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Vitamin D Status in Women with Uterine Fibroids: A Cross-sectional Study

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Abstract

BACKGROUND: Uterine fibroids (UFs) affect women of reproductive age and lead to major morbidity in premenopausal women. Identifying modifiable risk factors could help develop new UF prevention and treatment strategies.

OBJECTIVES: The purpose of this research was to investigate the relationship between serum Vitamin D3 levels and UF in women seeking gynecological services.

METHODS: This case–control design was conducted in September 2018 at the outpatient gynecology clinic of Shahid Beheshti University of Medical Sciences, Tehran, Iran. Cases had at least one ultrasound confirmed fibroid lesion with an average volume of 2 cm or greater. The outpatient clinic has enrolled a control group of patients without UF, based on transvaginal ultrasonography or any other gynecologic pathology. Radioimmunoassay techniques were applied to measure serum Vitamin D [25(OH) D3] levels.

RESULTS: A total of 148 patients met inclusion criteria, 71 women were had at least one UF and the remaining 77 participants showed normal, UF-free uterine structure. The mean serum concentration of 25-hydroxyvitamin D3 was lower in UF patients (21.37 ± 7.49 ng/mL) than without (24.62 ± 9.21 ng/mL) ($p = 0.02$). A modified odds ratio derived from a backward logistic regression model for 25-hydroxyvitamin D3 that included positive family history, age, body mass index, bleeding volume, physical activity, sun exposure, and history of abortion was 0.92 (95% CI, 0.88–0.98) ($P = 0.02$).

CONCLUSION: For women with UFs, the serum level of 25-hydroxyvitamin D3 was significantly lower than in controls. Vitamin D3 deficiency is a potential risk factor for UFs to occur.

Introduction

One of the most common pathologies in women of reproductive age is uterine leiomyomas (fibroids), a benign neoplasm of uterine smooth muscle cells [1]. Based on clinical diagnosis or diagnostic tests reports, the incidence of premenopausal women varies from 30% to 70% and rises with increasing age [2].

Uterine fibroids (UFs) lead to major morbidity in premenopausal women, regardless of their inherently benign neoplastic character [3]. Several studies have shown that fibroids have negative effects on iron deficiency anemia, stomach disorders, female pregnancy, and obstetric complications such as miscarriage and preterm labor [4], [5], [6].

Although the etiology of fibroids is unknown, there have been a sizeable scientific literature pertaining to the main factor inducing age, parity, race, obesity, family history, and serum micronutrients [7]. Over the past decade, it has been hypothesized whether Vitamin D deficiency is associated with fibroid. In 2008, Baird *et al*. demonstrated that women with Vitamin D insufficiency have a higher chance of developing UFs compared to women with normal levels of Vitamin D. Furthermore, the prevalence of UFs was significantly higher in Blacks rather Whites (75% vs. 51%; $p < 0.001$). As a result, more skin pigmentation tends to be correlated with lower Vitamin D status and higher UFs prevalence [8]. Although this association was seen in several studies, it has not been repeated in different populations, and the results are still contradictory [9], [10], [11], [12]. Multiple population-based studies have been carried out about the prevalence of Vitamin D deficiency in Iran [13], [14], [15], [16]. The meta-analysis of 48 studies including 18, 531 participants estimated the high prevalence of Vitamin D deficiency among male (45.64%, 95% CI: 29.63–61.65), female (61.90%, 95% CI: 48.85–74.96), and pregnant women (60.45%, 95% CI: 23.73–97.16) [17]. Along with the high prevalence of Vitamin D deficiency, a high prevalence of reproductive morbidity and health-seeking activity among women of reproductive age in Iran has been identified [18]. This study, therefore, was aimed to explore the relationship between serum Vitamin D3 levels and UFs in Iranian women seeking gynecological services.

Methods

This case–control study was conducted in September 2018 at the Shahid Beheshti University of Medical Sciences, Tehran, Iran's outpatient gynecology clinic. There was no treatment or intervention involved in this study, and this study was approved by the Medical School Ethical Committee.

Subjects

Case subjects in the present study were recruited among the women referred to Emam Hossein Hospital, for surgical removal of the UF or hysterectomy, and had at least one lesion with an average volume of 2 cm or more that was detected with transvaginal or abdominal ultrasound scan. Total uterine volume and total volume of fibroid were measured for each subject. Using a General Electric Voluson E8 BT 13 with transvaginal I-C 5-9 D probe and abdominal RAB 4-8 D probe, the ultrasound examination was performed by a gynecologist qualified for genital tract sonography. In the case of large fibroids, the area of the pelvis minor and the abdominal area (including the uterus and the adnexa) were investigated. UF is defined as a symmetrical area with distinct margins, hypoechogenic, and heterogeneous.

The outpatient clinic has enrolled a control group of patients without UF, based on transvaginal ultrasonography (TVU) or any other gynecologic pathology. Control patients were asked for consent during their visits to the clinic to take part in the study (for cervical smears, control ultrasounds, etc.). Eligible candidates obtained written informed consent before participation in the study.

