique based on the age, gender, and regionwise population statistics from the 2021 Census. The variables impacting 25(OH)D3 status were evaluated using a model for multinomial logistic regression analysis. In the model, the dependent variables were vitamin D sufficiency and coded vitamin D deficit or insufficiency.

6. RESULTS

A total of 2450 youngsters were enrolled in the research; 1223 were boys and 1227 were girls. 1319 of both sexes lived in metropolitan regions. Out of all the states, the following had the most participants: Madhya Pradesh with 335, Tamil Nadu a with 475, Punjab with 420, Uttar Pradesh with 405, Assam with 370, and Rajasthan with 445.

Table 1 shows the average anthropometric measures and vitamin D concentrations of the study participants broken down by gender and region. The study population had standard deviations of 11.5 ± 3.1 years for height, − 0.4 ± 1.0 for weight, − 0.5 ± 1.0 for BMI, and − 0.5 ± 1.0 for age and gender standardized Z-scores, respectively. With the exception of

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 03, 2024

females from Punjab (no differences between urban and rural girls, p > 0.05) and subjects from Tamil Nadu (no differences between urban and rural subjects, p > 0.05), urban subjects were heavier and taller than rural ones. The study population had an average vitamin D content of 45.8 ± 23.9 nmol/l. In general, the average vitamin D levels of males in urban and rural areas were noticeably greater than those of girls (p < 0.05). With the exception of Uttar Pradesh, where urban boys had higher mean vitamin D concentrations than rural counterparts, the difference was not statistically significant (p > 0.05), but the rural population had considerably higher vitamin D concentrations overall (p < 0.05) compared to the urban population. “The average vitamin D concentrations of urban Rajasthani boys and girls were much lower than those of their corresponding counterparts in other ethnic groups, with the exception of Assamese boys (p > 0.05 for both groups showed no significant difference in vitamin D concentrations) and Punjabi girls (p > 0.05 for both groups showed no significant difference in vitamin D concentrations). The mean vitamin D concentrations of rural boys and girls were not statistically different from their counterparts (p > 0.05), with the exception of rural Telangana boys, who had considerably higher concentrations than rural Uttar Pradesh boys.

Table 1. Demographic and anthropometric variables, vitamin D concentrations, and the research population's state, geography (rural/urban), and gender.

States	**Madhya Pradesh**		Tamil Nadu		**Punjab**		**Uttar Pradesh**		**Assam**		**Rajasthan**	
	Urban	**Rural**	**Urban**	**Rural**	**Urban**	**Rural**	**Urban**	**Rural**	**Urban**	**Rural**	**Urban**	**Rural**
Boys												
Age (years)	10.6 ± 3*t, p,c	11.6 ± 2.9	11.9 ± 2.9 g	11.4 ± 3.5	12.6 ± 3.6* m,a, g	11.6 ± 3.2	12.1 ± 2.4*g,	12.1 ± 3.6 m	11 ± 3 a	11.1 ± 3.4	11.2 ± 2.7p	10.5 ± 2.7 c
Height Z-Score	− 0.4 ± 1.1* c,m	− 0.7 ± 1.1 t,c	− 0.1 ± 1	0.1 ± 1.3m, p,c,a,g	− 0.4 ± 1.1* m,c	− 0.8 ± 1 t	0.1 ± 1* p,a,g	− 1.3 ± 1 t,a,g	− 0.5 ± 1*m, c	− 0.8 ± 1.1t, c	0.1 ± 1*p,a, g	− 1.1 ± 0.9 t
Weight Z-Score	− 0.4 ± 1.1* c	− 1.1 ± 1t	− 0.3 ± 1 c	− 0.3 ± 1 m,p,c, a,g	− 0.5 ± 1*c	− 1 ± 1 t,c	0.3 ± 1.1*m, t,p,a,g	− 1.5 ± 0.8 t,p,a	− 0.5 ± 0.9* c	− 0.8 ± 0.9 m,t, c	− 0.3 ± 1.1*c	− 1.4 ± 1t, a
BMI Z-Score	− 0.3 ±	− 1 ± 0.9	− 0.3 ±	− 0.4 ± 1 m,p,c,	− 0.4 ± 1*c	− 0.8 ±	0.3 ± 1.1*m, t,p,a,g	− 1.1 ± 0.7t,a	− 0.4 ± 0.9c	− 0.6 ±	− 0.4 ± 1.2*c	− 1.2 ±

