

Investigation of Serum Vitamin D3 and Prolactin as Potential Biochemical Biomarkers for Uterine Fibroids in Premenopausal Women: A Case-Control Study from Andhra Pradesh

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Abstract

Uterine fibroids (UFs) represent a significant clinical burden, affecting a vast majority of women during their reproductive years. Emerging evidence suggests that 1,25-dihydroxy vitamin D3 acts as a potent modulator of leiomyoma growth, while ectopic prolactin (PRL) secretion from fibroid tissue may contribute to systemic hormonal imbalances. This study evaluated the relationship between serum vitamin D3, PRL, and fibroid characteristics in a cohort from Andhra Pradesh. Following STROBE guidelines, this cross-sectional study analyzed 210 premenopausal women (105 cases with UFs ≥ 2 cm and 105 age- and BMI-matched controls). Serum was separated within 30 minutes of collection by centrifugation at 3000 rpm for 10 minutes and stored at -80°C until biochemical analysis. Vitamin D3 was quantified via ELISA, and PRL levels were determined through electrochemiluminescence immunoassay. Cases demonstrated significantly lower mean serum vitamin D3 levels compared to controls (10.85 ± 3.34 ng/mL vs. 19.64 ± 5.50 ng/mL; $P < 0.001$). Conversely, mean PRL levels were significantly higher in women with fibroids (27.79 ± 8.19 ng/mL vs. 13.96 ± 4.09 ng/mL; $P < 0.001$). We observed a strong negative correlation between vitamin D3 and fibroid number ($r = -0.513$; $P < 0.0001$) and a positive correlation with PRL levels ($r = 0.453$; $P < 0.0001$). Neither marker showed a statistically significant correlation with fibroid size. Our findings indicate that hypovitaminosis D and elevated serum prolactin are distinctive biochemical hallmarks in women with uterine fibroids. These parameters may serve as effective biomarkers for monitoring disease progression and informing therapeutic strategies.

Keywords: Uterine fibroids, Serum vitamin D3, Serum prolactin, Premenopausal, Biomarkers, Leiomyoma.

Introduction

Uterine fibroids, clinically referred to as leiomyomas, represent the most prevalent form of benign neoplastic growth among women within the reproductive demographic[1]. These monoclonal tumors are characterized by their non-malignant nature and arise specifically from the smooth muscle architecture of the uterus[2]. In India, UFs are highly prevalent, especially in women aged 30–40 but can occur at any age, posing a significant burden on women's health and the healthcare system [4], UFs can cause reproductive problems, including infertility, miscarriage, adverse pregnancy outcomes, and reduced quality of life [5]. Multifactorial etiopathology with hormonal factors, African-American race, nulliparity, obesity, and a positive history of fibroids are the risk factors for high rate of leiomyoma. They may be asymptomatic or can cause abnormal bleeding, pelvic pressure symptoms, infertility and growth or regress throughout the life[3]. In an in-vivo model of leiomyomas in rats, Halder et al demonstrated that 1,25 dihydroxyvitamin D3 causes a dramatic reduction in the dimension of the lesions [4,5]. Several UF risk factors were identified including, most importantly, black race, older age, vitamin D deficiency, obesity, family history, low parity, long period since last labor, food additives or soybean milk consumption, and hypertension [6,7]. Although commonly benign, UFs are associated with significant morbidity. Although some UF patients may be asymptomatic, 25–50% of them may present a wide range of severe and chronic symptoms, such as abnormal uterine bleeding (AUB), anemia, pelvic pain and pressure, gastrointestinal problems, subfertility, and various obstetric complications [8-10]. Numerous UF-derived symptoms may be explained by cytokine influence [11,12]. Transforming growth factor β (TGF- β) appears to be one of the most involved growth factors in UFs considering its role in fibrosis [11,13]. Additionally, the inflammatory process highly contributes to tumor biology via several cytokines [11,14]. Prolactin (PRL), a polypeptide hormone synthesized within the

anterior pituitary, is primarily recognized for its regulatory role in mammary tissue maturation and the initiation of lactation. Beyond its systemic pituitary origin[15], PRL expression has been identified in various peripheral tissues, including uterine leiomyomas[16]. Current evidence indicates that these fibroids may function as an autonomous, extra-pituitary site of prolactin secretion. Such ectopic production by the tumor tissue can lead to pathologically high serum PRL levels[17], which are clinically associated with impaired fertility[18].

