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Low Serum Vitamin D Level and Occurrence of Uterine Leiomyoma: A Case-Control Study

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A B S T R A C T

Objective: Uterine fibroids (UFs) is a benign disorder that affects women of reproductive age. Vitamin D is thought to play an important role in regulating cell proliferation and differentiation. This study aimed to investigate the role of vitamin D in uterine fibroid-related problems .

Methods: This case control study was done on 72 women with fibroid lesion (case group) who referred to Amir-al Momenin hospital, Zabol, Iran between April 2022 and December 2022. Moreover, 38 women with normal uterine morphology on ultrasonography were considered control group within the age range of 20 to 45 years. Blood samples were taken for measuring 25-hydroxyvitamin D levels. Demographic and other related clinical information were collected from the patients .

Result: The ratio of BMI in the case group (73.52%) was higher than the control group (63.15%). This difference was statistically significant (P=0.002). Vitamin D deficiency was common in the case group (54.90%) as compared to controls (6.7%) while sufficiency was common among controls (67.8% vs. 27.45%); the difference was statistically significant ($p < 0.05$). Women in the control group were exposed to sunlight more often than case group which was statistically significant (P=0.002). Women with uterine fibroids, 94.11% had low exposure to sunlight compared to women in the control group (73.68%), which was statistically significant (P=0.002). Ten patients (13.88%) of case group mentioned a family history of uterine fibroids, while none of the women in the control group had this item. This difference was statistically significant (P=0.014).

Conclusion: The present study showed that patients with UFs had significantly lower serum levels of vitamin D. Vitamin D deficiency is a potential risk factor for UFs.

Keywords: Serum vitamin D levels, leiomyomas, Uterine fibroid, Medical biotechnology

➢ **How to cite this paper**

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Introduction

Uterine fibroids (UFs), also referred to as uterine leiomyoma, are benign tumors located in the myometrium that result in significant morbidity such as menorrhagia and pelvic pain, often necessitating medical or surgical intervention [1]. The prevalence of UFs is reported to be as high as 77% [2]. Furthermore, women of color experience a considerably higher incidence, with at least twice the likelihood compared to white women, of developing this severe condition [3]. UFs can manifest as abnormal uterine bleeding, abdominal and pelvic pain, infertility, and obstetric complications including miscarriage and preterm labor, anemia, and gastric disorders such as bloating, constipation, and urinary symptoms [4]. The identification of risk factors for UFs has garnered increased attention as a means of preventing their occurrence. Multiple studies have demonstrated that a deficiency in vitamin D elevates the risk of UFs [4- 6]. Vitamin D, known for its anti-tumor properties, likely plays a significant role in the biology and prevention of UFs [4].

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Vitamin D is a precursor hormone that is synthesized in the skin through a reaction triggered by sunlight, and subsequently converted into its active form, 1.25-dihydroxyvitamin D3, primarily in the liver and kidneys [7]. Its biological effects are mediated by its interaction with the cellular receptor known as the vitamin D receptor, which in turn modulates the transcription rates of target genes involved in various physiological responses [8]. Vitamin D receptors are present in multiple human tissues including the skin, colon, brain, monocytes, macrophages, and uterine fibroid (UF) tissues throughout the menstrual cycle [7]. The active form of vitamin D possesses the ability to impede cellular growth and facilitate the differentiation of diverse cell types [9]. Numerous investigations have demonstrated that vitamin D plays pivotal roles in various biological processes beyond regulating calcium levels and promoting bone health (10-12). Additionally, there have been indications of a potential association between vitamin D and UFs [13]. A study conducted in 2018, identified vitamin D as a safe and effective way to manage and prevent fibroids [14]. In biological experiments, it has been observed that vitamin D can stop the growth of fibroid cells and promote cell death (apoptosis) in these cells [15]. Also, in animal tests, this vitamin was able to reduce the size of fibroids by about 75% [16].