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 03, 2024

e	1.1* c	t,a	1.1c	g		0.8 t				0.7 m,c, g		1.1 t,a
DB 25OHD (nmol/L)	46.7 ± 27m	50.2 ± 22.5	51.7 ± 19.2 m,a	56.6 ± 33.1c	45.9 ± 23m	47.8 ± 32.8	48.4 ± 27.3 m	46.2 ± 30t	42 ± 22.3 *,t	50.8 ± 18.9	36.5 ± 17.4*t ,c,p,g	56.3 ± 16.8
Girls												
Age (years)	10.6 ± 2.9* t,p,c	11.5 ± 3	12 ± 3 g	11.2 ± 3.5	12.7 ± 3.7m ,g,a	11.8 ± 3.4	11.9 ± 2.5 g	12.3 ± 3.9m ,a	10.8 ± 3p	11 ± 3c	11.4 ± 2.7*p	10.4 ± 2.7 c
Height Z-Score	− 0.3 ± 1*c	− 0.5 ± 1t, c	0.1 ± 0.9 p,a	0.1 ± 1m,p,c ,a,g	− 0.5 ± 1 m,t,c	− 0.6 ± 0.9 t,c	0.2 ± 0.9*p, a,g	− 1 ± 0.9m ,p,t,g	− 0.4 ± 0.9* m.t,c	− 0.8 ± 0.9t	0.2 ± 0.9*p, a	− 0.5 ± 0.8 t,c
Weight Z-Score	− 0.5 ± 0.9*	− 0.9 ± 1t	− 0.3 ± 0.9p	− 0.2 ± 1.1m, p,c,a,g	− 0.7 ± 0.9m ,t,c	− 0.9 ± 0.8 t	− 0.2 ± 1*p	− 1.1 ± 0.8t,a	− 0.4 ± 1*	− 0.7 ± 0.9t, c	− 0.2 ± 0.9*p	− 1 ± 0.9 t
BMI Z-Score	− 0.4 ± 1*	− 0.8 ± 1.1 t	− 0.5 ± 1	− 0.3 ± 1.1m, p,c,g	− 0.6 ± 0.9*	− 0.8 ± 0.8 t	− 0.3 ± 1*	− 0.8 ± 0.7t	− 0.3 ± 1	− 0.5 ± 0.9 m	− 0.4 ± 1.1*	− 1 ± 1t, a
DB 25OHD (nmol/L)	41.5 ± 24.1 m	45.9 ± 21.4	46.7 ± 17.2 m,p	50.1 ± 25.6	38.2 ± 15.4 *t	46.8 ± 30.9	43.3 ± 22.1m	45.2 ± 28.7	41 ± 17.6 *m	47.8 ± 19.9	33.7 ± 18.7*t ,c,a,g	49.4 ± 15.2

In the end, only 36.8% of the people tested had vitamin D levels high enough (> 50 nmol/L). It was found that 26.2% of the population had vitamin D deficiency (< 30 nmol/L), whereas 37% had inadequacy (30-50 nmol/L). According to the endocrine society clinical practice guidelines22, lower cut-offs for vitamin D concentrations were found; 10% of patients had adequate levels (> 75 nmol/L), 63% were deficient (< 50 nmols/L), and 27% had inadequate levels (between 50 and 75 nmol/L). Among all groups, vitamin D insufficiency was substantially greater in girls than boys (28.4% vs 23.9%) and in urban areas (30.2% vs 21.6%), with a p-value less than 0.05 for each. Gender and rural/urban location did not significantly differ in the percentage of insufficiency (p > 0.05). Subjects from Tamil Nadu

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 03, 2024

had the greatest percentage of vitamin D sufficiency overall, which was noticeably different from all other states save Uttar Pradesh. Subjects from Rajasthan had the greatest percentage of vitamin D insufficiency + deficiency (< 50 nmol/L), which was also seen in Punjab, Assam, and Madhya Pradesh, with the exception of Tamil Nadu and Uttar Pradesh. Twenty percent of the population lacked enough vitamin D, and 38 percent had inadequate levels, according to the survey's weighting.

