Original Article

Prevalence of Vitamin D Deficiency in Sub-fertile Women Visiting IVF Centers Pan India: A Cross-Sectional Study

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Abstract

Background: Vitamin D, essential for bone health and overall well-being, plays a pivotal role in reproductive health. This study aimed to assess the prevalence of vitamin D deficiency among infertile women in India, considering regional and demographic factors. **Methods:** This retrospective study involved 34,844 infertile women seeking treatment at various centers across India. Exclusions were made for women with kidney or liver disorders and those on medications affecting vitamin D metabolism. Serum 25(OH)D levels were measured through immunoassay techniques, and the data were analyzed using statistical software. **Results:** This study analyzed the vitamin D levels of 34,844 individuals across 21 states in India. The overall prevalence of vitamin D levels revealed that 24.70% had normal levels, while 45.10% had insufficiency, and 21.30% had deficiency. Few individuals (8.80%) had borderline high levels, and only a small fraction (0.10%) showed signs of vitamin D toxicity. Significant regional disparities were observed, with states like Goa exhibiting high normal levels while Delhi had high rates of both deficiency and insufficiency. **Conclusion:** This study unveils a substantial prevalence of vitamin D deficiency among infertile women in India, potentially affecting their fertility and reproductive health. While evidence suggests that optimizing vitamin D status may enhance reproductive outcomes, further research is required to establish clear guidelines for vitamin D supplementation in infertility treatment.

Keywords: fertility treatment, infertility, prevalence, reproductive health, serum 25(OH)D levels, vitamin D deficiency

Introduction

Vitamin D, a liposoluble hormone, is known to play a major role in bone and skeletal health. It acts by absorption of calcium and phosphorus from the intestine. It acts through the vitamin D receptor (VDR).[1] Vitamin D is synthesized by the skin through ultraviolet B rays. In food, it is obtained from natural sources like eggs, fish, mushrooms, cod liver oil, green vegetables, and fortified foods like cereals, milk, and yogurt.[2,3] When the metabolism of vitamin D takes place, it is converted into 1,25-dihydroxy vitamin D or calcitriol.[4]

Levels in the body are measured by checking the 25(OH) vitamin D levels, as it has a longer half-life. There is no consensus regarding the reference ranges, hence different clinicians consider different reference ranges.[5]

Apart from the skeletal benefits, various studies have shown its relationship with various neurological, cardiovascular, tumor, and reproductive health issues. VDR is present in various tissues related to the reproductive system, such as the hypothalamus, ovaries, granulosa cells, endometrium, placenta, decidua in females, and the testis and spermatogenetic cells in males.[6]

Various studies have shown an association between vitamin D and polycystic ovarian disease (PCOS) and endometriosis. Around 65–85% of women with PCOS are associated with low vitamin D levels. Many works of literature have shown the correlation between Vit-D with androgen levels, AMH, Insulin resistance, and follicular response. These improvements ultimately lead to improved clinical pregnancy rates. In a recent publication, the expression of

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VDR receptors in granulosa cells and vitamin D levels in the follicular fluid are decreased in PCOS women.[7]

In endometriotic patients, vitamin D levels were found to be decreased. Studies have shown the presence of VDR in ectopic endometrium, peritoneal endometriosis, just like in normal endometrium.[8]

Vitamin D supplementation has also been shown to have a positive effect on AMH expression. Studies have also shown a positive correlation between follicular fluid 25(OH) vitamin D levels and fertilization rate. Blastocyst secretes Interleukin-1B (IL-1B) which increases the production of calcitriol from the decidua and helps in embryo implantation by regulating the immunological cells in the endometrium. It is also known to regulate the expression of the *HOXA10* gene, which helps in implantation.[9]

Vitamin D deficiency is associated with various adverse maternal and fetal outcomes, such as increased risk of preeclampsia, gestational diabetes mellitus, low birth weight, neonatal tetany, and impaired skeletal development.[10]

India,[6] a country marked by diverse geographical, cultural, and socioeconomic heterogeneity, poses unique challenges concerning maternal health. Despite its significant population, comprehensive studies investigating the prevalence of vitamin D deficiency among Indian reproductive women remain limited. Given the various regions spanning northern latitudes with decreased sunlight exposure, coupled with dietary habits that might not always provide sufficient vitamin D, a comprehensive assessment of the prevalence of vitamin D deficiency in India is imperative and crucial for developing targeted interventions and public health strategies. These interventions can encompass dietary recommendations and potentially vitamin D supplementation regimens. Moreover, this knowledge can aid healthcare professionals in identifying high-risk groups.