Women were not eligible to participate in this study if they had: (1) Previous myomectomy or hysterectomy; (2) active pregnancy or pregnancy within 6 months of study commencement, (3) currently lactating or lactating within 6 months before the beginning of the study, (4) candidates who have had an abortion or miscarriage within 6 months of the start of the study, and (5) diabetes(6) used a vitamin supplement or hormonal therapy during or within 6 months of enrolment.

Blood assay

During the study visit, blood samples (about 12 mL) were also obtained from each participant. Radioimmunoassay techniques described previously [19], [20], [21] were applied to measure serum Vitamin D3 [25(OH) D3] levels. 25(OH) D3 was assessed using a direct, competitive chemiluminescence immunoassay at Heartland Assays, Inc. (Ames, IA) with DiaSorin Liaison 25(OH) D3 Total Testing platform [20], [21]. The sensitivity for this assay is 2.5 ng/mL, and the coefficients of variation between intra-assay are 11.2% and 8.1%, respectively. Endogenous 25(OH) D recovery is 100% [19]. Vitamin D3 was categorized based on the definition of Ritu and Gupta, so that, Vitamin D deficiency is defined as 25(OH) D <20 ng/mL, insufficiency as 20–29 ng/mL, and sufficiency as ≥30 ng/mL [22].

Physical activity was categorized according to metabolic equivalents (METs) questionnaire. The thresholds were defined as light, <3.0 METs; moderate, 3.0–5.9 METs; and vigorous ≥6.0 METs [23]. In addition, we categorized sun exposure according to self-administered short-term SEM-Q questionnaire [24]. The questionnaire asks about time (min) spent outdoors, weather, clothing, use of sunscreen, sun protection practices, use of multivitamin, and skin tone.

Statistical analysis

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 was 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 UF 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; IBM Corporation, Armonk, NY, USA).

Sample size

The sample size was determined using an estimated 25-hydroxyvitamin D3 concentration in 20.4 controls (SD = 11.8 ng/mL) [25] and stating as clinically relevant 30% lower concentration in women with leiomyomas. Type I and II errors were set at 0.05 and 0.20, respectively, roughly 71 cases and 77 controls are calculated for women to be recruited.

Results

A total 148 patients met inclusion criteria and were included in the study. TVU confirmed that 71 of these participants had at least one UF (with a volume of 2 cm^3 or greater) (Table 1). The remaining 77 participants displayed no UFs of normal uterine structure.

The age of participants ranged from 16 to 77 years (mean age, 41.15 ± 10.98 years). The mean age of the women with fibroids (42.71 ± 8.07 years)

Table 1: Baseline characteristics of women with and without uterine fibroids

Characteristics	Uterine fibroids (n=71)	Control (n=77)	p-value
Age, year	42.71 ± 8.07	39.75 ± 12.95	0.010
BMI, kg/m^2	28.24 ± 5.41	26.96 ± 4.61	0.13
Age at first menstruation, year	13.09 ± 1.19	12.89 ± 1.20	0.33
Age at first delivery, year	23.86 ± 19.34	21.77 ± 5.26	0.45
Family history, yes (%)	18 (25.35)	12 (15.59)	0.07
Bleeding volume			
Low (%)	5 (7.04)	13 (16.88)	
Moderate (%)	33 (46.48)	49 (63.64)	
High (%)	33 (46.48)	15 (19.48)	0.001
History of infertility, yes (%)	10 (14.08)	6 (7.79)	0.22
History of abortion, yes (%)	24 (33.80)	17 (22.08)	0.09
Sun exposure			
Low (%)	32	35	
Moderate (%)	34	29	
High (%)	5	13	0.09
Physical activity			
Low (%)	18 (25.35)	17 (22.08)	
Moderate (%)	35 (49.30)	44 (57.14)	
High (%)	18 (25.35)	16 (20.78)	0.64
History of myometrial, yes (%)	33 (46.4)	10 (13)	<0.0001
Serum level 25-hydroxyvitamin D_3, ng/ml	21.37 ± 7.49	24.62 ± 9.21	0.02
Deficiency (%)	30 (42.25)	17 (22.08)	
Insufficiency (%)	23 (32.40)	20 (25.97)	
Sufficiency (%)	18 (25.35)	40 (51.95)	0.003

BMI: Body mass index.

was slightly higher than the normal subjects, but not significantly different (39.75 ± 12.95 years; p = 0.10). In contrast, the fibroid group's mean body mass index (BMI) (28.24 ± 5.41) was higher than that of the control subjects, but it was not significant (26.96 ± 4.61; p = 0.13).

The number of women with 1, 2, and 3 or more fibroids was 89 (60.14%), 22 (14.86%), and 37 (25%), respectively. Overall, women had 238 lesions; 96 (65%) intramural, 37 (25%) subserosal, and 15 (10%) submucosa. The mean diameter of the fibroids was 21 mm (SD = 14), and the mean diameter of the larger lesion per woman was 24 mm (SD = 15). Table 1 displays the baseline characteristics of the two study groups.