In the past few years, there have been multiple research studies examining the potential preventive or long-term treatment effects of vitamin D on UFs. However, the outcomes of these studies have exhibited inconsistencies and have not yielded conclusive results (17-19). Furthermore, few studies have been done about this illness in Iran, and because of the covering of women who are forced to wear hijab (lack of exposure to sunlight), the level of vitamin D among women is lower (20, 21) and because of the high prevalence of this disease and the possibility of hysterectomy in Iran, and its importance in quality of life, we aimed to explore and investigate the role of vitamin D in addressing issues associated with UFs among patients with fibroid lesion in Amir-al Momenin Hospital, Zabol, Iran.

Methods

This study adopted a case-control design and mainly focused on premenopausal women within the age range of 20 to 45 years old. The participants were selected by convenience sampling method based on their presentation of clinical symptoms and their subsequent referral for transabdominal ultrasound by a gynecologist with expertise in genital tract sonography. The data collection period extended from April 2022 to December 2022 (spring-winter periods). The case group comprised women diagnosed with uterine body fibroids (excluding cervical fibroids), while the control group consisted of healthy women whose uterus exhibited no fibroids. To ensure the validity of the study, premenopausal women meeting any of the following criteria were excluded: (1) utilization of vitamin D or calcium supplements within the six months preceding study enrollment, (2) pregnancy or lactation, (3) presence of serious ailments such as psoriasis (skin disease), myocardial infarction or cerebral infarction (cardiovascular diseases), abnormal parathyroid gland (endocrine disease), tuberculosis (infectious disease), Type 1 diabetes mellitus or systemic lupus erythematosus (autoimmune disorders), malignancies affecting any organ, hepatic or renal diseases, and digestive system disorders related to malabsorption, and (4) history of previous myomectomy or hysterectomy. It is pertinent to note that this research did not involve any intervention or treatment and did not consider the different food intake effect on vitamin D. Prior to their participation in the study, all involved individuals provided written informed consent. The study was approved by the Ethical Committee of Zabol University of Medical Sciences (Ethics Code: IR.ZBMU.REC.1400.082).

Ultrasound measurements

Transvaginal ultrasound screening was conducted using ultrasound systems (GE VolusonTM E6, E8 or E10) from GE Medical Systems, an American company, to examine all participants in the study for the presence of UFs. Ultrasound assessments were performed to determine two key parameters related to UFs in each patient: the number of fibroid lesions and the size of the largest fibroid. A cutoff point of 3 cm was selected as the fibroid size index [22]. Based on this index, the participants were categorized into three groups for analysis and comparison purposes: (1) a group without fibroids, (2) a group with small fibroids (with a diameter less than 3 cm), and (3) a group with large fibroids (with a diameter greater than or equal to 3 cm).

Blood assay

All study participants provided a blood sample that was collected from the cubital vein during early morning. Serum 25-hydroxyvitamin D (25OHD) levels were tested in samples from all study participants. Blood samples were drawn, processed and stored at -20°Cprior to analyses. Electrochemiluminescence immunoassay was used to evaluate serum 25OHD levels with a Cobas e601 fully automated analyzer and associated reagent kits (Roche

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Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's instructions. The ranges of total serum 25OHD concentration indicating vitamin D deficiency [<20 ng/mL (<50 nmol/L)], suboptimal status [20–30 ng/mL (50–75 nmol/L)], and optimal concentration [30–50 ng/mL (75–125 nmol/L)] were confirmed according to guidelines for preventing and treating vitamin D deficiency [23].

A comprehensive assessment of the patients included obtaining a detailed history covering various aspects such as demographic features, physical activities, clinical symptoms, underlying diseases, and menarche status. Particular attention was given to capturing information on the duration of sunlight exposure during the time frame of 10:00 AM to 3:00 PM from the selected women. Physical activity levels were evaluated utilizing the metabolic equivalents (METs) questionnaire, which classified activity into three categories: low (<3.0 METs), moderate (3.0-5.9 METs), and high (≥6.0 METs) [24]. Additionally, sun exposure was categorized based on responses to the self-administered short-term SEM-Q questionnaire with reference period of one day (current day) with a grid divided in one hour intervals from sunrise to sunset [25]. This questionnaire inquired about several factors, including time spent outdoors (in minutes), weather conditions, clothing choices, use of sunscreen, sun protection practices, multivitamin usage, and skin tone.