Therefore, this study aims to investigate the prevalence of vitamin D deficiency among women across different regions of India, considering various socio-demographic, dietary, and lifestyle factors. By shedding light on the magnitude of the problem and identifying contributing factors, this research endeavors to provide a foundation for evidence-based interventions and policies aimed at enhancing maternal and fetal well-being in the context of vitamin D deficiency.

Methods

Study design

This retrospective study was conducted among 34,844 women visiting different centers of Indira IVF Hospital for their fertility treatment between the age group of 19 and 21 years during the period Sep 2022–July 2023. The study is approved by the Indira IVF Hospital Private Limited Institutional Ethics Committee (ECR/1614/Inst/RJ/2021).

Sample selection

The study incorporated women seeking fertility treatment, irrespective of their parity. Exclusion criteria were women with kidney and liver disorders and women who were on medications that could potentially interfere with calcium and vitamin D metabolism. They cover different geographic and cultural regions with different professions and lifestyles across India.

Data collection

All patient data related to study was collected from the central registry of Indira IVF Group of Hospitals. The process of collecting medical records involves systematically gathering information from medical records.

Laboratory analysis

Sample collection

A blood sample is collected from the study participants using established phlebotomy procedures. This sample is typically collected in a serum separator tube (SST) or plain tube, which allows for the separation of serum from the whole blood after centrifugation. The blood sample is then subjected to centrifugation, a process that separates the serum from the cellular components of the blood. Centrifugation leads to the formation of distinct layers, with the serum rising to the top. Once the serum is separated, it is carefully extracted from the SST or plain tube and prepared for analysis. Serum samples are often stored at low temperatures to prevent degradation of the 25(OH)D metabolite. The most common laboratory methods for measuring serum 25(OH)D levels involve immunoassays, which are techniques that use antibodies to detect specific molecules. There are two primary types of immunoassays used for measuring 25(OH)D.

Competitive binding assays

Competitive binding assays use an antibody that binds to the serum's 25(OH)D and a labeled form of 25(OH)D. The labeled form competes with the natural 25(OH)D in the serum for binding to the antibody. The amount of labeled 25(OH)D that binds is inversely proportional to the concentration of 25 (OH)D in the sample.

Chemiluminescent assays

Chemiluminescent assays use a specific antibody that binds to 25(OH)D. This binding is then detected through a chemical reaction that produces light. The intensity of the light emitted is proportional to the concentration of 25(OH)D in the serum.

Before analyzing the study samples, the laboratory instruments are calibrated using known standards of 25 (OH)D. Quality control samples with known concentrations of 25(OH)D are also run alongside the study samples to ensure the accuracy and reliability of the assay. The results of the immunoassay are provided as the concentration of 25(OH)D in the serum, measured in

nanograms per milliliter (ng/mL) or nanomoles per liter (nmol/L). These results are interpreted to determine whether an individual is vitamin D deficient, insufficient, or sufficient based on established cutoff values. The final 25 (OH)D measurements are reported, allowing them to categorize participants' vitamin D status for further analysis.

Overall, the laboratory methods for measuring serum 25(OH) D levels involve sophisticated immunoassay techniques that provide accurate and reliable assessments of an individual's vitamin D status. These measurements are crucial for understanding the relationship between vitamin D status and various health outcomes.

Statistical analysis

All patient data related to the study was collected from the central registry of Indira IVF Group of Hospitals. Categorical variables were expressed as frequency (percentages) and continuous variables were expressed as mean ± standard deviations (SD). All p-values < 0.05 were considered statistically significant. Statistical analyses were performed using Statistical Package for Social Science (IBM SPSS), version 28.0.

Results

There are various guidelines for measuring 25(OH) vitamin D levels but as per Institutional practise and pathology Lab (Neuberg) we have followed the levels as – levels <10 ng/mL are considered deficient, levels between 10 and 20 ng /mL are considered insufficient, levels between 20 and 32 ng/mL are considered normal, and levels 33–159 ng/mL are considered borderline. While values above 160 ng/mL were considered vitamin D toxicity or hypervitaminosis D.

