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Anti-ricket deficiency status among Indian adolescents

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Abstract

Vitamin D or the sunshine vitamin is a very critical nutrient and the best mode to obtain this vital factor is through direct exposure to the UVB rays of sunlight. However, deficiency of this easily obtainable nutriment is very high in our country. Inspite of being tropical, which receives sunlight all through the year, many factors have fueled vitamin D deficiency among all strata of the society. Data of a total of 517 adolescents were included for analysis. Serum 25-OH vitamin D levels were tested using liquid chromatography - mass spectrometry platform and reported. Along with the total serum vitamin D levels, individual levels of vitamin D2 as well as vitamin D3 was also recorded for analysis. Our report highlights total prevalence of vitamin D deficiency to be as high as 61%, with it being higher among adolescent girls at 63% compared to boys at 56%, though the difference is not statistically significant. Also, average levels of 25-OH vitamin D has been detected to be 13.04 ng/mL among the deficient subjects, while levels of vitamin D2 and D3 have been detected to be at 1.64 ng/mL and 11.43 ng/mL respectively. The finding highlights prevalence of high frequency of deficients among adolescents. This enforces the need to generate massive awareness among Indians, as deficiency of this vitamin in young age, becomes a pedestal for development of many more clinical ailments.

Key words : Indian, Adolescents, Anti-ricket, Deficiency, Vitamin D

INTRODUCTION

Micronutrient deficiency is a growing clinical concern world over and the burden of clinical conditions like anemia, osteoporosis, etc. is ever increasing. One of the major reasons being lack of awareness and also unavailability of many statistical references. Vitamin D deficiency is one such condition which has affected majority in the country inspite of being native residents of a tropical country which receives abundant sunlight throughout the year. This crucial vitamin is synthesized in the skin on exposure to UVB rays of early morning sunlight. A pro-hormone, vitamin D in serum is a mixture of both vitamin D2 (ergocalciferol) and D3 (cholecalciferol).

The active pro-hormone vitamin D is synthesized in the liver and kidney after a two step hydroxylation procedure. The first hydroxylation occurs in the liver to generate 25-hydroxyvitamin D [25(OH)D], which is subsequently converted to $1,25(OH)_2D$ or calcitriol primarily in the kidneys and released into the blood. The 25(OH)D or calcidiol forms the major component of the circulating vitamin D in the blood. Calcitriol in the blood exerts its functions through the vitamin D receptors (VDR) present on most cells of the body post binding with the vitamin D binding protein (DBP) [1].

Vitamin D levels in human body is very important as it is known to confer protection against multiple clinical conditions like osteoporosis, autoimmune conditions like multiple sclerosis, rickets, diabetes, cardiovascular conditions and even cancer. Levels of vitamin D in the serum also influences absorption of calcium and phosphorous to regulate and maintain bone health.

Few studies in Indian population have documented low serum levels contributing to hypovitaminosis D, especially in pregnant women and adolescents. This can be attributed to multiple factors like lifestyle, socio-economic conditions and lack of awareness. Also, the traditional clothing of certain communities, restricts exposure of skin to sunlight fueling deficiency. Certain unavoidable conditions like staying in higher altitudes, dark skin, old age, etc. adds upto weakening the process of vitamin D synthesis.

Hypovitaminosis is not a clinical issue only in India and has been reported from many countries. In Bangladesh, deficiency has been documented to be 38% in high income group women, while it is upto 50% in women of the low income group [2]. A study on post-menopausal women, reported the frequency of hypovitaminosis D to be 49% in Malaysia, 90% in Japan, 82% is South Korea and 47% in Thailand [3].

The state of vitamin D deficiency in India today has risen to epidemic proportions, with news paper reports claiming 69% Indians to be deficient. Many studies have been published pertaining to prevalence of deficiency in the Indian population. However such frequency determination in a mixed cohort and pan-India levels are very few. Also, determination of deficiency status has been majorly studied in adults, and very few on adolescents and that also on girls (from both low economic and middle income groups). These studies have documented only the status of total vitamin D deficiency status and frequency of insufficiency and sufficiency as well as toxicity largely remains unknown.

