

# Vitamin D Deficiency Picture: An Indian laboratory Retrospective Study of Over 10,000 Subjects

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## ABSTRACT

**Aim:** Vitamin D deficiency is a clinical condition prevalent world over and frequency studies estimate the same to be around 40% to 90%. Though diet and lifestyle have a major contribution towards blood levels of vitamin D, studying the deficiency prevalence demography-wise is important to chart specific remedial recommendations. **Materials and Methods:** Our study is aimed at presenting vitamin D status in a large cohort of over 10,000 Asian Indians. The study cohort consisted of a total of 10,379 Indians, inclusive of 4470 males and 5909 females. Serum vitamin D estimation was done using the analytical platform of Liquid Chromatography Mass Spectrometry (LC-MS). Levels of < 20 ng/mL was considered deficient while levels between 21 – 30 ng/mL was reported to be insufficient. **Result:** The frequency of vitamin D deficiency was detected to be 54.5%. The prevalence was detected to be higher in males at 56.7% as compared to females at 52.8% and the difference was found to be statistically significant. Age-wise analysis detected maximum frequency of deficiency in the 10 - 25 years group at 69.7%. **Conclusion:** Vitamin D deficiency is a public health concern in India and routine screening of this micronutrient is crucial among Indians wherein the prevalence has been detected to be high. **Key words:** Vitamin D, Indians, Deficiency, Insufficiency, Micronutrient.

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## INTRODUCTION

Vitamin D is an essential nutrient for maintaining bone health as it is associated with calcium and phosphorous homeostasis as well as skeletal growth. Discovery of vitamin D is associated with bone disease of the childhood; rickets. The association of rickets with lack of sunlight exposure was first identified by Sniadecki in 1822. Use of cod liver oil as a treatment for rickets in the mid-1800's further led to the detection of vitamin D in the cod liver oil to contribute towards the antirachitic properties by Mellanby and McCollum. This initiated the strategy of fortification in milk and other foods by the 1930s leading to eradication of deficiency in Europe and North America. Clinically vitamin D has two forms

*viz.* vitamin D<sub>2</sub> or ergocalciferol and vitamin D<sub>3</sub> or cholecalciferol. Though supplementation can be done with both vitamins D<sub>2</sub> and D<sub>3</sub>, the latter is also produced in the skin in response to the ultraviolet B radiation from the sun and hence vitamin D is also called the sunshine vitamin.<sup>[1]</sup>

Vitamin D apart from bone health has also been associated with stimulating insulin production, angiogenesis, inhibition of cellular proliferation, etc.<sup>[2,3]</sup> Physiological role of vitamin D and the adverse effects of insufficiency have been proven by many interventional, case-control as well as retrospective population studies.<sup>[4]</sup> Vitamin D Deficiency (VDD) has been identified as one of the most common nutrition deficiency world over and the major causes which have been attributed include intake of certain medications like anticonvulsants, obesity, inadequate exposure to sunlight, presence of conditions like lymphoma or primary hyperparathyroidism, etc.<sup>[3]</sup> Both vitamins D<sub>2</sub> and D<sub>3</sub> undergo metabolism in the liver and kidney, wherein first it is converted into 25-hydroxyvitamin D [25(OH)D] in the liver. Further, it is hydroxylated to the biologically active

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form of 1,25-dihydroxyvitamin D [ $1,25(\text{OH})_2\text{D}$ ] in the kidney. Diagnosis for VDD involves measurement of levels of  $25(\text{OH})\text{D}$  in the serum, while the active form  $1,25(\text{OH})_2\text{D}$  is involved in calcium absorption as well as mobilization.<sup>[1]</sup>

Estimation of vitamin D [ $25(\text{OH})\text{D}$ ] involves a lot of commercial assays available for platforms like Enzyme Immunoassay (EIA), Chemiluminescent Immunoassay (CLIA) or the analytical platform of High-pressure Liquid Chromatography (HPLC).<sup>[5,6]</sup> Considering many laboratory options being available for vitamin D estimation, the analytical platforms have been labeled to be a desirable reference method as separate quantification of vitamin  $\text{D}_2$  and  $\text{D}_3$  becomes a possibility. However, use of analytical technology requires reproducible method development making CLIA or EIA a practical alternative. Studies have also identified HPLC with ultraviolet quantification to be effective for use in routine estimations.<sup>[7]</sup> Many studies have also documented using Liquid Chromatography Mass Spectrometry (LC-MS) which has been shown to bear high specificity and sensitivity in comparison to RIA.<sup>[8]</sup>