Statistical analyses

Descriptive data were presented as mean, standard deviation (SD), and percentage. For qualitative characteristics, the Chi-square independence test was used to determine the differences between the groups. The independent t-test and Chi-squared test were used to compare serum Vitamin D levels and quantitative variables between the two study groups. A backward logistic regression model was applied to select the variables for measuring the association between serum levels of Vitamin D and UFs adjusting for variables known to be associated with UF. $p < 0.05$ was considered statistically significant. Data analysis was carried out by statistical software (SPSS, v 24; SPSS Inc., Chicago, IL, USA).

Results

A total of 110 participants were included in the study, comprising 72 women with fibroid and 38 healthy women without fibroids.

Baseline and demographic characteristics of study population were shown in Table 1. The mean age of women in cases and controls were 40. 11 ± 8.68 and 33.36 ± 9.57respectively and age ranges were 19 to 56 years. The mean age of patients in the case group was significantly higher than the control group which was statistically significant (P=0.001). Among the studied patients, 38.23% of the case group and 36.84% of the control group had a diploma or higher educational level. The remaining patients in both groups had an educational level lower than a diploma. No significant statistical difference was observed in this field between the two groups (P=0.529).

The results showed that 87.5% of patients with UFs had moderate to high physical activity and only 12.5% of them had low physical activity. However, in the control group, moderate to high and low daily activity was reported in 93.75% and 6.25%, respectively. These differences were not statistically significant (P=0.488). The highest ratio of BMI in the case group was between 25-29.9 (73.52%) and <25(17.64%), while the BMI recorded for women of the control group was the most frequent in the range of 25-29.9(63.15%) and 30 -34.9 (31.57%). None of the people in the control group had a BMI above 35, while this BMI was 2.94% in the case group. Also 17.64% of the case group had a BMI below 25, while this rate was only 5.26% in the control group. These differences were statistically significant (p<0.05), which means that patients with UFs had a significantly lower BMI than the control group.

According to the results of Table 1, 94.11% of women with uterine fibroids had low exposure to sunlight compared to women in the control group (73.68%). Also, 5.88% of women in the case group and 21.05% of the control group were moderately exposed to sunlight. None of the women with fibroids had high exposure to sun, while 5.26% in the control group had high exposure. Therefore, the results of this study showed that women in the control group were exposed to sunlight more than women with uterine fibroids which was statistically significant (P=0.002). Ten patients (13.88%) with UFs mentioned a family history of uterine fibroids, while none of the women in the control group had a family history of uterine fibroids. This difference was statistically significant (P=0.014).

The mean serum 25-hydroxy vitamin D level among cases was 14.52 ± 7.89 ng/mL, while in the control group, it was 26.6 ± 14.36 ng/mL, and the difference was statistically significant ($p < 0.05$). On further categorical analysis, it was observed that vitamin D deficiency was more common in the case group (54.90%) as compared to healthy controls (6.7%) while sufficiency was more common among controls

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(67.8% vs. 27.45%), the difference being statistically significant ($p < 0.05$).

Table 1: Baseline demographic characteristics of study population

Variable	Uterine fibroids (n=72, %)	Control (n=38, %)	*P*-value
Mean Age (year)	40.11± 8.68	33.36± 9.57	0.001[b]
Level of education			
>Diploma (%)	44(61.17	24(63.15	0.529[a]
≤Diploma (%)	28(38.23	14(36.84)	
Physical Activity			
Low (%)	10(12.5	5(6.25)	0.488[a]
Moderate/high (%)	62(78.5	33(93.75)	
BMI(kg/m2)			
<25 (kg/m2)	13(17.64	2(5.26	
25-29.9 (kg/m2)	51(73.52	24(63.15)	0.002[a]
30-34.9 (kg/m2)	5(5.88)	12(31.57	
≥35 (kg/m2)	3(2.94	0(0	
Sun Exposure			
Low (%)	66(94.11)	28(73.68)	
Moderate (%)	6(5.88)	8(21.05)	0.002[a]
High (%)	0(0	2 (5.26)	
Family history of uterine fibroids			
Yes (%)	10(13.88	0(0	0.014[a]
No (%)	62(86.11)	38(100)	