Vitamin D testing is routinely done in our laboratory for blood samples obtained from all over the country. This makes available pan-India specimen, the data from which has been analyzed for this report. This report specifically projects the frequency of vitamin D deficients among a mixed cohort of Indian adolescents in both the genders. Our study is one of the few to highlight the frequency of all four clinical variants of vitamin D among Indian adolescents.

MATERIALS AND METHODS

Cohort

The sample cohort considered for the analysis in this study includes a total of 517 adolescents, inclusive of 333 girls and 184

boys. The age group considered was from 10 - 19 years for analysis and the mean age of the cohort was 15 ± 3 years for both the gender.

Methods

Estimation of serum vitamin D was done using the sensitive analytical platform of Liquid Chromatography - Mass Spectrometry (LC - MS/MS) validated and set for routine analysis of steroids. The laboratory developed protocol for analysis, involved extraction using acetonitrile followed by separation using an HPLC (Nexera UHPLC) with a C8 column (LUNA, 30 X 3 mm) in isocratic mode using deionized water, methanol and 0.1% formic acid as mobile phase. A sample of 50µL was injected within the column maintained at 40°C and the run time for separation of both VD2 and D3 was set at 6 minutes. Post separation, the analytes passed through the dissolvation line into the MS (Shimadzu LCMS-8040), supported by an APCI (Atmospheric Pressure Induced Chemical Ionization) source. The generated ions were filtered by the quadrupole system followed by detection by a PMT (Photomultiplier Tube).

Quality control

Assessing quality control materials and linearity before each batch of sample analysis is followed as a routine practice in the laboratory. For vitamin D analysis, Lypocheck Immunoplus Controls from Bio-Rad was used (all three levels). The reference material was treated the same way as patient samples and analyzed. Also linearity check using prepared standards was done before each analysis batch. Post the mandated accuracy and precision checks, the prepared samples were analyzed.

Chromatogram analysis

The resulting chromatogram obtained after 6 minutes of HPLC run followed by ion segregation and analysis, highlighted a sharp VD2 peak at RT of between 3.42 - 3.45 min, while VD3 is eluted between 3.37 - 3.4 min. The transitions captured for final analysis was the 413.4 > 107, 123 for VD2 and that for VD3 is 383.4 > 365.2. The representative images of the chromatogram are shown in Figures 1 and 2 respectively.

RESULTS

A total of 517 serum samples from adolescents were analyzed to identify their vitamin D status. Total vitamin D levels were taken into consideration while clinically classifying the reports as either being deficient (< 20 ng/mL), insufficient (20 - 30 ng/mL), sufficient (30 - 100 ng/mL) or toxic (> 100 ng/mL). The serum vitamin D levels was calculated considering the individual levels of VD2 and VD3 assessed in the cohort.

In case of total vitamin D levels, the prevalence of deficiency in our cohort of adolescents was detected to be at 61%. The deficiency among girls was detected to be at 63%, while in boys it was 56%. The data analyzed by the Fisher's exact test however did not detect the difference in the frequency between both the gender to be statistically significant. The data depicting all the four

Table 1: Summary of all four clinical variants detected in this study

Table 1 - Vitamin D clinical presentation				
Classification	**Deficient**	**Insufficient**	**Sufficient**	**Toxic**
Total	315 (61%)	116 (22%)	82 (16%)	4 (0.7%)
Females	211 (63%)	69 (21%)	50 (15%)	3 (0.9%)
Males	104 (56%)	47 (25%)	32 (17%)	1 (0.5%)

Table 2: Mean serum values of vitamin D detected in each clinical variant

Table 2 - Total Vitamin D [Mean values - ng/mL (SD)]				
Classification	**Deficient**	**Insufficient**	**Sufficient**	**Toxic**
Total	13.04 (4.2)	24.14 (2.7)	42.22 (16.8)	127.53 (33.4)
Females	12.9 (4.2)	24 (2.7)	45.14 (19.6)	111.7 (12.7)
Males	13.32 (4.3)	24.34 (2.7)	37.65 (9.7)	175.12
Vitamin D3 [Mean values - ng/mL (SD)]				
Classification	**Deficient**	**Insufficient**	**Sufficient**	**Toxic**
Total	11.43 (4.2)	22.28 (3.2)	37.8 (15.7)	125.62 (32.1)
Females	11.4 (4.3)	22.25 (3.3)	40.67 (18.5)	110.47 (13)
Males	11.51 (4.2)	22.34 (3.1)	33.3 (8.3)	171.07
Vitamin D2 [Mean values - ng/mL (SD)]				
Classification	**Deficient**	**Insufficient**	**Sufficient**	**Toxic**
Total	1.64 (1.5)	1.85 (1.5)	4.42 (10)	1.91 (1.5)
Females	1.55 (1.4)	1.75 (1.5)	4.46 (11.9)	1.2 (0.6)
Males	1.81 (1.6)	2 (1.6)	4.34 (6.2)	4.05

