International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

A Study on Biological Reference Interval of Vitamin D in Western Indian Population

Dr. Ruchita Chauhan[1], Dr. Shilpa Jain[2], Dr. Neeta Malukar[3]

[1]Third Year Resident
Corresponding Author Email: *chauhanruchita191[at]gmail.com*

[2]Professor
Email: *jinshilpa73[at]gmail.com*

[3]Associate Professor
Email: *nmalukar[at]ymail.com*

Abstract: ***Background**: Vitamin D plays a vital role in calcium-phosphorus metabolism and bone health. Its deficiency is a global health issue, with significant prevalence in India despite ample sunlight. This study aims to evaluate the Biological Reference Interval (BRI) of vitamin D levels in the Western Indian population and assess its adequacy using Chemiluminescence Immunoassay (CLIA). **Methods**: A cross-sectional study was conducted on 482 healthy participants (188 males, 294 females) aged 1–80 years at Sir Sayajirao Hospital, Gujarat, from April to September 2024. Serum 25-hydroxyvitamin D (25-OH vit D) levels were measured using the Abbott i-1000 SR analyzer. Data on diet, sunlight exposure, and lifestyle factors were collected via questionnaires. Statistical analysis included unpaired t-tests and percentile-based categorization. **Results**: Vitamin D deficiency (<20 ng/mL) was observed in 54.98% of participants, with 24.48% classified as insufficient (21–29 ng/mL) and 20.54% as sufficient (>30 ng/mL). Females had significantly lower mean levels (19.54 ± 9.25 ng/mL) compared to males (23.46 ± 11.16 ng/mL; p< 0.0001). The observed range (7.5–46 ng/mL) aligned with manufacturer-provided BRIs(6.6-49.9ng/ml). Hypovitaminosis D was prevalent across all age groups, with higher deficiency rates in females and older adults. **Conclusions**: Although the manufacturer's BRI was applicable, this study highlights the need to refine deficiency thresholds specific to the Western Indian population. The findings underscore the importance of tailored public health strategies to address the high prevalence of vitamin D deficiency and its associated health risks.*

Keywords: Vitamin D, Biological reference interval, Hypovitaminosis D, chemilumionescence

1. Introduction

Vitamin D, or calciferol, is not strictly classified as a true vitamin because it can be synthesized in the skin when an individual is adequately exposed to sunlight [1]. It can also be obtained through certain dietary sources, such as fatty fish, fortified dairy products, and egg yolks. Despite its importance, vitamin D deficiency has become a global public health concern, affecting both children and adults across different age groups and ethnicities.[2] Typically, 80-90% of vitamin D is produced in the skin from 7-dehydrocholesterol in the epidermal malpighian layer following sun exposure [3]. Dietary intake becomes necessary only when sunlight exposure is insufficient, contributing 10-20% of the total vitamin D levels [1,3]. Vitamin D plays a crucial role in bone and tooth development and supports muscle function. It enhances the absorption of calcium and phosphate in the intestine and kidneys. Deficiency in vitamin D leads to rickets in children and osteoporosis in adults [3]. Additionally, vitamin D has been linked to conditions such as obesity, hypertension, diabetes, and cancer. Vitamin D receptors are involved in either activation or repression of transcription of target genes, regulating bone and calcium homeostasis, inflammation, cell mediated immunity, cell cycle progression, and apoptosis. Vitamin D deficiency increase cancer risk. Vitamin D Receptor(VDR) signaling influences proliferation, differentiation, and apoptosis of keratinocytes, cutaneous immune responses and also useful in maintaining homeostasis of oral epithelium and oral immunity.[4]

Clinical documentation of vitamin D deficiency and excess has been reported both in India and globally[5]**.** A review of over 195 studies from 44 countries examining global vitamin D levels revealed that 88.1% of the samples had vitamin D concentrations below 75 nmol/L (30 ng/mL), the threshold for adequacy [6]. Studies in India indicate a widespread deficiency, with prevalence rates between 70% and 100% in various populations [7]. Puri S et al. reported that 90.8% of schoolgirls in Delhi exhibited biochemical hypovitaminosis D, while Shah P et al. reported a 94.94% prevalence among 500 apparently healthy staff members at Fortis Hospital, Mumbai [8,9]. This underscores the need to establish a Biological Reference Intervals (BRI) tailored to the Indian population, considering the unique geographical diversity across the country. This study aims to evaluate the BRI for its adequacy for the Western Indian population by using Chemiluminescence Immunoassay (CLIA). Given these findings, Indian patients are currently assessed for vitamin D status using BRI [10] based on western data:Deficiency(<20 ng/ml), Insufficiency(21-29 ng/ml) and Sufficiecy(>30 ng/ml).