Table 2. Survey Study population characteristics weighted by vit D deficient category (n = 2500, all variables survey-weighted): age, anthropometric Z scores, vitamin D concentrations, prevalence of vit D deficiency/insufficiency, and sufficiency.

Vitamin D status	**Measures of anthropometry and vitamin D insufficiency that were weighted in the survey**			
	Deficient (< 30 nmol/L)	**Insufficient (30–50 nmol/L)**	**Sufficient (> 50 nmol/L)**	**Total**
Na	8,822,572	16,346,782	18,037,571	43,206,925
Age (years)b	12.5 ± 2.2	12.4 ± 2.2	12.8 ± 2.2	12.6 ± 2.2
Height Z-Scoreb	− 0.3 ± 1.1	− 0.5 ± 1.1	− 0.5 ± 1.1	− 0.5 ± 1.1
Weight Z-Scoreb	− 0.5 ± 1.1	− 0.7 ± 1	− 0.7 ± 1	− 0.7 ± 1
BMI Z-Scoreb	− 0.6 ± 1.1	− 0.6 ± 1	− 0.7 ± 1	− 0.6 ± 1
Vitamin D (nmol/L)b	20.7 ± 7.2	39.7 ± 5.8	69.6 ± 19.5	48.3 ± 23.6
Percent population	20	38	42	100

Reflects the descriptive traits of a sample of the population that underwent anthropometric, body composition, and nutritional examinations in addition to survey weighting. When looking at the population using the modified Kuppuswami classification23, we can see that most people were either middle class or lower class. Of the kids surveyed, 66% spent more than 30 minutes each day playing outside. The vitamin D deficient (VDD) group of children had substantially higher body composition measures, including BMI Z-score, TSFT, and body fat % Z-scores, suggesting that they weighed more than the other two groups. Additionally, the energy intakes of the VDD group were noticeably greater than those of the other two groups ($p < 0.05$). The VDD groups also had a substantially greater calcium density per 100 kcal compared to the groups with inadequate or sufficient vitamin D levels ($p < 0.05$).

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 03, 2024

Table 3. Vitamin D concentrations as a proxy for socioeconomic level, anthropometry, body composition, and lifestyle variables in a sample of 672 youths (all data are survey-weighted)

Parameters	Survey weighted a			
	Deficient (< 30 nmols/L)	**Insufficient (30–50 nmols/L)**	**Sufficient (> 50 nmols/L)**	**Total**
Na	3,609,460	6,137,148	5,570,475	15,317,083
Age in years	12.8 ± 1.9	13 ± 2	13.6 ± 2.2	13.2 ± 2.1
Height Z-Score	− 0.1 ± 0.9	− 0.4 ± 1	− 0.5 ± 1.1	− 0.3 ± 1
Weight Z-Score	− 0.3 ± 0.9	− 0.6 ± 0.7	− 0.7 ± 0.9	− 0.6 ± 0.8
BMI Z-Score	− 0.4 ± 0.9	− 0.6 ± 0.7	− 0.7 ± 0.7	− 0.6 ± 0.8
TSFT (mms)	12 ± 7	9 ± 6	9 ± 5	10 ± 6
TSFT Z-Score	− 0.6 ± 1.3	− 1.1 ± 1.2	− 1.3 ± 1.2	− 1 ± 1.3
Fat percent	19 ± 10	17 ± 9	16 ± 9	17 ± 9
Fat percentage Z-score	− 0.4 ± 1.0	− 0.6 ± 0.9	− 0.7 ± 0.9	− 0.6 ± 0.9
Muscle mass percent	77 ± 10	79 ± 9	80 ± 9	79 ± 9
Muscle mass percent Z-score	0.3 ± 1.0	0.6 ± 0.9	0.7 ± 0.8	0.6 ± 0.9
Energy (kcal/day)	1940 ± 620	1750 ± 600	1860 ± 630	1830 ± 620
Proteins (g/day)	49 ± 18	44 ± 18	47 ± 19	46 ± 19
Fat (g/day)	62 ± 26	53 ± 25	56 ± 27	56 ± 27
Calcium (mg/day)	630 ± 380	460 ± 320	490 ± 330	510 ± 340
Calcium-Density (mg/100 kcal)	30 ± 13	27 ± 14	26 ± 13	27 ± 13
Phosphorous(mg/day)	1100 ± 430	960 ± 380	1000 ± 410	1000 ± 400
Calcium: Phosphorus	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2
LES (% N)	45	49	54	50
MES (% N)	48	45	40	46
Playing in sun (% N)	65	65	67	66