Our research highlights a significant gap, as no previous investigations have concurrently analyzed the synergistic association of vitamin D3 and prolactin with the presence of uterine fibroids. To address this lack of data, the present study was designed to systematically evaluate the correlation between serum vitamin D3 concentrations, prolactin levels, and the specific clinical characteristics namely the count and dimensions of fibroids in premenopausal women.

Materials and Methods

Study Design and Setting: Our study following STROBE guidelines, we conducted a cross-sectional study across rural community health centers and outreach fertility clinics in South India. Data collection spanned six months, from January to June 2023.

Eligibility and Recruitment: In our study, Premenopausal women aged 18–50 were screened. Inclusion criteria for the case group required the presence of at least one fibroid ≥ 2 cm on transvaginal ultrasonography (TVS). Exclusion criteria included pregnancy, lactation, history of miscarriage within six months, recent use of hormonal contraceptives or Vitamin D supplements, and systemic conditions like chronic renal failure or pituitary disorders.

The study involved a total of 210 premenopausal women recruited from rural community health centers and outreach fertility clinics in South India. Participants were divided into two equal groups: a case group (n=105) consisting of women with at least one uterine fibroid ≥ 2 cm confirmed by transvaginal ultrasonography, and

a control group (n=105). Anthropometric measurements included height, weight, BMI, age, and age of menarche. Detailed clinical data regarding daily physical activity, duration of sun exposure, family history of UFs, diet, and menstrual cycle regularity were gathered through a combination of structured interviews and medical record reviews.

Sample Collection and Processing: After obtaining written informed consent, venous blood samples were collected from all participants under aseptic conditions. Serum was separated within 30 minutes of collection by centrifugation at 3000 rpm for 10 minutes and stored at -80°C until biochemical analysis. Serum vitamin D3 was subsequently quantified via ELISA, and PRL levels were determined through electrochemiluminescence immunoassay.

Statistical analysis

This statistical methodology outlines a standard quantitative approach where data managed in MS Excel was analyzed via SPSS v16.0, using Mean $\pm$ SD for continuous variables and frequencies for categorical ones. To determine relationships, the researchers employed Chi-square tests for categorical associations and independent t-tests to compare group means, while utilizing Pearson and Spearman correlations to evaluate linear and non-parametric relationships, respectively. The significance threshold was set at $P < 0.05$, ensuring that any identified associations or

differences had a 95% probability of not being due to random chance.

Ethical consideration

The study was conducted in strict adherence to the International Council for Harmonization (ICH) "Guidance for Good Clinical Practices," the Indian Council of Medical Research (ICMR) "Guidelines for Biomedical Research on Human Participants," and the Declaration of Helsinki. The study protocol received formal approval from the **Institutional Ethics Committee (IEC)**. Prior to enrollment, all participants were fully informed about the study's objectives, and **written informed consent** was obtained from each individual. The authors certify that patient confidentiality was maintained throughout the data collection and analysis process.

Results

A total of 436 women were assessed for eligibility across the rural community health centers and outreach fertility clinics in Andhra Pradesh. Following the screening and enrollment process, 226 women were excluded for not meeting inclusion criteria, declining participation, or incomplete clinical testing. Consequently, 210 premenopausal women were recruited and allocated into two equal cohorts: the case group (n=105) and the matched control group (n=105). All 210 participants were included in the final analysis, with no exclusions from the analysis stage.

Table 1: General characteristics of the case and control groups (N=210)

Characteristics	Case (n=105)	Control (n=105)	Total (n=210)	P-value
Age (years), (Mean±SD)	35.67 ± 5.06	34.26 ± 5.15	34.96 ± 5.15	0.08*
Age at menarche (year), (Mean±SD)	12.82 ± 1.52	13.02 ± 1.45	12.92 ± 1.49	0.40*
Body Mass Index (kg/m ²), (Mean±SD)	25.24 ± 3.54	24.76 ± 3.28	25.00 ± 3.42	0.37*
Weight (kg), (Mean±SD)	62.4 ± 8.1	60.8 ± 7.9	61.6 ± 8.0	0.15*
Height (cm), (Mean±SD)	157.2 ± 5.4	156.8 ± 5.1	157.0 ± 5.3	0.58*
Family History of UFs, n (%)	28 (26.7)	12 (11.4)	40 (19.0)	0.005**
Menstrual Cycle Status				
Irregular, n (%)	13 (12.4)	7 (6.7)	20 (9.5)	0.17**
Regular, n (%)	92 (87.6)	98 (93.3)	190 (90.5)	
Physical Activity (hrs/week)				
Low (< 1 h.), n (%)	45 (42.9)	64 (60.9)	109 (51.9)	<0.001**

