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Original Article

Biochemistry Section

A Study on the Biological Reference Interval of Vitamin D in North-East India

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ABSTRACT

Introduction: Vitamin D is the generic name given to all steroids that exhibit the biological activity of Cholecalciferol and derived from 7-dehydrocholesterol. Vitamin D has a major role in maintenance of healthy bones and teeth, which are of considerable public health importance. Vitamin D is normally synthesised in the skin in adequately sun exposed individuals and a minor portion is derived from the diet. Deficiency of vitamin D is prevalent worldwide. Vitamin D levels range from 30-100 ng/mL in healthy individuals. The Indian population is being evaluated for vitamin D using Biological Reference Interval (BRI) derived from western studies.

Aim: To evaluate the BRI set by two reagent manufacturers, Ortho Clinical Diagnostics and Biomerieux to assess whether they were adequate for the North-Eastern Indian population.

Materials and Methods: The study consisted of 678 participants, comprising of healthy attendants accompanying patients presenting at two tertiary level super-speciality hospitals in Guwahati, Assam, India. Samples were collected all through one year, from July 2017 to June 2018. Both male and female participants were assessed for 25-hydroxy vitamin D levels by Chemiluminescence and Enzyme-linked Fluorescence techniques, the two most common techniques employed in the region. The values obtained were statistically analysed by unpaired Student's t-test. The p-value<0.05 were considered statistically significant.

Results: For 678 participants aged 1-82 years, BRI of vitamin D was observed to be 8.0-56.3 ng/mL, these values overlapped with data from both the reagent manufacturers' studies. Only 241 (35.5%) participants were found to have vitamin D sufficiency (>30 ng/mL). In our study, we also found that vitamin D levels decreases with age, with highest levels in the age group 1-10 years and lowest levels in the geriatric age group (> 60 years age). Statistically significant difference was found between vitamin D levels between male and female participants (p<0.001).

Conclusion: The vitamin D levels observed in the present study were in a range that was in between the ranges in the studies undertaken by the reagent manufacturers. Also, the values that we obtained were on the lower side. These observations shows that the criteria for defining the status of insufficiency and deficiency of vitamin D need to be further evaluated for the North-Eastern population.

Keywords: 25-hydoxy, Chemiluminescence, Cholecalciferol, Enzyme-linked fluorescence assay, Public health

INTRODUCTION

Vitamin D, also commonly known as calciferol, is not strictly a true vitamin, since it can be synthesised in the skin of an adequately sun exposed individual [1]. Under normal circumstances, 80-90% of Vitamin D is synthesized in the skin from 7-dehydrocholesterol present in the malpighian layer of epidermis on exposure to sunlight [2]. Only when sunlight exposure is inadequate, a dietary source of vitamin D becomes necessary which contributes to 10-20% of the total vitamin D pool [1,2]. Vitamin D has a major role in the development of bones and teeth and also in modulating the functions of muscles. It stimulates absorption of calcium and phosphate from the intestine and kidney. Its deficiency causes rickets in children and osteoporosis in adults [2]. Vitamin D is also thought to be associated with other conditions and diseases like obesity, hypertension, diabetes and cancer. Vitamin D deficiency as well as excess are well documented in clinical conditions both in India and else where in the world [3-9]. In a study to examine the pattern of vitamin D levels across the world, more than 195 studies conducted in 44 countries were reviewed to determine differences by age, sex and region. It was found that 88.1% of samples presented in the review study had vitamin D less than 75 nmol/L (30 ng/mL), which is the cut-off for vitamin D adequacy [9]. Similarly, in studies undertaken in India, researchers have found vitamin D deficiency is epidemic all over the country, with prevalence of deficiency ranging from 70% to 100% in the Indian population [10]. Puri S et al., in their study have found a prevalence of biochemical hypovitaminosis D among school girls of Delhi in the range of 90.8% and Shah P et al., found prevalence of 94.94% among 500 apparently healthy staff of Fortis Hospital, Mumbai [11,12]. In this setting, Indian patients are being evaluated for Vitamin D status using Biological Reference Interval (BRI) [Table/Fig-1] [13,14] derived from western studies. This necessitates the establishment of a BRI for the Indian population, keeping in consideration the unique geographical location of the different regions in the country. This study attempts to evaluate the BRI set by the two reagent manufacturers and assess whether it is adequate for the North-Eastern Indian population using the two most common methodologies used for estimating vitamin D levels in the region – Chemiluminescence Immunoassay (CLIA) and Enzyme Linked Fluorescent Assay (ELFA).