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Mean Serum 25OHD level, ng/ml	14.52 ± 7.89	26.6 ± 14.36	< 0.05[b]
Deficiency (%)	44 (54.90)	8 (6.7)	
suboptimal status (%)	10 (17.64)	11 (25.5)	< 0.05[b]
optimal concentration (%)	18 (27.45)	19 (67.8)	

[a]*Chi-square test*, [b]*independent t-test.*

As Table 2 displays, 77.77% of patients with UFs complained of bleeding, while this rate was only 5.26% in the control group. This difference was statistically significant (P＜0.05). Also, patients with uterine fibroids had more abdominal (16.66%) and pelvic pain (27.77%) than the control group, and this difference was statistically significant (P<0.05). Other clinical symptoms in two groups did not have statistically significant differences .

The mean menarche age of the control group (10.82±0.86 years) was less than case group. The results showed that the control group and patients with small fibroids had a significantly lower menarche age than patients with large fibroids. This difference was statistically significant (P=0.030). The mean vitamin D in the studied population was 24.17 ± 12.16. The mean of vitamin D in patients with large fibroids was 6.98 ± 17.72, while this average was 13.23 ± 25.22 and 12.17 ± 26.68 in the group of patients with small fibroids and the control group, respectively. As a result, the results indicated that patients with large fibroids had significantly and significantly lower serum levels of vitamin D than patients with small fibroids and the control group (P=0.016).

Table 2: Clinical symptoms of studied groups

Variables	**Uterine fibroids (n=72(%))**	**Control (n=38(%))**	***P*-value**
Vaginal Bleeding			
Yes (%)	56(77.77)	2(5.26	0.000
No (%)	16(22.22	36(94.73	
Stomach ache			
Yes (%)	12(16.66	0(0)	0.008
No (%)	60(83.33	38(100	
Pelvic pain			
Yes (%)	20(27.77	0(0	0.000
No (%)	52(72.22	38(100	
IDA			

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Yes (%)	26(36.11	8(21.05	0.131
No (%)	46(63.88	30(78.94)	
Endometriosis			
Yes (%)	2(2.77)	0(0	0.544
No (%)	70(97.22	38(100)	
PCOS			
Yes (%)	0(0)	2(5.26	0.117
No (%)	72(100	36(94.73)	
Infertility			
Yes (%)	16(22.22)	4(10.52	0.193
No (%)	56(77.77)	34(89.47	
Amenorrhea			
Yes (%)	2(2.77)	0(0)	0.544
No (%)	70(97.22)	38(100)	
Abortion			
Yes (%)	0(0)	2(5.26)	0.117
No (%)	72(100	36(94.73)	
Cystic			
Yes (%)	2(2.77)	0(0	0.544
No (%)	70(97.22)	38(100)	

Note: IDA: Iron Deficiency Anemia,Chi-square test

Discussion

UFs are the most prevalent noncancerous tumors occurring in women, exerting a substantial detrimental effect on their overall well-being [26]. Depending on a woman's reproductive intentions, pharmacological and/or surgical interventions are frequently warranted, resulting in significant healthcare expenditure. Furthermore, the utilization of existing drug therapies may engender adverse effects [15].

This investigation unveiled that patient diagnosed with UFs frequently experienced symptoms such as bleeding, abdominal discomfort, and pelvic pain. These findings align with prior research indicating that bleeding and pelvic pains are prevalent symptoms associated with UFs [27, 28). Additionally, the UFs group demonstrated a significantly elevated likelihood of having a positive family history compared to the control group. The presence of UFs among first-degree relatives appears to constitute a prominent risk factor [29]. Specifically, individuals with a family history of UFs face twice the risk of developing the condition [30]. Similarly, a study discovered that a positive family history coupled with a marked increase in BMI augments the likelihood of developing UFs [31]. Contradicting the aforementioned study, the current investigation observed that patients with uterine fibroids exhibited a significantly

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lower BMI compared to the control group, with the majority falling within the normal BMI range.