2. Materials and Methods

According to the Clinical and Laboratory Standards Institute (CLSI) guidelines for BRI determination, the minimum number of volunteers required for a study should be 120[11]. This cross-sectional study was conducted on 482 participants, who were healthy attendants accompanying patients presenting at SSG hospital, tertiary level hospitals in Vadodara, Gujarat. Samples were collected for 6 months, a period starting from April 2024 to September 2024. In total 188 male and 294 female participants aged 1-80 years were assessed for 25-hydroxy vitamin D (25-OH vit D) levels using Abbott i-1000 SR, fully automated chemiluminescent analyzer. Participants were divided into 3 groups according to their vitamin D status: Group 1:Deficiency(<20 ng/ml), Group 2: Insufficiency(21-29 ng/ml) and Group 3:Sufficiecy(>30 ng/ml).[10]

Exclusion Criteria: Exclusion criteria were applied during sample collection to minimize bias. Individuals with dermatological, hepatic, renal or endocrine disorders that could impact vitamin D levels were excluded. Additionally, pregnant and lactating women, children, alcoholics, cigarette smokers, and those with acute or terminal illnesses or on medications that could influence vitamin D levels were also excluded from the study.

Questionnaire: The self-reported questionnaire included questions about the type and amount of food consumed over the previous 24 hours (vegetarian or non-vegetarian and consumption of vitamin D rich like fatty fish, fortified milk, egg yolk), time spent outdoors during work or commuting, duration of sunlight exposure between 10 a.m. and 2 p.m., use of sunscreen, hat or umbrella for sun protection and the type of occupation. History for dietary supplementation, previous history of deficiency and any chronic health conditions was also taken. History of medication was taken like anticonvulsants, glucocorticoid. History for physical activity or exercise was taken.

Sample Collection: A four-milliliter blood sample was collected from participants under aseptic conditions from the median cubital vein. The samples were placed in properly labeled plain vacutainers and centrifuged at 3000 rpm for 15 minutes. The collected serum was biochemically analyzed within 8 hours of collection. Samples not processed immediately were stored in a sample storage refrigerator at 2-8°C.

Estimation Of Vitamin D by CLIA: The 25-OH Vitamin D Total test was performed using the Abbott 25-OH Vitamin D Reagent Pack and the Abbott 25- OH Vitamin D Calibrators on the Abbott i-1000 SR fully automated chemiluminescent Immuno assay analyzer. A chemi leuminesence micro partical immunoassay(CMIA) technique was used to determine the concentration of 25-OH vitamin D present in the patient sample. [10]

Statistical Analysis: Statistical analysis was performed using Med CalC. All data was expressed in Mean±SD or in Median. All graphs were prepared using Microsoft Excel 2013. Statistical significance for unpaired Student's t-test was defined as p-value <0.05.

3. Results

The reagent kit literature for Abbott architect i-1000 SR define the vitamin D insufficiency level at <20 ng/ml. The vitamin D range of Abbott architect kit insert is 6.6-49.9 ng/ml. Values below 6.6 ng/ml and values above 49.9 are excluded. With reference to this criteria, 54.98% of the test subjects in the present study fall in the vitamin deficiency category, 24.48% fall in the vitamin insufficiency and 20.54 % have sufficient level of vitamin D.

Table 1: Showing vitamin D status of study subjects

Classification	Vitamin D level	No of subjects	Percentage
Group 1: Deficiency	<20 ng/ml	265	54.98 %
Group 2: Insufficiency	21-29 ng/ml	118	24.48 %
Group 3: Sufficiency	>30-49.9 ng/ml	99	20.54 %
		Total: 482	100 %

The statistical analysis of the demographical data shows that the values obtained in the present study lies in between the values obtained from the reference studie.The observed range of vitamin D level in the present study was 7.5-46 ng/mL (2.5th to 97.5th percentile).