Vitamin D Status	Vitamin D level
VITROS	
Deficient	<20 ng/mL
Insufficient	20-<30 ng/mL
Sufficient	30-100 ng/mL
Potential toxicity	>100 ng/mL
VIDAS	
Deficient	<20 ng/mL
Insufficient	20-29 ng/mL
Sufficient	30-100 ng/mL
Potential toxicity	>100 ng/mL

[Table/Fig-1]: Biological Reference Interval of Vitamin D (Vitros and Vidas) [13,14].

Materials and Methods

As per Clinical and Laboratory Standards Institute (CLSI) guideline for BRI determination minimum number of volunteers under study should be 120 [15]. This cross-sectional study consisted of 678 participants, who were healthy attendants accompanying patients presenting at Hayat Hospital and Pratiksha Hospital, two tertiary level superspeciality hospitals in Guwahati. Samples were collected all throughout the year for a period starting from July 2017 till June 2018. In total 255 male and 423 female participants aged 1-82 years were assessed for 25-hydroxy vitamin D (25-OH vit D) levels. 25-OH vit D was assessed at Hayat hospital using Vitros ECi platform of Ortho Clinical Diagnostics and at Pratiksha hospital on the Mini Vidas platform of Biomerieux.

Exclusion criteria

Certain exclusion criteria were applied during sample collection to avoid biasness. Individuals with hepatic, renal, dermatological and endocrine disorders that affect vitamin D levels were excluded. Additionally, pregnant and lactating women, children, alcoholics, cigarette smokers with any acute or terminal illness or on any medications which could affect levels of vitamin D were also excluded from the study.

Ethical approval

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional committees and with the 1975 Helsinki declaration and its later amendments or comparable ethical standards. Permission for collecting the samples and their analysis was obtained from the Ethics Committee of both the hospitals (Letter No. HH/CL/Ethics/173,PRAT/ADMIN/EC/105). Informed consent was obtained from all the participants under the study. Enrolment was purely on a voluntary basis.

Questionnaire

The details regarding the diet consumed and the average duration of sunlight exposure were collected with the help of a self-reporting questionnaire. The questionnaire incorporated questions regarding the type and amount of food consumed in the last 24 hours, time spent outdoors during work or commuting to and from office, duration of sunlight exposure between 10 am and 2 pm and the type of occupation.

Sample Collection

Four millilitres blood sample was drawn from the participants under aseptic conditions from the median cubital vein. It was collected in properly labelled vacutainers and then centrifuged at 3000 rpm for 15 minutes. The serum thus obtained was subjected to biochemical analysis within 8 hours of collection of blood. Samples not immediately processed were stored at 2-8 ºC in sample storage refrigerator.

Estimation of Vitamin D by CLIA

The VITROS 25-OH Vitamin D Total test was performed using the VITROS 25-OH Vitamin D Total Reagent Pack and the VITROS 25-OH Vitamin D Total Calibrators on the VITROS ECi Immunodiagnostic System. A competitive immunoassay technique was used to determine the concentration of 25-OH vitamin D present in the patient sample [13].

Estimation of Vitamin D by ELFA

Enzyme Linked Fluorescent Assay (ELFA) technique is used in MiniVidas for the determination of 25-hydroxyvitamin D in human serum or plasma using the VIDAS 25 OH Vitamin D TOTAL kit. The ELFA principle combines an enzyme competitive immunoassay method with a final fluorescent detection to quantify the analyte at 450 nm. The intensity of the fluorescence is inversely proportional to the concentration of vitamin D antigen present in the sample. At the end of the procedure, the instrument calculates the results from the stored calibration curve [14].

STATISTICAL ANALYSIS

Statistical analysis was performed using IBM Statistical Package for Social Sciences (SPSS) Statistics Program Version 20. 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.

RESULTS

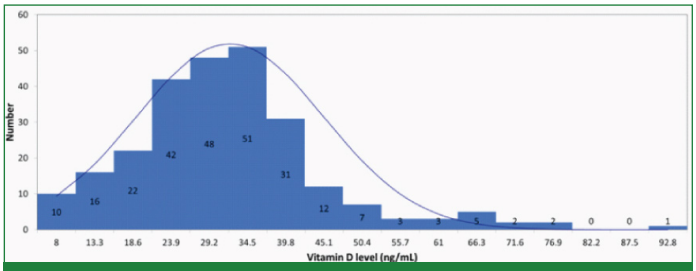
The reagent kit literature for both Vitros and Vidas define the vitamin D insufficiency level at <30 ng/mL [14, 15]. With reference to this criteria, 64.5% of the test subjects in the present study fall in the vitamin insufficiency category.