Moderate (1–2 h.), n (%)	26 (24.8)	9 (8.6)	35 (16.7)	
High (> 2 h.), n (%)	34 (32.3)	32 (30.5)	66 (31.4)	
Sun Exposure (h/day)				
Low (< 1 h.), n (%)	77 (73.3)	29 (27.6)	106 (50.5)	<0.001**
Moderate (1 h.), n (%)	15 (14.3)	47 (44.8)	62 (29.5)	
High (> 1 h.), n (%)	13 (12.4)	29 (27.6)	42 (20.0)	
Dietary Habit				
Vegetarian, n (%)	54 (51.4)	37 (35.2)	91 (43.3)	0.03**
Non-vegetarian, n (%)	51 (48.6)	68 (64.8)	119 (56.7)	

*Independent t-test, **Chi-square test. SD: Standard deviation. All values adapted for N=210

The statistical analysis confirms that the case and control groups were effectively matched, as evidenced by the lack of significant differences in age ($P=0.08$), age at menarche ($P=0.40$), and BMI ($P=0.37$). This parity is crucial for minimizing confounding variables when assessing the relationship between Vitamin D3, Prolactin, and uterine fibroids. However, the data reveals a strong association between uterine fibroids and lifestyle choices, specifically significantly lower sun exposure ($P<0.001$) and distinct physical activity patterns ($P<0.001$) in the case group compared to controls. Additionally, family history appears to be a notable risk factor, with a significantly higher prevalence in cases (26.7%) than in controls (11.4%).

Table 2: Comparative Distribution of Serum Markers and Clinical Parameters (N=210)

Characteristics	Case (n=105)	Control (n=105)	Total (n=210)	P-value
Serum Vitamin D3 (ng/mL), (Mean±SD)	10.85 ± 3.34	19.64 ± 5.50	15.25 ± 6.31	<0.001*
Vitamin D3 Status, n (%)				
Deficient (<10 ng/mL)	49 (46.7)	7 (6.7)	56 (26.7)	<0.001**
Insufficient (10–19.9 ng/mL)	56 (53.3)	35 (33.3)	91 (43.3)	
Sufficient (≥ 20 ng/mL)	0 (0.0)	63 (60.0)	63 (30.0)	
Serum Prolactin (ng/mL), (Mean±SD)	27.79 ± 8.19	13.96 ± 4.09	20.87 ± 9.47	<0.001*
Prolactin Level, n (%)				
Normal (≤ 23.3 ng/mL)	25 (23.8)	104 (99.0)	129 (61.4)	<0.001**
High (> 23.3 ng/mL)	80 (76.2)	1 (1.0)	81 (38.6)	
Number of Fibroids, (Mean±SD)	2.4 ± 1.2	-	-	-
Total Fibroid Size (cm), (Mean±SD)	4.8 ± 2.1	-	-	-
Haemoglobin (Hb) (g/dL), (Mean±SD)	10.2 ± 1.4	12.1 ± 1.1	11.2 ± 1.3	0.002*

*Independent t-test, **Chi-square test. SD: Standard deviation.

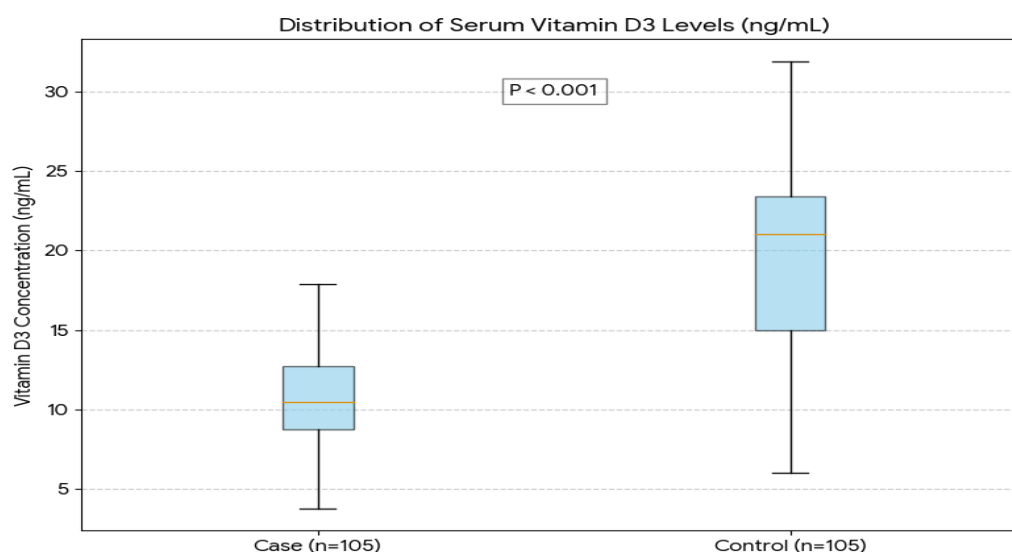
At this stage our study in case of The biochemical analysis reveals a highly significant disparity between the two groups, with the case group displaying markedly lower mean serum Vitamin D3 levels (10.85 ± 3.34 ng/mL) compared to controls (19.64 ± 5.50 ng/mL; $P<0.001$). Notably, 100% of the case group fell into the deficient or insufficient Vitamin D3 categories, whereas 60% of the control group maintained sufficient levels. Conversely, serum prolactin levels were significantly elevated in the case group (27.79 ± 8.19 ng/mL) relative to the healthy controls (13.96 ± 4.09 ng/mL; $P<0.001$). These results, combined with the slightly lower haemoglobin levels in cases, suggest that Vitamin D3 deficiency and hyperprolactinemia are significant biochemical features in premenopausal women with uterine fibroids in this Andhra Pradesh cohort.