details the variables impacting vitamin D insufficiency and deficiency in relation to vitamin D sufficiency using a multinomial logistic regression model. Two independent models were used to conduct the regression analysis. Model 2 utilized the Indian states as predictors, with Rajasthan used as a reference category (because of its low percentage of vitamin D sufficiency) and other lifestyle and body composition variables, whereas Model 1 used area of residence (rural/urban) as a predictor.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 03, 2024

Table 4. Model 1 includes demographic, anthropometric, body composition, and lifestyle variables, such as urban/rural location, and uses multinomial logistic regression to identify factors that predict vitamin D status in a subset of the study population (n = 672).

Independent variables#	**Model 1**			
	Vit D deficient		**Vit D insufficient**	
Distribution (%N)		19%		34%
	OR	95% CI	OR	95% CI
Age (years)	0.75*	0.67–0.85	0.78*	0.78–0.93
Calcium density mg/100 kcal	1.00	0.96–1.05	0.98	0.98–1.06
Calcium:phosphorus ratio	0.81	0.04–16.79	0.01	0.01–1.58
Girls	2.18*	1.37–3.47	0.89	0.89–1.84
Boys	Reference		Reference	
Low socio class	0.53	0.22–1.28	0.40	0.40–1.92
Medium socio class	0.67	0.27–1.64	0.60	0.60–2.98
High socio class	Reference		Reference	
No playing in Sun	1.0	0.63–1.63	0.88	0.88–1.88
Playing in Sun	Reference		Reference	
Overweight BMI Z	2.39*	1.25–4.55	0.66	0.66–2.24
Normal weight BMI Z	Reference		Reference	
High TSFT Z	2.37	0.81–6.89	0.51	0.51–4.62
Low TSFT Z	Reference		Reference	
Urban	2.03*	1.25–3.30	0.82	0.82–1.75
Rural	Reference		Reference	
Constant	2.33		2.19	
Nagelkerke R2	0.13		0.13	
Correct predicted %				52

Table 5. Model 2 (demographic, anthropometric, body composition, lifestyle, and residency state) used multinomial logistic regression to identify factors that predicted vitamin D status in a subgroup of the study population (n = 672).

Independent variables#	**Model 2**			
	Vit D deficient		**Vit D insufficient**	
Distribution (%N)		19%		34%
	OR	95% CI	OR	95% CI
Age (years)	0.87*	0.76–1.00	0.91*	0.83–0.99
Calcium density mg/100 kcal	1.00	0.95–1.05	1.01	0.97–1.05
Calcium:phosphorus ratio	1.45	0.05–46.91	0.31	0.02–5.18
Girls	2.27*	1.36–3.77	1.29	0.88–1.87
Boys	Reference		Reference	
Low socio class	0.70	0.26–1.88	0.96	0.42–2.21
Medium socio class	0.65	0.23–1.79	1.35	0.58–3.14
High socio class	Reference		Reference	

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 03, 2024

No playing in Sun	0.63	0.37–1.09	1.14	0.76–1.72
Playing in Sun	Reference		Reference	
Overweight BMI Z	2.44*	1.18–5.05	1.22	0.65–2.30
Normal weight BMI Z	Reference		Reference	
High TSFT Z	1.12	0.33–3.81	0.95	0.29–3.07
Low TSFT Z	Reference		Reference	
Madhya Pradesh	0.54	0.24–1.26	0.82	0.38–1.8
Telangana	0.07*	0.03–0.16	0.35*	0.19–0.67
Amritsar	0.04*	0.01–0.10	0.13*	0.06–0.26
Lucknow	0.03*	0.01–0.09	0.30*	0.16–0.56
Assam	0.24*	0.10–0.57	0.55	0.26–1.16
Rajasthan	Reference		Reference	
Constant		2.22		2.07
Nagelkerke R2	0.30		0.30	
Correct predicted %				59