The correlation between the age of menarche and the prevalence of UFs has been a subject of discussion among various authors [29, 30]. In our study, both the control group and patients with a small fibroid demonstrated a notably lower age of menarche compared to patients with large fibroids. A multicenter prospective cohort study, conducted between the years 2001 and 2011, focused on screening and monitoring fibroid presence in early pregnancies. Researchers discovered a connection between the age of menarche and the occurrence of leiomyoma. Specifically, a one-year increase in the age of menarche showed an inverse association with fibroid presence (adjusted risk ratio: 0.87, 95% CI: 0.82-0.91) [31]. It has been theorized by experts that the relationship between menarche age and fibroids may be attributed to the duration and level of exposure to endogenous sex steroid hormones [32]. Two prevailing hypothesis proposed that a longer exposure to estradiol and progesterone, due to an earlier age of menarche, and a unique hormonal environment associated with early menarche, may heighten the risk of fibroid development [33].

The findings of our study indicate that patients with multiple fibroids exhibited significantly lower serum vitamin D levels compared to patients with small fibroids and the control group. Moreover, as the severity and size of uterine fibroids increased, there was a notable and significant decline in serum vitamin D levels. These results are in line with the study by Farzaneh et al. [34] which showed that for women with UFs, the serum level of 25-hydroxyvitamin D3 was significantly lower than in controls in Iranian region. Moreover these results align with the study conducted by Sabry et al. [35], which also demonstrated an inverse association between low serum vitamin D levels and the occurrence of UFs, suggesting that vitamin D deficiency may be a potential risk factor for developing UFs. In a previous study by Halder et al. [36], it was observed that vitamin D treatment effectively reduced the size of uterine fibroids in an animal model known as the Eker rat. This reduction in size was attributed to the suppression of cell proliferation, possibly facilitated by the overexpression of the 24-hydroxylaze enzyme, which may contribute to the presence of hypovitaminosis D [37]. Additionally, as previously mentioned, vitamin D was found to inhibit Wnt/β-catenin activation and downregulate the expression of mTOR signaling [16]. Furthermore, Ciebiera et al. reported that low serum vitamin D levels in mice were associated with increased expression of sex steroid receptors in the myometrium, as well as elevated expression of genes associated with proliferation, fibrosis, and inflammation [38].

Additionally, the findings of our study indicate that the amount of sunlight exposure plays a crucial role in determining the serum vitamin D levels. Interestingly, the evaluations conducted in our study revealed that women with UFs had significantly lower sunlight exposure compared to women in the control group. This finding aligns with the study by Kaplan et al. [39], where they attributed the high prevalence of vitamin D deficiency to seasonal variations in sun exposure and the practice of wearing covering clothing. Their study, conducted during the winter-spring period, highlighted the significant association between wearing covering clothing and vitamin D deficiency. They emphasized that outdoor activities, sunlight exposure, clothing style, and seasonal changes contribute to variations in vitamin D levels.

Limitations

It is important to acknowledge that our study has certain limitations, such as the relatively small sample size and its single-center nature. Furthermore, this study was conducted during one year (from spring to winter) but it was not possible condition to measure the effect of different seasons on vitamin D measurement. To draw more comprehensive conclusions, further studies involving multiple clinical centers with larger samples are warranted.

Conclusion

Consistent with previous studies, this study demonstrates that patients with UFs have significantly lower serum levels of vitamin D compared to the control group. Furthermore, as the severity and size of uterine fibroids increase, there is a notable decline in vitamin D levels. These findings suggest that vitamin D deficiency could be a potential risk factor for the development of uterine fibroids. However, due to the limited availability of evidence from clinical studies, further clinical studies are needed to explore the utility of vitamin D for the treatment of UFs.

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Contributions

Conception and design: Shila Tayyeb Ghasemi; Administrative support: Mania kaveh; Provision of study materials or patients: Shila Tayyeb ghasemi; Collection and assembly of data: Shila Tayyeb ghasemi; Data analysis and interpretation: Mania kaveh; Manuscript writing: All authors and Final approval of manuscript: All authors.

Conflict of interests

The authors declare that they have no conflict of interests.

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