Table 3: Correlation between Serum Markers and Fibroid Characteristics in the Case Group (n=105)

Variables	r / P-value	Serum Vitamin D3 (ng/mL)	Serum Prolactin (ng/mL)	Number of Fibroids	Size of Fibroids (cm)
Serum Vitamin D3	r	—	-0.099	-0.513***	0.006
	P-value	—	0.383	<0.0001	0.961
Serum Prolactin	r	-0.099	—	0.453***	0.154
	P-value	0.383	—	<0.0001	0.172
Number of Fibroids	r	-0.513***	0.453***	—	0.215
	P-value	<0.0001	<0.0001	—	0.056

*Pearson correlation coefficient is significant at the 0.01 level (2-tailed).

The study now proves that serum Vitamin D3 levels share a strong, highly significant **negative correlation** with the number of fibroids ($r = -0.513$, $P < 0.0001$), signifying that as Vitamin D3 levels decrease; the number of uterine fibroids tends to rise. On the other hand, serum Prolactin (PRL) levels exhibit a strong **positive correlation** with the number of fibroids ($r = 0.453$, $P < 0.0001$), indicating that upper prolactin concentrations are associated with a greater fibroid count. Interestingly, neither Vitamin D3 nor Prolactin indicated a statistically significant correlation with the specific size of the fibroids in this our study ($P > 0.05$).

**Fig1:** Box Plot represents the distribution of vitamin D3 levels in case and control groups.

In above graph, the distribution of serum vitamin D3 levels across the study (N=210) in Andhra Pradesh, which underlines a profound biochemical difference between the cohorts. The case group (n=105) displays significantly lower median vitamin D3 levels (10.45 ng/mL) compared to the healthy control group (n=105), which reveals a significantly higher median concentration of 21.0 ng/mL ($P < 0.001$). As illustrated, the interquartile range for cases is entirely confined within the deficient and insufficient zones, whereas the control group displays a broader distribution getting into the sufficient range. This graphical representation confirms that hypovitaminosis D is a distinctive clinical feature in premenopausal women with uterine fibroids compared to matched controls in this regional setting.