Many geographical studies have described vitamin D status across different regions. In Europe, vitamin D insufficiency has been described to be common among children according to the ALSPAC birth cohort study in UK involving 7560 children. Causes of low socioeconomic status, advanced pubertal age, less exposure to sunlight, non-white ethnicity, as well as female gender had been attributed towards deficiency.<sup>[9]</sup> Studies in Middle-East regions like Turkey, Jordan and Saudi Arabia indicated deficiency to be common irrespective of high levels of sunshine due to the clothing attire; hijab.<sup>[10-12]</sup> In India, deficiency estimates have identified frequency of 50% - 90% and according to a report by the International Osteoporosis Foundation in 2009, in North India 96% of the neonates, 91% of healthy school girls and 84% of pregnant women are vitamin D deficient.<sup>[13,14]</sup>

Our retrospective study presents the picture of vitamin D insufficiency as well as deficiency in a pan-Indian cohort of over 10,000 individuals who have undergone routine preventive healthcare screening from our accredited clinical reference laboratory.

## MATERIALS AND METHODS

### Study Cohort

The retrospective analysis included a total of 10,379 individuals inclusive of 4470 males and 5909 females respectively. The data was collated from analysis done as part of preventive healthcare test in our CAP and

NABL accredited reference laboratory and hence did not involve any informed consent as it was not a hospital setting. Apart from age and gender no other patient identifiers were utilized for analysis in this report. The cohort characteristics have been highlighted in Table 1.

### Vitamin D Estimation

Serum vitamin D levels were measured using LC-MS/MS technology on the LCMS/MS 8040 tandem mass analyzer (Shimadzu, Kyoto, Japan). Extraction involved use of acetonitrile followed by separation using the HPLC (Nexera UHPLC) with a C8 column (LUNA, 30X3 mm) in isocratic mode with deionized water, methanol and 0.1% formic acid as mobile phase. Run time for both vitamin  $\text{D}_2$  and  $\text{D}_3$  was set at 6 mins and further subjected to MS analysis for detection.

## RESULTS

The clinical reference range for vitamin D status were; VDD for levels  $<20$  ng/mL, Vitamin D Insufficient (VDI) for levels between  $21 - 30$  ng/mL and Vitamin D Sufficient (VDS) for levels  $>30$  ng/mL. The mean vitamin D levels of the study cohort were assessed to be  $20.3 \pm 11.3$  ng/mL.

The frequency of VDD was detected to be 54.5% with the frequency among males being higher at 56.7% compared to females at 52.8% and the difference was statistically significant at  $p < 0.0001$ . Age-group wise analysis, detected difference in gender-wise frequency of VDD in cohort of  $25 - 50$  years and above  $50$  years to be statistically significant at  $p < 0.0001$  and  $p < 0.05$  respectively. The average levels of vitamin D in the VDD cohort were detected to be  $11.7 \pm 3.9$  ng/mL. In case of VDI, though the frequency among females was detected to be 24.4% compared to males at 23.3%, there was no statistical significance. Age-group wise analysis detected frequency between males and females in  $10 - 25$  years and  $25 - 50$  years cohort to be statistically significant at  $p < 0.05$  for both. The average levels of vitamin D detected in the VDI cohort were  $24.7 \pm 3.2$  ng/mL. In case of VDS, a total frequency of 21.6% was detected and the difference between males at 20.0%

**Table 1: Study cohort characteristics.**

| Gender | N     | Mean Vitamin D levels (ng/mL) |
|--------|-------|-------------------------------|
| Male   | 4470  | 19.9 +/- 11.1                 |
| Female | 5909  | 20.6 +/- 11.5                 |
| Total  | 10379 | 20.3 +/- 11.3                 |

Further, analysis was also done considering different age-groups like  $0 - 10$  years,  $10 - 25$  years,  $25 - 50$  years and above  $50$  years.