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 studies [Table/Fig-2].The observed range of vitamin D level in the present study was 8.0-56.3 ng/mL (2.5^{th} to 97.5^{th} percentile).

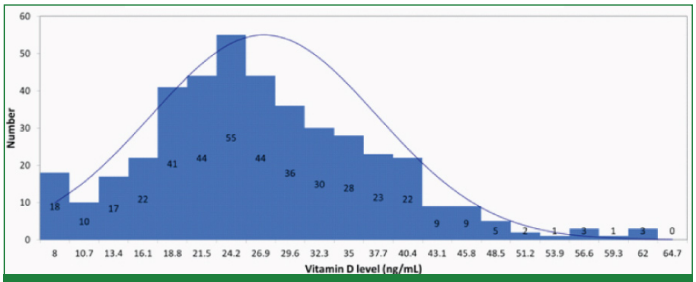
	Age group (years)	Number of test Subjects	Observed Range 2.5^{th} to 97.5^{th} per-centile (ng/mL)	Median (ng/mL)
Present Study	1-82	678	8.0-56.3	26.2
Population Study, Vitros	21-79	399	14.7-68.3	33.4
Population Study, Vidas	Not Available	140	9.3-48.5	23.1

[Table/Fig-2]: Comparative demographical and statistical data of the test subjects of the present study in comparison to those of Vitros and Vidas.

The frequency distribution of vitamin D in the study population showed that only 30.7% of females and 43.1% of males had adequate vitamin D levels above 30 ng/mL, indicating that the majority of the individuals under study had insufficient vitamin D levels. Vitamin D levels decreased progressively with age [Table/Fig-3,4].



[Table/Fig-3]: Distribution of Vitamin D levels in the overall male population studied by both CLIA and ELFA combined (Mean 29.41±12.99 ng/mL).

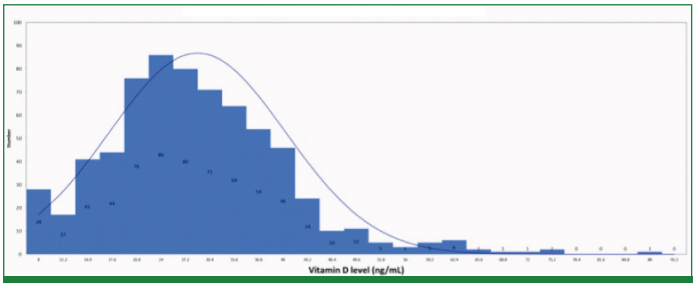


[Table/Fig-4]: Distribution of Vitamin D levels in the overall female population studied by both CLIA and ELFA combined (Mean 25.85±10.37 ng/mL).

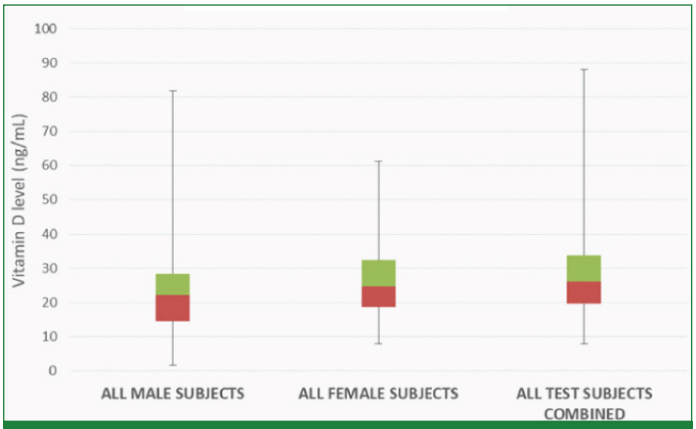
The analysis through interquartile range and Box Plots of vitamin D levels in male and female subjects irrespective of the method of estimation and the vitamin D levels in all the test subjects combined irrespective of gender was done [Table/Fig-5-7]. The difference in the medians was found to be statistically significant (p=0.0019).

	All Male Subjects (n=255)	All Female Subjects (n=423)	All Test Subjects Combined (n=678)	
Minimum (ng/mL)	8	8	8	
1st Quartile (ng/mL)	20.85	18.75	19.6	
Median (ng/mL)	28.4	24.7	26.15	p-value= 0.0019*
3rd Quartile (ng/mL)	34.8	32.35	33.78	
Maximum (ng/mL)	88	61.3	88	

[Table/Fig-5]: Interquartile range of Vitamin D levels separately in Male and Female subjects irrespective of the method of estimation and the Vitamin D levels in all the Test subjects combined irrespective of gender.
Unpaired Students' t-test, *p-value<0.05 Statistically Significant



[Table/Fig-6]: Distribution of Vitamin D levels in all the Test subjects irrespective of the method of determination (Mean 27.19±11.54 ng/mL).