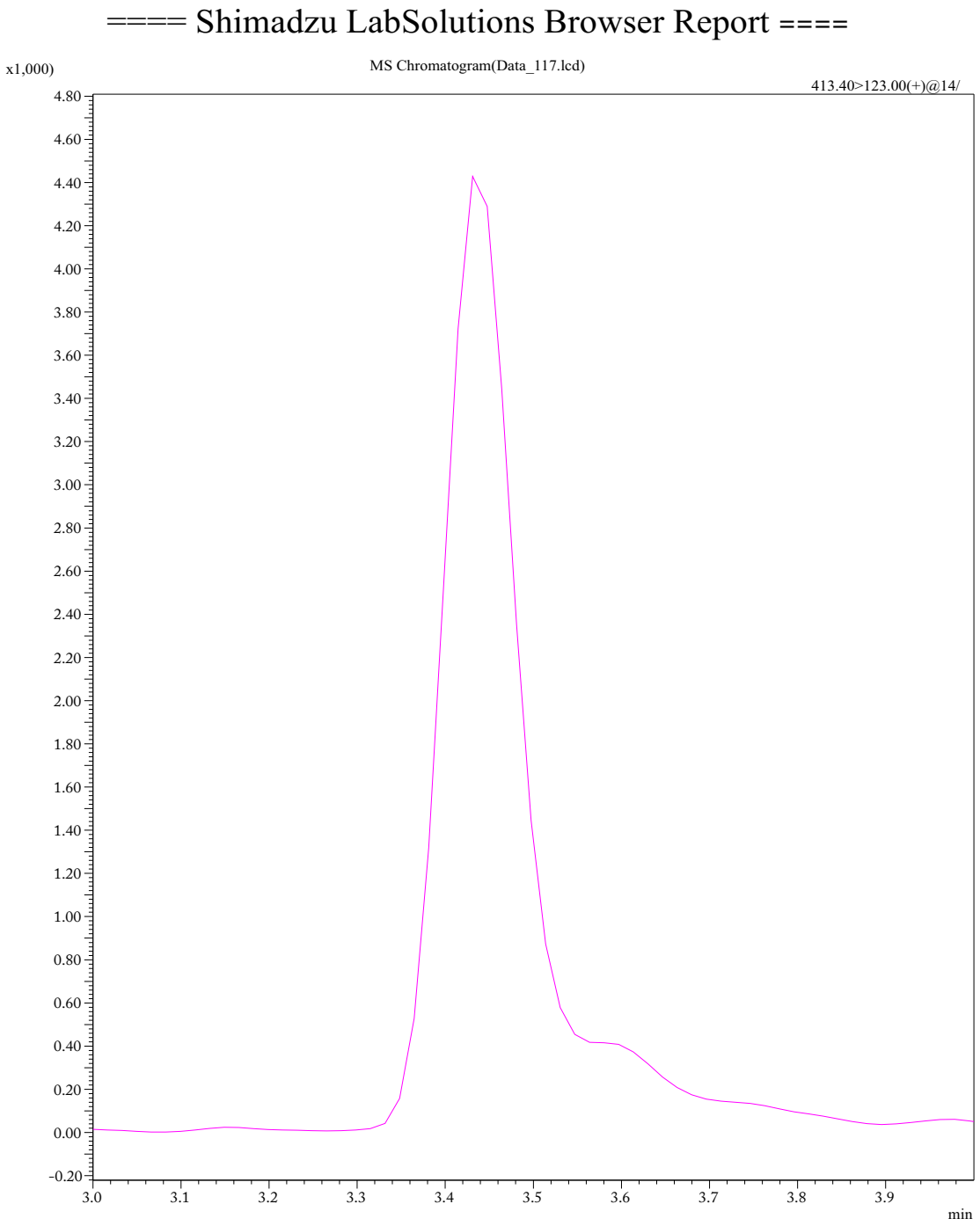

Figure 1: Chromatogram of vitamin D2 . Transition - 413.4 > 107 , 123; RT - 3.42 - 3.45 min

clinical references for total vitamin D is summarized in Table 1.