**Table 2: Age-group wise analysis for frequency of status of VDD and VDI.**

| Status of Vitamin D | VDD (<20 ng/mL)               |                               |                               | VDI (21-30 ng/mL)             |                               |                               |
|---------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
|                     | Age group (years)             | Total Frequency (%)           | Frequency in Males (%)        | Frequency in Females (%)      | Total Frequency (%)           | Frequency in Males (%)        |
| 0 – 10              | 55.6%<br>(12.9 +/- 3.5 ng/mL) | 51.3%<br>(13.3 +/- 3.3 ng/mL) | 60.4%<br>(12.6 +/- 3.6 ng/mL) | 23.1%<br>(24.5 +/- 3.2 ng/mL) | 25.2%<br>(24.7 +/- 3.1 ng/mL) | 20.7%<br>(24.2 +/- 3.4 ng/mL) |
| 10 – 25             | 69.7%<br>(11.0 +/- 3.8 ng/mL) | 66.6%<br>(11.3 +/- 3.8 ng/mL) | 71.7%<br>(10.8 +/- 3.8 ng/mL) | 16.5%<br>(24.3 +/- 3.2 ng/mL) | 20.0%<br>(24.7 +/- 3.5 ng/mL) | 14.2%<br>(23.9 +/- 3.0 ng/mL) |
| 25 – 50             | 58.9%<br>(11.6 +/- 3.9 ng/mL) | 62.0%<br>(11.6 +/- 3.9 ng/mL) | 56.7%<br>(11.6 +/- 4.0 ng/mL) | 22.4%<br>(24.5 +/- 3.2 ng/mL) | 21.1%<br>(24.5 +/- 3.1 ng/mL) | 23.4%<br>(24.6 +/- 3.2 ng/mL) |
| > 50                | 41.0%<br>(12.2 +/- 3.8 ng/mL) | 44.7%<br>(12.3 +/- 3.8 ng/mL) | 37.9%<br>(12.2 +/- 3.9 ng/mL) | 29.2%<br>(25.0 +/- 3.1 ng/mL) | 28.1%<br>(25.0 +/- 3.2 ng/mL) | 30.1%<br>(25.0 +/- 3.1 ng/mL) |

and females at 22.8% was detected to be significant at  $p < 0.05$ . Age-group analysis detected difference in gender frequency to be significant in the 25 – 50 years and > 50 years cohort at  $p < 0.05$ . The age-group wise cohort findings with relevance to VDD and VDI have been summarized in Table 2.

## DISCUSSION

Vitamin D is the most common nutrient found deficient in the majority world over. VDD has been associated with increased prevalence of cardiovascular conditions, diabetes mellitus, polycystic disease and other co-morbidities like autoimmune disorders, respiratory diseases like asthma, chronic obstructive lung disease and cancer.<sup>[13]</sup> The major source of vitamin D includes exposure to sunlight mainly the UV-B rays which leads to endogenous synthesis in the skin apart from fortified foods and fish.<sup>[15]</sup> Though endogenous vitamin D synthesis is sunlight associated, VDD has been recorded both in sunshine sufficient and deficient countries and also has been found to be independent of age, gender and geography by many studies.<sup>[15,16]</sup>

Relevance of studying VDD in the Indian scenario has been established by many publications and reports each focused on different demography, region or age-group. Our report is an attempt to present the status of VDD among different age-groups in a pan-India cohort irrespective of geography, diet or any other socio-economic factors. A total of 10379 individuals were assessed including 4470 males and 5909 females of different age groups from below 10 years to above 50 years. The aim is to present a complete age-group wise vitamin D profile among Asian Indians.

Serum vitamin D levels were measured using the analytical technology of LC-MS/MS and as per the US Endocrine Society classification levels of <20 ng/mL were considered deficient. Cohort analysis detected the average vitamin D levels to be around 20.3 +/- 11.3 ng/mL. The samples considered for analysis were those which had been processed in a reference laboratory thus minimizing the scope for any sample selection bias. Data from all the samples processed in one year were included for analysis. Among analysis for VDD and VDI apart from VDS, the frequency for VDI was detected to be highest at 54.5% and the difference between males at 56.7% and females at 52.8% was found to be significant at  $p < 0.0001$ . Many community-based studies among apparently healthy controls have recorded prevalence between 50 – 94%, while hospital-based studies record prevalence from 37 – 99%. High frequency has been noted throughout the country by geography-specific studies.<sup>[17-21]</sup>

Age-group wise analysis for VDD detected the frequency to be 41.0% in the above 50 years cohort with difference between males at 44.7% and females at 37.9% to be significant at  $p < 0.05$ . A similar Indian study which assessed vitamin D status among healthy Indians (n = 1346), of over 50 years of age, detected prevalence of VDD to be 91.2%, while VDI was detected to be a mere 6.8%. This study concluded hypovitaminosis D to be universal among North Indians over 50 years of age and also identified absence of parathyroid hormone response in more than 50% of VDD individuals.<sup>[19]</sup> Analysis for VDI in the over 50 years age-group cohort in our case detected frequency to be 29.2%. This difference in finding between our report and the previous published one could be the geographical inclusions

considered wherein the published report studied North Indians, while ours pan-India.