[Table/Fig-7]: Box plot of Vitamin D levels separately in Male and Female subjects irrespective of the method of estimation and the Vitamin D levels in all the test subjects and combined results irrespective of gender (p-value=0.0019 by unpaired students t-test).

DISCUSSION

In this study, we estimated the serum vitamin D levels in different sections of the North-eastern Indian population in an attempt to verify whether the biological reference range established by the reagent manufacturers can be projected on the general population. The values obtained were in overlap to that of the two studies undertaken by the manufacturers. However, a statistical test to assess the significance between the medians among the three studies cannot be used because no additional data from the other two studies was available. The BRI set by the reagent manufacturers [Table/Fig-1] can be applied to the population under study. But it also has to be taken into consideration that the majority of the population under study have hypovitaminosis D. Even though we are in agreement with the Biological Reference Range set by the reagent manufacturers, we feel that the criteria for defining the status of insufficiency and deficiency of Vitamin D need to be further evaluated for the North-Eastern population. This is because, we have found that only 35.5% of the test subjects in our study satisfy the criteria for vitamin D sufficiency.Thus, interpretation of test results have to be made keeping in mind the high prevalence of vitamin D deficiency without any obvious symptoms. Guwahati is located at 26° 06'N and 91° 35'E with an elevation of 54 metres. It receives sunshine for 2276.9 hours annually, which comes to an average of 6.23 hours of sunshine daily [16]. It has been found in studies done previously that there is a high prevalence of vitamin D deficiency in the Indian population with rates of prevalence of deficiency greater than 90% in some areas irrespective of location of the population [17-24]. In the same studies, it was found that in the whole India, the prevalence of deficiency of vitamin D has been found to be 94.4% in north India [17], 85.6% in south India [20], 92.5% in eastern India [22] and 97.5% in western India [23]. This can be ascribed to various factors like lifestyle and sociocultural factors which do not facilitate adequate sun exposure, inadequate diet and absence of food fortification [10-12].

In our study, we also have found that the levels of vitamin D show a decreasing trend with age. Advanced age leads to multiple alterations in the metabolism of vitamin D and its precursors which ultimately lead to its deficiency. This has been attributed to the fact that synthesis of vitamin D declines with age due to decline in synthesis by skin, decrease in absorption from food or by decline in renal function [25, 26]. The reason for hypovitaminosis D in elderly may not simply be age alone, but may also be due to differences in sun exposure and differences in fat and vitamin D intake. These lower levels may also be encountered during periods of chronic illness, immobility or long term hospitalization in the elderly [25]. A similar observation has been reported elsewhere by other scientists who have observed that children and young adults have significantly higher levels of vitamin D than older adults irrespective of gender, race or ethnicity [25, 27, 28]. In our study, we have found statistically significant difference between the median values of vitamin D between male and female genders. Vitamin D deficiency is more prevalent among females. It probably reflects decreased exposure to sunlight among women due to the use of sunscreens or sunshades as protection from sunburn or it may be due to their work indoors. Because systemic vitamin D status is determined primarily by the amount of vitamin D produced in skin due to sunlight exposure and not only on dietary sources alone, people who habitually participate in outdoor activities due to work tend to have higher vitamin D levels than those who do not participate in such activities [29].

LIMITATION

A limitation of the present study was that we could not establish Biological Reference Ranges for individual age groups because of lack of study subjects. A study on that would be our next course of action.

CONCLUSION

Vitamin D deficiency is prevalent in the North-Eastern population. Deficiency is more in females and levels tend to decline as age advances. Our aim was to find out whether a new BRI is needed to assess the Vitamin D levels of the North-eastern Indian population. We found that the BRI of the reagent manufacturers are adequate for the population under study, even though vitamin D levels are on the lower side as compared to the two studies.