The mean values of total serum vitamin D levels analyzed in our cohort along with vitamin D3 and D2 levels analyzed is summarized in Table 2.

DISCUSSION

Among nutritional deficiencies in India, vitamin D was never imagined or considered to be a competing factor till the 1990s as maximum studies then were concentrated on analyzing serum levels of calcium and alkaline phosphatase in Indians [4]. Serum vitamin D [25(OH)D] has been shown to be the most robust marker to diagnose vitamin D deficiency. High frequency of vegetarians in the country coupled with socioeconomic conditions and lack of awareness regarding this condition as well as its treatment through supplements were and still continue to fuel deficiency.

Studies pertaining to assessing the prevalence of vitamin D deficiency among Indians only began in the 2000s. Few of the most recent study in Indian adolescents documented have been summarized in Table 3.

Almost all the studies reported till date have analyzed and recorded frequency of vitamin D deficiency majorly among adolescent girls residing in certain specific regions of the country. Our study, is one of the first to have analyzed the clinical status of vitamin D deficiency in a mixed cohort. Also, our study has

Table 1: Recent Indian studies on Vitamin D deficiency prevalence among Indian adolescents

Sr No	Area	Cohort	N	Deficiency (%)	Mean serum levels [25 (OH)D] in ng/mL	Reference
1	Delhi*	Adolescents (Girls and boys)	1829	96.9	8.3 (5.2)	5
2	Delhi	College girls	96	100	5.16 (3.08)	6
3	Delhi	Urban adolescent females (low and high income group)	404	90.8	12.74 (6.17)	7
4	Pune	Premenarchal school girls (low income group)	214	34.2	24.6 (10.4)	8
5	Barabanki (Lucknow)	Rural female adolescents	121	88.6	13.32 (6.4)	9
6	Tirupati*	Rural children	70	M - 76.5 F - 72.2	M - 17 (1.3) F - 19 (1.59)	10
		Urban children	69	M - 81.5 F - 62.9	M - 15.57 (1.2) F - 18.5 (1.66)	
5	Pan - India*#	Adolescents (Girls and boys)	517	61	13.04 (4.2)	This study

* - Three studies which have recorded along with the frequency of deficients, also the others *viz.* insufficient and sufficient. *# - Our study which has recorded all the four clinical variants along with values of VD2 and VD3 assessed using superior analytical platform of LC-MS/MS.