There were significant factors that contributed to vitamin D deficiency, including being younger, being female, being overweight, living in an urban area, and residing in the state of Rajasthan. The odds ratios for these factors were 0.75 (0.67-0.84), 2.18 (1.37-3.47), 2.38 (1.25-4.55), 2.03 (1.24-3.30), and $p < 0.05$, respectively." Triceps skinfold thickness, dietary calcium intakes (adjusted for 100 kcal/day), socioeconomic status, sunshine exposure, and the calcium to phosphorus ratio were not associated with deficiency or insufficiency status in the study population.

DISCUSSION

We are unaware of any previous multicenter studies that have used the LCMS/MS technique to determine vitamin D levels in children and adolescents from India. Only around 42% of Indian children and adolescents (aged 5–18) were found to have adequate vitamin D levels (25(OH)D > 50 nmol/L) in this multi-state research. Consequently, 58% of the population had vitamin D insufficiency or deficiency—defined as having 25OH D levels below 50 nmol/L, 20% being deficient, and 38% being inadequate. The average 25(OH)D3 vitamin D concentration was less than 50 nmol/L. Using the endocrine society guidelines[26] as a benchmark, we find that only 10% of individuals exhibited adequate vitamin D concentrations (> 75 nmol/L). Vitamin D insufficiency is more common in certain states than others. Vitamin D deficiency and insufficiency were most common in the western Indian state of Rajasthan, while the southern Indian states of Tamil Nadu and Uttar Pradesh had the highest vitamin D sufficiency rates. Not only that, vitamin D insufficiency was more common in urban areas than in rural areas (30.2% vs.21.6%) and among females (28.4% vs.23.9%), according to the study.

There is a significant global incidence of vitamin D insufficiency. Some 5.9% of the population in the USA, 7.4% in Canada, and 13% in Europe is insufficient, while 24%, 37%,

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 03, 2024

and 40% of the populations in those regions are inadequate.[27,28] This is true even in the most industrialized nations. In contrast to a research that found deficiency + insufficiency in 70% of individuals aged 1-21 years, another American study on teenagers aged 11-18 years found concentrations < 50 nmol/L in 42% of subjects. Vitamin D deficiency in children has been documented in studies conducted in Germany (12.5% deficiency and 32.7% insufficiency) and England (12.1% severe deficiency < 25 nmol/L in 4-10 years and 20-24% in 11-18 years). [29,30]

Despite lower dietary calcium intake, higher percentages of body surface area exposed, and higher daily sun exposure among girls from lower socioeconomic strata, a Delhi study found that girls from upper socioeconomic status were more likely to suffer from vitamin D deficiency owing to their substantially higher body mass index and body fat percentage. [31,32]

In their research on school-aged children in Rajasthan, Mandlik et al. found that the amount of time spent in the sun had a substantial impact on vitamin D concentrations. Equally important in determining vitamin D level in 11–18 year olds was the amount of time spent in the sun, according to research from Ethiopia. [33] Having said that, we did not find that time spent in the sun significantly predicted vitamin D status. This is likely due to the fact that kids and teens in cities spend more time inside than their rural counterparts, who are said to get more sunshine. Vitamin D status may not have been independently predicted by living in an urban or rural area, as the former probably reflected the amount of time spent in the sun. [34,35]

CONCLUSION

In conclusion, we describe vitamin D status in children and adolescents in India utilizing the LCMS/MS technology and standardized methodology. Our multi-state research found that over 50% of the youths surveyed, including those living in both urban and rural areas, lacked adequate vitamin D levels. We detail variations in vitamin D levels across states and between urban and rural areas. Vitamin D insufficiency is a major public health issue in India, particularly among young women, urbanites, and overweight children and adolescents, according to our research.

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