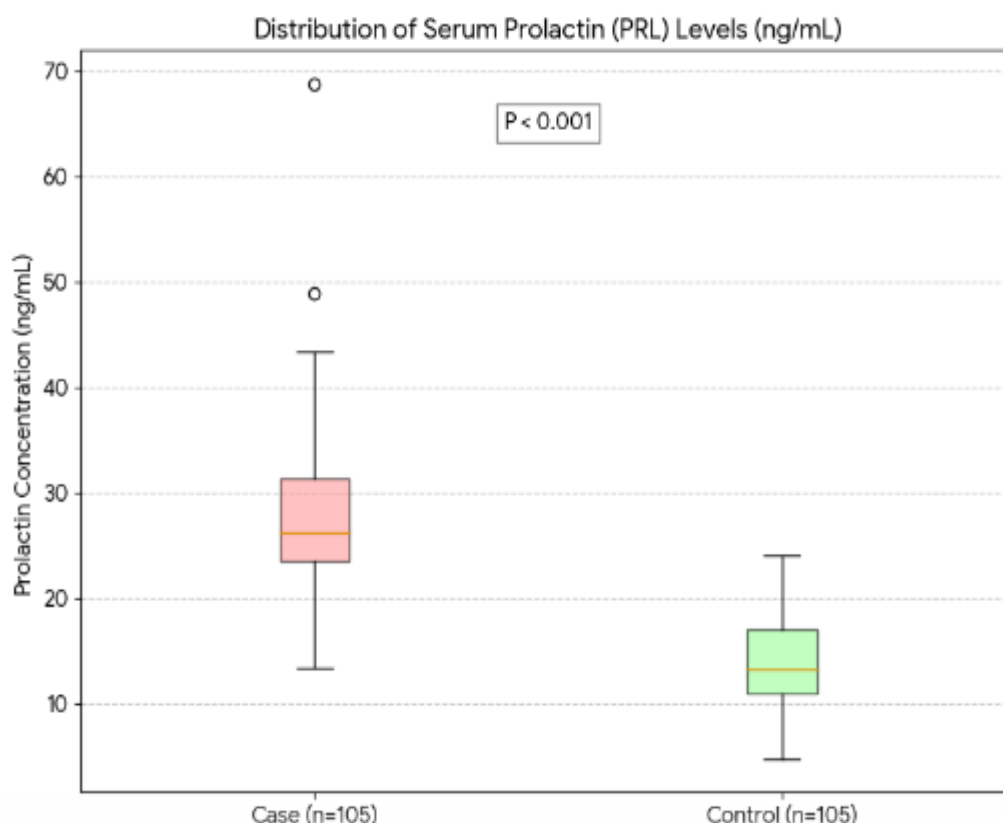


Figure2 : Box plot represents the distribution of serum prolactin levels in case and control groups.

In above graph, the distribution of serum prolactin (PRL) levels across the study population (N=210) in Andhra Pradesh is illustrated in Figure 3, revealing significantly higher concentrations in the case group compared to healthy controls. We observed that, The mean serum PRL level in premenopausal women with uterine fibroids was markedly elevated at 27.79 ± 8.19 ng/mL, while the control group maintained a significantly lower mean of 13.96 ± 4.09 ng/mL ($P < 0.001$). As shown in the box plot, the case group exhibits a median value of 26.185 ng/mL with a distinct shift toward hyperprolactinemia, including clinical outliers reaching concentrations as high as 68.75 ng/mL. In contrast, the control group's distribution remains concentrated within the normal physiological range with a median of 13.27 ng/mL, endorsing that the elevated PRL levels observed in the case cohort are statistically significant and also it represent a key biochemical characteristic of the disease within this regional inhabitants.

Discussion

As mentioned by Mohamed et al. in 2016, the routine screening of pregnant patients for vitamin D deficiency was conducted by large group of clinicians and studies showed the benefits of vitamin D supplementation in those women [19]. Similarly, we believe it will soon become the daily practice of clinicians who treat women with UFs. Notably, the response to vitamin D medications might differ among populations and even in the same person [20].

The clinical findings of this study demonstrate a profound biochemical imbalance in premenopausal women from Andhra Pradesh diagnosed with uterine fibroids (UFs). Our data indicate that the mean serum vitamin D3 levels were markedly lower in the case group compared to healthy, matched controls (10.85 ± 3.34 ng/mL vs. 19.64 ± 5.50 ng/mL). This observation is consistent with recent Indian research, such as the study by Srivastava et al. [21] in Lucknow, which similarly identified significantly reduced 25-hydroxy vitamin D3

levels in women with fibroids. The high prevalence of deficiency in our case group—where not a single patient reached the "Sufficient" threshold of ≥ 20 ng/mL—supports the hypothesis that hypovitaminosis D is a critical risk factor for leiomyoma development.

A study by Paffoni et al. [22] who investigated similar research query in 126 women with fibroid and 256 controls visiting two infertility clinics in Italy. They found lower mean serum concentration of 25-hydroxyvitamin D3 in women with fibroid compared to controls (18.0 ± 7.7 vs. 20.8 ± 11.1 ng/mL, respectively, $p = .010$). Moreover, they reported that the crude odds ratio (OR) for the presence of fibroids in women with serum levels of 25-hydroxyvitamin D3 below 10 ng/mL compared with those with 25-hydroxyvitamin D3 ≥ 10 ng/mL was 2.2 (95% confidence interval [CI] 1.1–4.3) ($p = .022$).