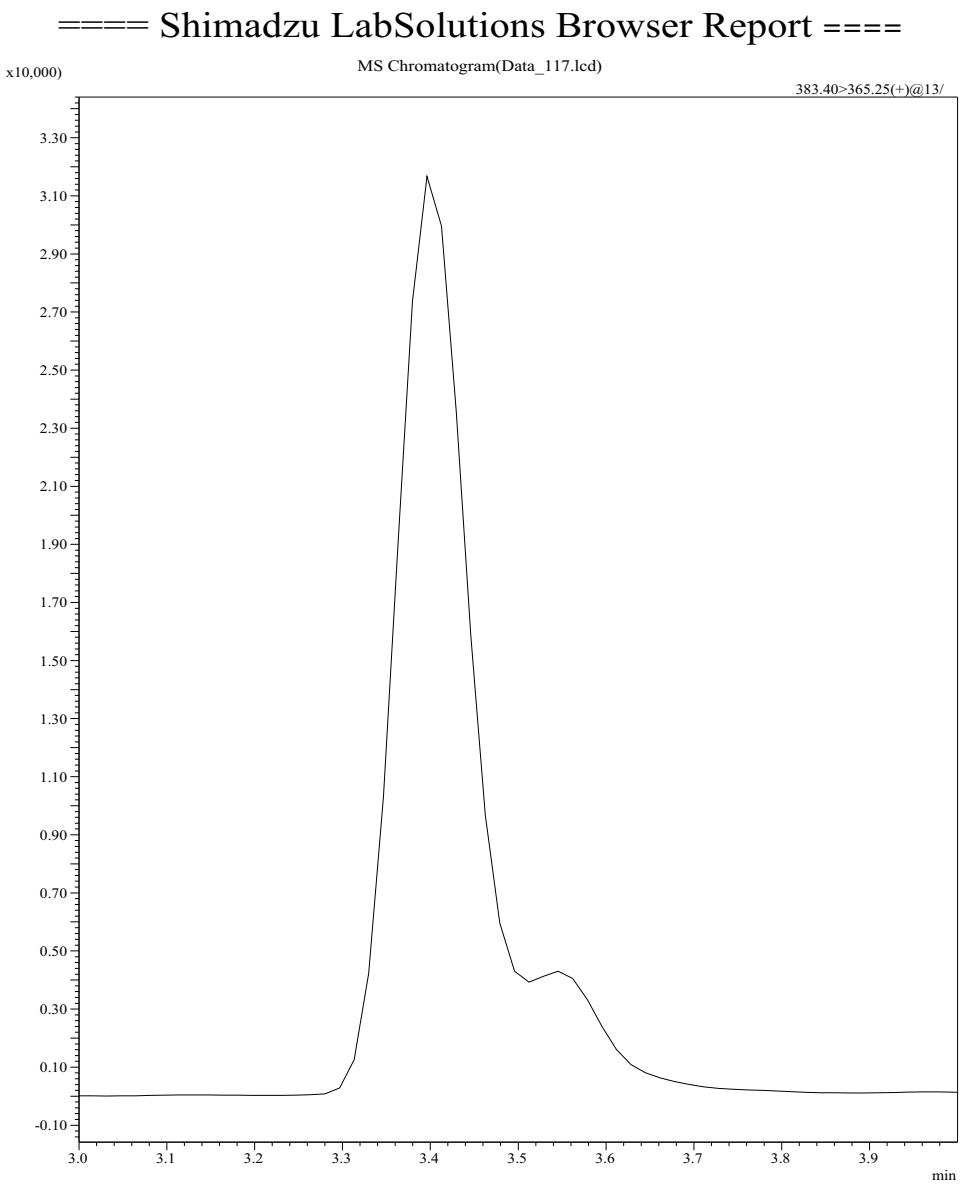

Figure 1: Chromatogram of vitamin D3. Transition - 383.4 > 365.2; RT - 3.37 - 3.4 min

documented the frequency of deficiency to be comparable in girls as well as boys and also the average serum values of both vitamins D2 and D3 in the cohort was analyzed using the superior LC-MS/MS platform. The results highlighted in the aforementioned tables, highlights the severity of this growing health scare in India, which needs to be tackled with awareness, mass screening camps and appropriate supplementation for treatment.

CONCLUSION

Our study is one of the very few to highlight mean levels of both vitamin D2 and D3 in a mixed cohort of Indian adolescents. Though the clinical reference for diagnosis and classification is limited to total vitamin D levels, separate analysis of vitamin D2 and D3 levels become important to monitor therapy. Supplements pertaining to both vitamin D2 and D3 are available in the market, and their individual assessment will aid in understanding efficacy as well as issues pertaining to absorption if any. Toxicity due to vitamin D supplementation is not unheard of in the medical community and hence awareness regarding availability of efficient diagnostic modalities is the need of the hour.

The deficiency of this anti-ricket factor among adolescents needs to garner more attention in India, as it is in this stage a therapeutic intervention can confer protection against development of osteoporosis along with other multiple associated disorders including cancer. It is the melanin in Indians which confer the dark skin tone, thereby naturally hampering adequate vitamin D synthesis even on exposure to sun. Also, use of sunscreens, long indoor working hours further adds to developing inadequacy. Thus, though the recommendation states direct skin exposure to early morning sun for more than 45 min as a requirement for adequate vitamin D synthesis, this alone may not work towards treating and managing hypovitaminosis D. Fortification and supplementation seem to be the only mode to tackle vitamin D deficiency in India.

Conflict of interest

None

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