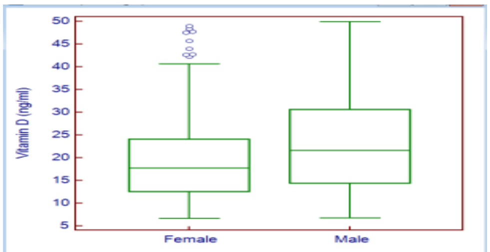

Figure 1: Shows that mean value of vitamin D is lower in females as compared to males

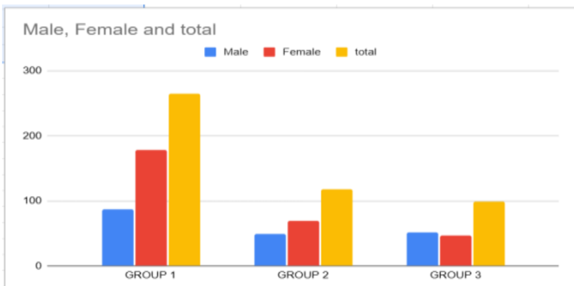

Figure 2: Shows gender distribution between group 1,2 & 3

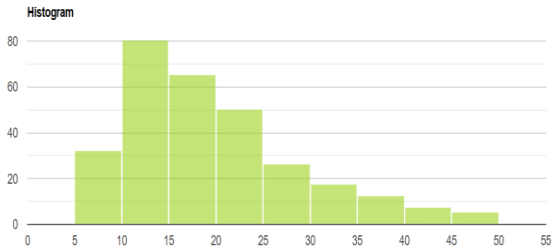

Figure 3: Shows Vitamin D level distribution in female individuals (Mean 19.54 $\pm$ 9.25ng/ml), Shapiro wilk test: W=0.92, p<0.001

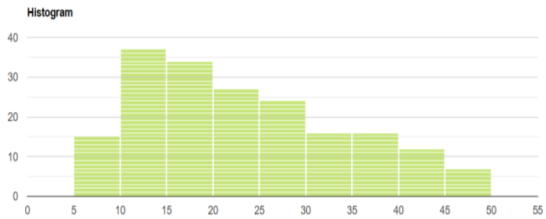

Figure 4: Shows Vitamin D level distribution in male individuals (Mean 23.46 $\pm$ 11.16 ng/ml), Shapiro wilk test:W=0.95, p<0.001

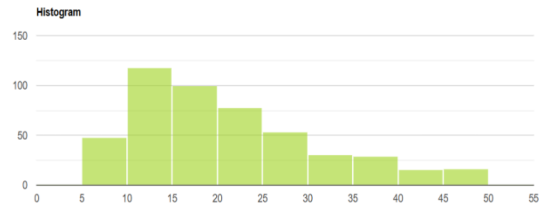

Figure 5: Shows Vitamin D level distribution in all individuals (Mean 21.07 + 10.21 ng/ml), Shapiro wilk test: W=0.93, p<0.001

Table 2: Interquartile range of Vitamin D levels according to gender and in all study subjects irrespective of method of estimation.

Vitamin D (ng/ml)	Male subjects (n=188)	Female subjects (n=294)	All study subjects (n=482)	
Minimun	6.7	6.6	6.6	P value <0.0001
1st Quartile	14.3	12.4	13	
Median	21.6	17.8	19	
3rd Quartile	30.5	24.1	26.2	
Maximum	49.9	49	49.9	
Unpaired student's t-test applied between male and female. *p value<0.05 Statistically Significant				

4. Discussion

Humans have historically relied on sunlight to meet their vitamin D needs (5). One possible reason for the evolution of melanin pigmentation was to enable individuals who migrated north and south of the equator to produce sufficient vitamin D in their skin (12). Recommendations to avoid all sun exposure have now put global populations at risk of vitamin D deficiency (13). This risk has become evident in Australia, where rising skin cancer rates led to campaigns promoting the avoidance of direct sun exposure without protection, such as clothing or sunscreen(14).