Studies correlating socioeconomic status and vitamin D status have also been recently published in the Indian scenario and one such cross-sectional study among 444 subjects, detected 46.4% to be deficient and the maximum subjects to be above 60 years of age. Moderate deficiency was recorded in 39.9% and maximum were detected from the 41–60 years cohort.<sup>[18]</sup> Our study detected maximum frequency of VDD among the 10 – 25 years cohort at 69.7%, but statistical significance in frequency difference between males and females at 62.0% and 56.7% was detected in the 25 – 50 years group at  $p < 0.0001$ .

The need for routine VDD screening has also been emphasized by another recent publication which studied prevalence and clinico-epidemiology of vitamin D deficiency in patients with type 2 diabetes mellitus and hypertension in a pan-India cohort. This study detected overall prevalence of VDD and VDI to be 83.7%, while it was 82% among the newly diagnosed. VDD and VDI frequency was specifically detected to be 60.9% and 22.9% respectively. High prevalence of VDD among the newly diagnosed indicates detection of deficiency being missed in a large proportion of patients with diabetes and hypertension.<sup>[22]</sup> Another community-based cross-sectional urban elderly study including 298 above 60 years individuals, detected VDD prevalence to be 56.3% and the mean levels to be 19.3 +/- 0.54 ng/mL. This study detected hypertension to be a significant risk factor and predictor of VDD apart from high body mass index and presence of metabolic syndrome.<sup>[23]</sup>

Vitamin D supplementation and outcome has also been recorded by many reports and one such meta-analysis by Endocrine Society detected a statistically significant risk reduction ( $p = 0.01$ ) towards experiencing at least one fall.<sup>[24]</sup> Effect of vitamin D supplementation has also been studied on the outcome of pulmonary tuberculosis. This study involving 1787 patients involved in active vitamin D supplementation with the standard anti-tuberculosis regimen detected increase in proportion of sputum smear and culture conversion and also improvement in plasma calcium, lymphocyte count and chest radiograph was noted.<sup>[25]</sup> Recent global reports also indicate vitamin D market to touch \$2.5 billion by the year 2020 and the Assocham Healthcare Committee revealed 8 in 10 people from Delhi region of the country to be affected by VDD.<sup>[26]</sup>

## CONCLUSION

The need for aggressive screening and monitored supplementation and treatment strategies are a necessity to tackle the global malady of vitamin D deficiency. Public health measures like educating, fortification, supplementation as well as mass screening programs form key pointers for a healthcare action plan. VDD continues to be a silent epidemic and publications which focus beyond specific gender and geography is a need to devise action points to manage mass public health concerns.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ABBREVIATIONS

**CAP:** College of American Pathologists; **CLIA:** Chemiluminescent Immunoassay; **EIA :** Enzyme Immunoassay; **HPLC:** High-Performance Liquid Chromatography; **25(OH)D:** 25-hydroxyvitamin D; **1,25(OH)2D:** 1,25-dihydroxyvitamin D; **LC-MS:** Liquid Chromatography-Mass Spectrometry; **NABL:** National Accreditation Board For Testing and Calibration Laboratories; **RIA:** Radioimmunoassay; **VDD:** Vitamin D Deficient; **VDI:** Vitamin D Insufficient; **VDS:** Vitamin D Sufficient.

## SUMMARY

One of the first few to assess vitamin D deficiency and insufficiency in a Pan-India cohort of over 10,000 individuals. Estimation of vitamin D2 and D3 enabled by Liquid Chromatography Mass Spectrometry. Age-group analysis also included; 0-10 years, 10 – 25 years, 25 – 50 years and >50 years. This retrospective analysis presents VDD in a wide range age cohort as well as among both the genders

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