The patients were considered between 21 and 49 years of age. Out of 35844 women, 24.7% (4001) were normal, 45.10% (15,719) were insufficient, 21.30% (7435). Very small number of patients 8.80% (3063) were borderline and 23 women had 0.10% toxicity of vitamin D levels. This data is crucial as it sets the context for understanding the prevalence of vitamin D-related issues in the study population.

Our study reveals notable variations in the prevalence of vitamin D levels among different Indian states. The overall prevalence of vitamin D deficiency is 21.30%, with Assam reporting the lowest deficiency rate at 7.60% and Goa having the highest proportion of individuals with normal levels at 66.70%. These findings highlight the regional disparities in vitamin D status. Assam has a lower prevalence of deficiency (7.60%) compared to other states, likely attributed to increased sun exposure. Goa has the highest percentage of individuals with normal vitamin D levels (66.70%), likely due to its coastal location and abundant sunlight. Delhi reports a high prevalence of deficiency (33.90%), emphasizing the need for public health initiatives in the capital. Odisha exhibits a lower prevalence of deficiency (4.00%) compared to other states, possibly linked to dietary habits and sun exposure [Table 1].

Overall, vitamin D toxicity levels are quite low across all clinics, which is a positive finding as excessive vitamin D can

Table 1: Prevalence of Vitamin D across different states

State	Normal	Insufficiency	Deficiency	Borderline high	Toxicity
Overall prevalence	24.70	45.10	21.30	8.80	0.10
Assam	45.60	38.60	7.60	8.20	0.00
Bihar	23.70	44.60	23.30	8.40	0.10
Chhattisgarh	38.40	40.80	9.00	11.80	0.00
Delhi	20.70	33.50	33.90	12.00	0.00
Goa	66.70	33.30	0.00	0.00	0.00
Gujarat	19.60	37.20	33.30	9.90	0.00
Haryana	16.70	45.30	29.70	8.30	0.00
Himachal Pradesh	24.40	44.10	23.60	7.90	0.00
Jammu and Kashmir	26.60	43.60	17.50	12.30	0.00
Jharkhand	31.30	48.90	11.50	8.30	0.00
Karnataka	28.80	45.70	16.50	8.90	0.10
Madhya Pradesh	24.40	50.50	20.00	5.10	0.00
Maharashtra	27.60	46.00	18.30	0.00	0.10
Odisha	38.40	52.20	4.00	5.40	0.00
Punjab	24.70	42.00	20.20	12.90	0.10
Rajasthan	23.10	49.70	20.10	6.70	0.50
Tamil Nadu	28.20	49.60	17.60	4.60	0.00
Telangana	38.60	43.00	7.00	11.40	0.00
Uttar Pradesh	21.40	45.70	24.10	8.80	0.10
Uttarakhand	23.60	50.80	19.50	6.20	0.00
West Bengal	27.50	48.40	15.50	8.60	0.00

have adverse effects. However, it is essential to monitor and educate patients to prevent potential toxicity cases.

The clinic-wise variation in vitamin D prevalence highlights the need for region-specific strategies to address vitamin D deficiency and optimize overall health outcomes. More research can help improve vitamin D status in populations with higher deficiency rates while maintaining awareness of potential toxicity risks.

Discussion

Our study revealed significant disparities in vitamin D prevalence among different states in India. Notably, the overall prevalence of vitamin D deficiency (21.30%) and insufficiency (45.10%) indicates a widespread issue that requires attention. Some key observations and implications can be drawn from the results.

Regional disparities

The striking regional disparities in vitamin D prevalence observed in this study were crucial for public health considerations. These disparities are influenced by a combination of geographical, environmental, lifestyle, and dietary factors, and they necessitate tailored interventions. Here are some key observations for specific states that stands out with the highest prevalence of individuals with normal vitamin D levels (66.70%). This could be attributed to its tropical climate, abundant sunlight, and dietary practices that include foods rich in vitamin D. In contrast, Delhi shows the highest prevalence of vitamin D deficiency (33.90%). This could be linked to factors such as high pollution levels, limited sun exposure due to urban lifestyles, and inadequate dietary habits. These states exhibit an alarmingly high prevalence of vitamin D deficiency. Factors contributing to this trend may include inadequate sunlight exposure, dietary habits that lack vitamin D-rich foods, and geographical location in some cases. These findings emphasize the critical need for targeted interventions, including public awareness campaigns and dietary guidance, in these regions. Kushay et al. (2018) emphasize the significance of regional disparities in vitamin D status in India. They recognize that different regions exhibit varying levels of deficiency and insufficiency, highlighting the need for targeted interventions. Our study extends beyond this study[1] by encompassing a more extensive list of Indian states and incorporating the aspect of vitamin D toxicity, which is not explored in study.