The biological mechanism underlying this association likely involves the role of 1,25-dihydroxy vitamin D3 as a potent inhibitor of cellular proliferation. Evidence from *in vitro* studies suggests that vitamin D3 downregulates key growth markers like proliferating cell nuclear antigen (PCNA) and B-Cell Leukemia (BCL-2) in human leiomyoma cells. Furthermore, vitamin D3 acts as an antifibrotic agent by suppressing the expression of extracellular matrix proteins, such as collagen type-1 and fibronectin, and reducing the profibrotic effects of transforming growth factor β -3 (TGF- β 3). Our findings of a strong negative correlation ($r = -0.513$) between vitamin D3 levels and the number of fibroids reinforce the clinical significance of these molecular pathways.

A cross sectional study conducted by Halder et al included 104 women with fibroid and 50 controls without the disease. They similarly reported lower mean serum vitamin D3 concentration among cases with fibroid [23].

Simultaneously, our study highlights the role of prolactin (PRL) as a significant biomarker. We observed that serum PRL levels were significantly elevated in the case group (27.79 ± 8.19 ng/mL) compared to controls (13.96 ± 4.09 ng/mL). Research suggests that UFs may serve

as an extra-pituitary source of PRL, contributing to a state of hyperprolactinemia that is independent of thyroid function. The positive correlation between PRL levels and the number of fibroids ($r = 0.453$) indicates that as the disease progresses and fibroid burden increases, serum PRL levels rise accordingly. This supports the theory that fibroids are not merely responsive to hormonal environments but are active endocrine-secreting tissues.

Beyond biochemical markers, lifestyle factors played a significant role in our regional cohort. A significant majority of women in the case group reported low sun exposure (73.3%) and reduced physical activity, both of which are strongly associated with lower vitamin D3 synthesis. These findings mirror those of Sabry et al. [24], who also noted that lower serum vitamin D3 levels inversely correlated with total fibroid volume. The increased risk observed in participants with a family history of UFs (26.7% in cases vs. 11.4% in controls) further emphasizes the multifactorial nature of this condition, combining genetic predisposition with environmental and biochemical triggers.

Establishing a definitive causal link would necessitate a rigorous, prospective longitudinal cohort study, involving serial measurements of vitamin D status alongside consistent patient monitoring to track the actual incidence of uterine fibroid development. However, implementing such an exhaustive analytical framework is resource-intensive and may require several years of observation, making it clinically and logistically challenging to execute.[25,26]

So implication of vitamin D deficiency in uterine fibroid will bring in a whole new therapeutic aspect and it will impart a huge impact in the outcome.[27,28] also, it is vital to find out whether or not vitamin D supplementation will help in regression of fibroid. In this regard, Halder and colleague reported reduction in the size of uterine fibroid in the Eker rat model after vitamin D3 supplementation [29]. The findings of this investigation demonstrate a clear inverse relationship between vitamin D deficiency and

the prevalence of uterine fibroids within this South Indian population. These results reveal a significant clinical opportunity for both the prevention and non-invasive management of leiomyomas in women of reproductive age. By establishing hypovitaminosis D as a modifiable risk factor, our data suggest that biochemical monitoring and targeted supplementation may play a pivotal role in managing disease burden in this regional cohort. However, further longitudinal studies are warranted to fully validate these associations and refine standardized therapeutic protocols.

Limitations and Future Directions

While our study is limited by its cross-sectional nature and relatively small regional sample size, it provides early and vital clinical data on the concurrent roles of vitamin D3 and prolactin in South Indian women. Given that randomized clinical trials have shown vitamin D3 supplementation can inhibit leiomyoma growth and prevent recurrence, our results suggest that monitoring and correcting these biochemical levels could be an effective, non-invasive management strategy for uterine fibroids.

Acknowledging the inherent constraints of this research is vital for a balanced interpretation of the biochemical data obtained from the Andhra Pradesh cohort. The primary limitation of this study resides in its cross-sectional design, which effectively identifies significant associations between hypovitaminosis D, hyperprolactinemia, and uterine fibroids but cannot definitively establish a temporal causal sequence. Furthermore, while the sample size of 210 participants provided sufficient statistical power to detect highly significant differences ($P < 0.001$), the findings are geographically specific to South Indian women and may be influenced by regional dietary or environmental factors that limit universal generalizability. Future research should prioritize longitudinal interventions, specifically randomized clinical trials, to evaluate whether therapeutic correction of hypovitaminosis D can actively reduce fibroid volume or prevent post-surgical recurrence. Additionally, expanding molecular

investigations into the extra-pituitary pathways of prolactin secretion within leiomyoma tissue would further clarify the endocrine role of these tumors and refine non-invasive diagnostic protocols.