In this study, we estimated serum vitamin D levels across Western Indian population to determine whether the biological reference range (BRI) established by reagent manufacturers can be applied to the general population. The obtained values were consistent the BRI provided by the reagent manufacturers so it can be applicable to the population studied. Nonetheless, it is important to note that the majority of the study population exhibited hypovitaminosis D. Although we agree with the BRI established by the reagent manufacturers, we believe that the criteria for defining vitamin D insufficiency and deficiency need further evaluation for the Western Indian population. This is highlighted by our finding that only 20.54% of the participants met the criteria for vitamin D sufficiency. Therefore, test result interpretations should consider the high prevalence of asymptomatic vitamin D deficiency.

The Indian Space Research Organization's solar energy mapping identified Gujarat as the region receiving the highest levels of solar radiation. [15]. Previous studies have indicated a high prevalence of vitamin D deficiency throughout India, with deficiency rates exceeding 90% in some areas, regardless of geographic location [5]. Reported deficiency prevalence includes 94.4% in North India [16], 85.6% in South India [17], 92.5% in Eastern India [18], and 97.5% in Western India [19]. Contributing factors include lifestyle and socio cultural practices that limit sun exposure, inadequate dietary intake, and lack of food fortification [7-9]. Jayanta Das et al. found a declining trend of vitamin D levels with increasing age [5]. Advanced age is associated with multiple changes in vitamin D metabolism, such as decreased synthesis in the skin, reduced dietary absorption, and declining renal function, which can contribute to deficiency [20,21]. Hypovitaminosis D in the elderly may not be attributed solely to age but may also result from differences in sun exposure and variations in dietary fat and vitamin D intake. Chronic illness, immobility, and prolonged hospitalization can further exacerbate vitamin D deficiency in older adults [20]. Similar findings have been reported by other researchers, who noted that children and young adults have significantly higher vitamin D levels compared to older adults, regardless of gender, race, or ethnicity [20,5]. Our study found a statistically significant difference in median vitamin D levels between males(21.6 ng/dl) and females(17.8 ng/dl), consistent with the findings of Jayanta Das et al(male-28.4ng/dl, female-24.7 ng/dl). In their study they found 35.5% participants met the criteria for vitamin D sufficiency.[5] Vitamin D deficiency is more prevalent among females, likely due to reduced sun exposure caused by the use of sun protection measures such as sunscreens or sunshades, or due to indoor work.[5] Since systemic vitamin D levels are primarily determined by skin synthesis from sunlight exposure rather than dietary sources alone, individuals engaged in outdoor activities tend to have higher vitamin D levels compared to those with predominantly indoor activities.[22]

5. Limitations

A limitation of the present study was the inability to establish distinct BRI for individual age groups due to an insufficient number of study participants. Future research focusing on age-specific BRIs that would enhance the accuracy of deficiency assessments. Expanding the study to include a larger and more diverse sample across various age groups is essential for generating comprehensive reference data.

6. Conclusion

Vitamin D deficiency is highly prevalent in the Western Indian population, with greater deficiency observed among females and decline in levels as age increases. Our findings indicate that the existing BRI provided by reagent manufacturers is generally applicable to the Western Indian population, despite observed vitamin D levels being lower compared to the manufacturer's baseline data. The high prevalence of vitamin D deficiency and considering the potential need for more specific criteria to assess insufficiency and deficiency accurately in this demographic.

References

[1] Bender DA. Ch 44, Micronutrients: Vitamins and Minerals. In: Murray RK, Bender DA, Botham KM, Kennelly PJ, Rodwell VW, Weil PA, editors. Harper's Illustrated Biochemistry. 30th ed. New York: McGraw-Hill Education; 2015. pp. 546-63.

[2] Holick MF. Vitamin D deficiency. **N Engl J Med.** 2007;357(3):266-81.