High prevalence of deficiency

Several states, including Bihar, Gujarat, Haryana, Madhya Pradesh, Punjab, Rajasthan, and Uttar Pradesh, exhibit a high prevalence of vitamin D deficiency. There are various studies,[11] which highlight the need for targeted interventions, including public awareness campaigns and dietary guidance. The variation in vitamin D levels among different clinics suggests that regional factors, including geographical location, climate, dietary habits, and lifestyle choices, may play a significant role in determining vitamin D status.

Toxicity levels

In general, the prevalence of vitamin D toxicity is negligible, but a few states like Rajasthan show a higher prevalence, which may require monitoring and intervention. While the overall prevalence of vitamin D toxicity is minimal, some regions stand out with a slightly higher prevalence, such as Rajasthan. The study reveals that Rajasthan has a relatively elevated percentage of individuals with vitamin D toxicity, approximately 0.50%. This observation may be attributed to factors specific to the region, such as dietary habits, cultural practices, and climatic conditions. The relatively higher prevalence in Rajasthan necessitates special attention and monitoring to ensure that individuals are not at risk of adverse health effects. In contrast, some regions in India demonstrate an extremely low prevalence of vitamin D toxicity. Most notably, Goa reports almost negligible toxicity, with only 0.00% of individuals experiencing this condition. This could be attributed to the region's tropical climate, ample sunlight, and dietary practices that provide an abundant natural source of vitamin D. Such low toxicity rates in Goa indicate a balanced approach to vitamin D intake, where individuals are less likely to exceed safe levels of the nutrient.

Our study shows the importance of vitamin D toxicity, shedding light on this less-explored aspect. In contrast, many related studies[12] primarily concentrate on vitamin D deficiency and insufficiency. This distinction highlights the comprehensive nature of our research, addressing both ends of the vitamin D spectrum.[13]

The prevalence of vitamin D insufficiency and deficiency across several states underscores the importance of incorporating vitamin D-rich foods into the diet. Promoting the consumption of foods such as fatty fish, fortified dairy products, and mushrooms can be an effective strategy. Meta-analyses and systematic reviews[12] compile data from multiple studies to provide a more comprehensive view of vitamin D prevalence globally. These studies often highlight the overall prevalence and help identify trends and regional variations. These studies[14,15] collectively emphasize the worldwide significance of vitamin D deficiency and insufficiency. They reinforce the importance of geographical, cultural, and lifestyle factors in determining vitamin D status and the need for tailored strategies to address this health concern on a global scale. Studies in Bagdad[5] highlight the importance of sun exposure and outdoor activities in maintaining adequate vitamin D levels. However, even in sunny Bagdad, specific population groups may still experience deficiencies. The comparative studies[16,17] highlight the significant role of regional factors, such as geography, climate, and dietary practices, in influencing vitamin D status. This underlines the importance of region-specific public health strategies.

This study is not devoid of limitations. It does not take into consideration age, dietary intake, and other factors. However, the major strength of this study is the big cohort spread representing our diverse country which needs to be considered. The data underscore the importance of routine vitamin D assessments and tailored interventions to address vitamin D-related health issues, especially in areas with higher deficiency rates.

Further research, including geographic and demographic analyses, can help identify specific factors contributing to variations in vitamin D status among clinics and regions.

Conclusion

In conclusion, this study highlights the pressing need for region-specific strategies to address vitamin D deficiencies in India. The prevalence of vitamin D deficiency is around 24.7%. Vitamin D deficiency has been implicated in infertility in women. The available evidence suggests that overall insufficiency of vitamin D with 45% optimizing vitamin D status may improve reproductive health outcomes. However, further research is needed to establish clear guidelines for vitamin D supplementation in infertility treatment.

Data Availability Statement

The dataset for this study will be made available on request.

Declaration

The submitted work has not been published before (neither in English nor in any other language) and that the work is not under consideration for publication elsewhere.

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Conflicts of interest

There are no conflicts of interest.

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