Conclusion

In conclusion, our study demonstrates that premenopausal women with uterine fibroids in Andhra Pradesh exhibit a distinct biochemical profile characterized by significantly lower serum vitamin D3 levels and elevated serum prolactin concentrations compared to healthy controls. The data reveal a strong negative correlation between vitamin D3 and the number of fibroids, suggesting that hypovitaminosis D may be a significant risk factor in the growth and progression of these benign tumors. Conversely, the positive correlation observed with prolactin levels supports the theory that fibroids may act as an extra-pituitary source of this hormone, potentially contributing to reproductive complications. Furthermore, the significant associations with lifestyle factors, such as reduced sun exposure and sedentary physical activity, highlight the importance of environmental and behavioral influences on the disease's prevalence in this regional cohort. These findings emphasize that monitoring serum vitamin D3 and prolactin levels could serve as essential clinical biomarkers for the early detection and management of uterine fibroids. Future long-term clinical trials with larger sample sizes are recommended to confirm these associations and to evaluate the therapeutic potential of vitamin D3 supplementation as a non-invasive treatment strategy.

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possible. We also acknowledge the Institutional Ethics Committee for their rigorous review and oversight of the study protocol.

Author statement

We declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

Data Availability

The dataset and primary evidence supporting the conclusions of this investigation will be available from the corresponding author upon reasonable request.

Ethical Approval and Participant Consent

The protocol for this research was formally reviewed and approved by the Institutional Review Board (IRB) of Nimra Institute of Medical Sciences (NIMS), Nimra Nagar, Jupudi (V), Ibrahimpatnam (M), Krishna Dist., Andhra Pradesh, India. All procedures were conducted in strict accordance with the ethical standards of the 1964 Declaration of Helsinki and its subsequent amendments, as well as the ICMR guidelines for biomedical research.

Written informed consent was obtained from all N=210 participants (comprising 105 cases and 105 controls) prior to data collection. All individuals were fully briefed on the study's objectives regarding the biochemical evaluation of uterine fibroids, the voluntary nature of their participation, and their right to withdraw at any stage without consequence. To protect participant privacy and minimize bias, all clinical data and serum samples were strictly anonymized and handled with the highest level of confidentiality throughout the biochemical analysis and storage process.

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Conflicts of Interest: The authors declare no conflicts of interest.

Declaration on the Use of Artificial Intelligence

The authors declare that artificial intelligence based tools were used solely for grammatical correction and language refinement of the manuscript. No AI tools were employed in the study design, data collection, data analysis, interpretation of results, or in drawing scientific conclusions.

References:

1. Vahdat M, Allahqoli L, Mirzaei H, Giovannucci E, Salehiniya H, Mansouri G, et al. The effect of vitamin D on recurrence of uterine fibroids: A randomized, double-blind, placebo-controlled pilot study. *Complement Ther Clin Pract* 2022;46:101536.
2. Arjeh S, Darsareh F, Asl ZA, Kutenaei MA. Effect of oral consumption of vitamin D on uterine fibroids: A randomized clinical trial. *Complement Ther Clin Pract* 2020;39:101159.
3. Laughlin SK, Schroeder JC, Baird DD. New directions in the epi- demiology of uterine fibroids. *Semin Reprod Med*. 2010;28:204-17.
4. Luk J, Torrealday S, NealPerry G, Pal L. Relevance of vitamin D in reproduction. *Hum Reprod*. 2012;27:3015-27.
5. Sabry M, Al-Hendy A. Innovative oral treatments of uterine leiomyomas. *Hum Reprod*. 2012;943635.
6. Stewart EA, Cookson CL, Gandolfo RA, Schulze-Rath R. Epidemiology of uterine fibroids: a systematic review. *BJOG*. 2017;124(10):1501–12.
7. Ciebia M, Włodarczyk M, Ślabuszeńska-Jozwiak A, Nowicka G, Jakiel G. Influence of vitamin D and transforming growth factor beta3 serum concentrations, obesity,