Paper ID: SR241122183834 DOI: https://dx.doi.org/10.21275/SR241122183834

[3] Mithal A, Wahl DA, Bonjour JP, Burckhardt P, Dawson-Hughes B, Eisman JA, et al. Global vitamin D status and determinants of hypovitaminosis D. OsteoporosInt. 2009;20(11):1807-20.
[4] R. A. G.Khammissa ,1 J.Fourie,1 M.H.Motswaledi,2 R.Ballyram,1 J. Lemmer,1 andL.Feller The Biological Activities of Vitamin D and Its Receptor in Relation to Calcium and Bone Homeostasis, Cancer, Immune and Cardiovascular Systems, Skin Biology, and Oral Health. BioMed Research International Volume 2018, Article ID 9276380, 9 pages https://doi.org/10.1155/2018/9276380
[5] Das, J., Patgiri, D., Mattack, N., & Jahir, E. T. (2019). A study on the biological reference interval of vitamin D in North-East India. *National Journal of Laboratory Medicine*, *8*(2), BO01-BO04. https://doi.org/10.7860/NJLM/2019/39537:2348
[6] Hilger J, Friedel A, Herr R, Rausch T, Roos F, Wahl DA, et al. A systematic review of vitamin D status in populations worldwide. British Journal of Nutrition. 2011; doi:10.1017/S0007114513001840.
[7] Ritu G, Gupta A. Vitamin D deficiency in India: Prevalence, causalities and interventions. Nutrients. 2014;6:729-75; doi:10.3390/nu6020729.
[8] Puri S, Marwaha RK, Agarwal N, Tandon N, Agarwal R, Grewal K, et al. Vitamin D status of apparently healthy schoolgirls from two different socioeconomic strata in Delhi: Relation to nutrition and lifestyle. British Journal of Nutrition. 2008; 99:876-82.
[9] Shah P, Kulkarni S, Narayani S, Sureka D, Dutta S, Vipat AS, etal. Prevalence study of vitamin D deficiency and to evaluate the efficacy of vitamin D3 granules 60,000 IU supplementation in vitamin D deficient apparently healthy adults. Indian Journal of Clinical Practice. 2013;23(12):827-32.
[10] Abbott architect Vitamin D kit insert
[11] Jones G, Barker A. Reference intervals. ClinBiochem Rev. 2008;29(i):S93-97.
[12] Loomis WF. Vitamin D, sunlight and natural selection. Science 1967; 157:501– 4.
[13] Wolpowitz D, Gilchrest BA. The vitamin D questions: how much do you need an how should you get it? J Am Acad Dermatol 2006;54:301–17.
[14] McGrath JJ, Kimlin MG, Saha S, Eyles DW, Parisi AV. Vitamin D insufficiency in south-east Queensland. Med J Aust 2001;174:150 –1.
[15] Fernandes S. ISRO study: Gujarat, not Rajasthan is the sunniest state. Hindustan Times [Internet]. [cited 2024 Nov 10]. Available from: https://www.hindustantimes.com/
[16] Holick MF. Phylogenetic and evolutionary aspects of vitamin D from phytoplankton to humans. In: Pang PKT, Schreibman MP, eds. Vertebrate endocrinology: fundamentals and biomedical implications. Vol 3. Orlando, FL: Academic Press, Inc (Harcourt Brace Jovanovich), 1989: 7– 43.
[17] Harinarayan CV, Ramalakshmi T, Venkataprasad U. High [20]prevalence of low dietary calcium and low vitamin D status in healthy south Indians. Asia Pac. J. Clin. Nutr. 2004;13:359-64.
[18] Baidya A, Chowdhury S, Mukhopadhyay S, Ghosh S. Profile of vitamin D in a cohort of physicians and diabetologists in Kolkata. Indian J. Endocrinol. Metab. 2012;16:S416-S17.
[19] Multani SK, Sarathi V, Shivane V, Bandgar TR, Menon PS, Shah NS, et al. Study of bone mineral density in resident doctors working at a teaching hospital. J. Postgrad. Med. 2010;56:65-70.
[20] Quesada JM, Jans I, Benito, Jimenez JA, Bouillon R. Vitamin D status of elderly people in Spain. Age and Ageing. 1989;18:392-97.
[21] Boucher BJ. The problems of vitamin D insufficiency in older people. Aging and Disease. 2012;3(4):313-29.
[22] Patgiri D, Rohman SM, Borah MB. A comparative study of vitamin D and serum total calcium levels in two socioeconomic groups in Guwahati Metropolitan City. Journal of Medical Science and Clinical Research. 2016;4(6).doi:http:// dx.doi.org/10.18535/jmscr/v4i6.57