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] 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.
[3] Heshmat R, Mohammad K, Majdzadeh SR, Forouzanfar MH, Bahrami A, Ranjbar Omrani GH, et al. Vitamin D deficiency in Iran: A multi-center study among different urban areas. Iranian J Publ Health, A supplementary issue on Osteoporosis and Bone Turnover. 2008;No.1:72-78.
[4] Elsammak MY, Al-Wossaibi AA, Al-Howeish A, Alsaeed J. High prevalence of vitamin D deficiency in the sunny Eastern region of Saudi Arabia: A hospital-based study. Eastern Mediterranean Health Journal. 2011;17(4):317-22.
[5] Yetley EA. Assessing the vitamin D status of the US population. Am J Clin Nutr. 2008;88(suppl):558S-64S.
[6] Mark S, Gray-Donald K, Delvin EE, O'Loughlin J, Paradis G, Levy E, et al. Low vitamin D status in a representative sample of youth from Quebec, Canada. Clinical Chemistry. 2008;54(8):1283-89.
[7] Vierucci F, Pistoia MD, Fanos M, Erba P, Saggese G. Prevalence of hypovitaminosis D and predictors of vitamin D status in Italian healthy adolescents. Italian Journal of Pediatrics. 2014;40:54.

[8] Absoud M, Cummins C, Lim MJ, Wassmer E, Shaw N. Prevalence and Predictors of vitamin D insufficiency in children: A great Britain population based study. PLoS ONE. 2011; 6(7):e22179. doi:10.1371/journal.pone.0022179.
[9] 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.
[10] Ritu G, Gupta A. Vitamin D deficiency in India: Prevalence, causalities and interventions. Nutrients. 2014;6:729-75; doi:10.3390/nu6020729.
[11] 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.
[12] 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.
[13] tVitD. REF 684 2894. [products insert]. Buckinghamshire, UK. Ortho-Clinical Diagnostics. 2012.
[14] VIDAS® 25 OH Vitamin D TOTAL (VITD). REF 30 463. [products insert]. Marcy-l'Etoile, France. bioMérieux SA. 2013.
[15] Jones G, Barker A. Reference intervals. ClinBiochem Rev. 2008;29(i):S93-97.
[16] National Oceanic and Atmospheric Administration (US). Guwahati Climate Normals 1971-1990 [Internet] [cited 2018 Nov 27]. Available from https://en.wikipedia.org/wiki/Guwahati
[17] Zargar AH, Ahmad S, Masoodi SR, Wani AI, Bashir MI, Laway BA, [17]et al. Vitamin D status in apparently healthy adults in Kashmir Valley of Indian subcontinent. Postgrad. Med. J. 2007;83:713-16.
[18] Tandon N, Marwaha RK, Kalra S, Gupta N, Dudha A, Kochupillai [18]N. Bone mineral parameters in healthy young Indian adults with optimal vitamin D availability. Natl. Med. J. India 2003;16:298-302.
[19] Goswami R, Gupta N, Goswami D, Marwaha RK, Tandon [19]N, Kochupillai N. Prevalence and significance of low 25-hydroxyvitamin D concentrations in healthy subjects in Delhi. Am. J. Clin. Nutr. 2000;72:472-75.
[20] 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.
[21] Harinarayan CV, Ramalakshmi T, Prasad UV, Sudhakar D. [21]Vitamin D status in Andhra Pradesh: A population based study. Indian J. Med. Res. 2008;127:211-18.
[22] Baidya A, Chowdhury S, Mukhopadhyay S, Ghosh S. Profile of [22]vitamin D in a cohort of physicians and diabetologists in Kolkata. Indian J. Endocrinol. Metab. 2012;16:S416-S17.
[23] Multani SK, Sarathi V, Shivane V, Bandgar TR, Menon PS, Shah [23]NS, et al. Study of bone mineral density in resident doctors working at a teaching hospital. J. Postgrad. Med. 2010;56:65-70.
[24] Beloyartseva M, Mithal A, Kaur P, Kalra S, Baruah MP, [24]Mukhopadhyay S, Bantwal G, Bandgar TR. Widespread vitamin D deficiency among Indian health care professionals. Arch. Osteoporos. 2012;7:187-192.
[25] Quesada JM, Jans I, Benito, Jimenez JA, Bouillon R. Vitamin D [25]status of elderly people in Spain. Age and Ageing. 1989;18:392-97.
[26] Boucher BJ. The problems of vitamin D insufficiency in older [26]people. Aging and Disease. 2012;3(4):313-29.
[27] Gallagher JC. Vitamin D and aging. Endocrinol Metab Clin North [27]Am. 2013;42(2):319-32.doi:10.1016/j.ecl.2013.02.004.
[28] Jain RB. Variability in the Levels of Vitamin D by Age, Gender, [28]and Race/Ethnicity: Data from National Health and Nutrition Examination Survey 2007-2010. Journal of Nutrition and Health Sciences. 2016; 3(2):203. doi:10.15744/2393-9060.3.203.
[29] Patgiri D, Rohman SM, Borah MB. A comparative study of [29]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.

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