- and family history on the risk for uterine fibroids. *Fertil Steril*. 2016;106(7):1787–92
8. Stewart EA, Laughlin-Tommaso SK, Catherino WH, Lalitkumar S, Gupta D, Vollenhoven B. Uterine fibroids. *Nat Rev Dis Primers*. 2016;2:16043.
 9. Parker WH. Etiology, symptomatology, and diagnosis of uterine myomas. *Fertil Steril*. 2007;87(4):725–36.
 10. Al-Hendy A, Myers ER, Stewart E. Uterine fibroids: burden and unmet medical need. *Semin Reprod Med*. 2017;35(6):473–80
 11. Ciarmela P, Islam MS, Reis FM, Gray PC, Bloise E, Petraglia F, et al. Growth factors and myometrium: biological effects in uterine fibroid and possible clinical implications. *Hum Reprod Update*. 2011;17(6):772–90.
 12. Protic O, Toti P, Islam MS, Occhini R, Giannubilo SR, Catherino WH, et al. Possible involvement of inflammatory/reparative processes in the development of uterine fibroids. *Cell Tissue Res*. 2016;364(2):415–27.
 13. Ciebiera M, Wlodarczyk M, Wrzosek M, Meczekalski B, Nowicka G, Lukaszuk K, et al. Role of transforming growth factor beta in uterine fibroid biology. *Int J Mol Sci*. 2017;18(11):2435.
 14. Ciebiera M, Wlodarczyk M, Zgliczynska M, Lukaszuk K, Meczekalski B, Kobierzycki C, et al. The role of tumor necrosis factor alpha in the biology of uterine fibroids and the related symptoms. *Int J Mol Sci*. 2018;19(12):3869.
 15. Freeman ME, Kanyicska B, Lerant A, Nagy G. Prolactin: Structure, function, and regulation of secretion. *Physiol Rev* 2000;80:1523–631.
 16. Ahmed AA, Deif OM, Al-Latif A, Saad S, Mohamed AM. Serum protein and prolactin in evaluation of uterine fibroids. *Egypt J Hosp Med* 2019;76:3653–8.
 17. Chapitis J, Riddick DH, Betz LM, Brumsted JR, Gibson M, Prior JC, et al. Physicochemical characterization and functional activity of fibroid prolactin produced in cell culture. *Am J Obstet Gynecol* 1988;158:846–53.
 18. Abdulla MM, Baban RS, Farid YY. Role of ectopic prolactin on thyroid hormones level in a sample of Iraqi infertile women with uterine fibroids. *Iraqi J Med Sci* 2012;10:334–8.
 19. Mohamed SA, Al-Hendy A, Schulkin J, Power ML. Opinions and practice of US-based obstetrician-gynecologists regarding vitamin D screening and supplementation of pregnant women. *J Pregnancy*. 2016;2016:1454707.
 20. Carlberg C, Haq A. The concept of the personal vitamin D response index. *J Steroid Biochem Mol Biol*. 2018;175:12–7.
 21. Srivastava P, Gupta HP, Singh RK, Pant P. Relationship of serum 25-hydroxy vitamin D levels with uterine leiomyoma: A case-control study. *J Obstet Gynaecol India*. 2020;70(1):67–73.
 22. Paffoni A, Somigliana E, Vigano' P, et al. Vitamin D status in women with uterine leiomyomas. *J Clin Endocrinol Metab*. 2013;98(8):E1374–8.
 23. Baird DD, Dunson DB, Hill MC, Cousins D, Schectman JM. High cumulative incidence of uterine leiomyoma in black and white women: ultrasound evidence. *Am J Obstet Gynecol*. 2003;188:100–7.
 24. Sabry M, Halder SK, Allah AS, Roshdy E, Rajaratnam V, Al-Hendy A. 2013. Serum vitamin D3 level inversely correlates with uterine fibroid volume in different ethnic groups: A cross-sectional observational study. *Int J Womens Health*;5:93–100.
 25. Flake GP, Andersen J, Dixon D. Etiology and pathogenesis of uterine leiomyomas: a review. *Environ Health Perspect*. 2003;111:1037–54.
 26. Kjerulff K, Langenberg P, Seidman J, Stolley P, Guzinski G. Uterine leiomyomas: racial differences in severity, symptoms and age at diagnosis. *J Reprod Med*. 1996;41:483–90.
 27. Bouyer J, Job-Spira N, Pouly JL, Coste J, Germain E, Fernandez H. Fertility following radical, conservative-surgical or

- medical treatment for tubal pregnancy: a population-based study. BJOG. 2000;107(6):714-21.
28. Pérez-López FR, Ornat L, Ceausu I, Depypere H, Erel CT, Lambrinoudaki I, et al. EMAS position statement: management of uterine fibroids. Maturitas. 2014;79(1):106-16.
29. Halder SK, Sharan C, Al-Hendy A. 1,25-dihydroxyvitamin D3 treatment shrinks uterine leiomyoma tumors in the Eker rat model. Biol Reprod. 2012;86:116