The analysis demonstrated that the serum levels of 25-hydroxyvitamin D_3 significantly differed in both groups (p = 0.02). The mean serum levels of 25-hydroxyvitamin D_3 were 21.37 ± 7.49 ng/mL and 24.62 ± 9.21 ng/mL, respectively, in patients with and without UF. Furthermore, Vitamin D3 deficiency was more prevalent for women with UF than control group, and the difference was statistically significant (p = 0.003).

A modified odds ratio (OR) derived from a backward logistic regression model for 25-hydroxyvitamin D3 that included positive family history, age, BMI, bleeding volume, physical activity, sun exposure, and history of abortion was 0.92 (95% CI, 0.88–0.98) (p = 0.02). Patients with a positive family history had a triple higher risk of developing UF (OR = 2.85, 95% CI, 1.16–6.99) compared to women with a negative family background. A one unit rise in BMI increased the chances of UF by 1.15 (OR = 1.15; 95% CI, 1.02–1.39). An increase in bleeding volume increases the risk for UF by 2.08 (OR = 2.08; 95% CI, 1.73–5.88). Table 2 reported the results of the logistic regression model.

Table 2: Logistic regression model for uterine fibroids

Variable	Odds ratio (95% CI)	p-value
Serum level 25-hydroxyvitamin D_3, ng/ml	0.92 (0.88–0.98)	0.01
Family history		
No	Reference	
Yes	2.85 (1.16–6.99)	0.03
BMI	1.15 (1.02–1.39)	0.04
Bleeding volume		
Low	Reference	
Moderate	2.08 (0.95–5.88)	0.35
high	5.89 (2.74–9.89)	0.002

BMI: Body mass index.

Discussion

To the best of our knowledge, this is a first study to evaluate the association of Vitamin D_3 status of Iranian women with UFs. The results of the current study showed that the serum level of 25-hydroxyvitamin D3 was significantly lower in women with UFs compared to controls. This association was also significant after adjusting for confounding variables, including family history of fibroids, BMI, and menstrual bleeding.

The results of our study are in line with those in different geographic regions and with different cultural contexts. Baird *et al*. conducted a prospective cohort study within a large health plan in Washington, DC between 1996 and 1999. Briefly, 1036 premenopausal women were screened for fibroids with transvaginal and transabdominal ultrasound. It was found that women with adequate 25(OH) D_3 circulation (>20 ng/ml) had lower levels of fibroids compared to those with insufficient Vitamin D_3 (adjusted OR = 0.68, 95% CI: of 0.48, 0.96), and there was no evidence of heterogeneity between Black and White women. Furthermore, the prevalence of UFs was significantly higher in Blacks rather Whites (75% vs. 51%; p < 0.001). Hence, they conclude that more skin pigmentation tends to be correlated with lower Vitamin D status and higher UFs prevalence [8]. In another cross-sectional study by Sabry *et al*., circulating 25 (OH) D_3 levels of 104 women who had at least one fibroid lesion compared to 50 women with a normal, fibroid-free uterine structure. They found an inverse association in all ethnic groups between 25(OH) D_3 deficiency (measured by radioimmunoassay) and the occurrence of uterine leiomyomas [9].

There are also a few studies that have not seen the association between Vitamin D and uterine leiomyomas. In a large cross-sectional study conducted between 2001 and 2006, there was no association between serum levels of 25(OH) D and self-reported UL diagnosis among 3590 premenopausal women. The authors acknowledged that the case finding method would result in differential misclassification resulting in an "underestimation" of the hypothesized association between 25(OH) D_3 deficiency and uterine leiomyomas occurrence [12].

Although there is no clinical trial to evaluate the effect of Vitamin D on fibroids, it has been proven in several *in vitro* studies. Blauer *et al*. found that

0.1 ng/mL physiologic level of 1, 25(OH) D_3 inhibited the growth of myometrial and leiomyoma cells nearly 12%. The inhibition of growth was dependent on concentration in both myometrial and leiomyoma cells. In both types of cells, the highest concentration of 1,25(OH)2D_3 (100 ng/mL) inhibited growth of 62% after rising doses of 1, 25(OH)2D_3 for 9 days [26]. In another study by Sharan *et al*., Vitamin D_3 suppressed fibroid cell growth at 1 ng/mL by 47 ± 0.03% and at 0.1 ng/mL by 38 ± 0.02% compared to control cells at 120 h [27]. The findings of aforementioned studies approved that 1,25(OH)2D_3 was able to suppress the growth of myometrial and leiomyoma at a concentration of 0.1 ng/mL within the physiological range of concentration (48–156 ng/mL) of 1,25(OH)2D3.

The precise molecular mechanism of 1,25(OH)2D3 on uterine leiomyoma remains unknown, although degradation of extracellular matrix (ECM) is one of the most proposed mechanism [28]. The ECM is a non-cellular component three-dimensional network of extracellular macromolecules, such as collagen, enzymes, fibronectin, laminins, proteoglycans, and glycoproteins which present within all tissues and provide structural and biochemical support of surrounding cells [29]. Creation of UFs is stimulated by increasing cell proliferation and ECM deposition. The growth of UFs is due to the increased proliferation of cells and the ECMs deposition. Abnormal deposition of ECM plays a crucial role in the pathophysiology of UFs [30].

Vitamin D3, by increasing the expression and activity of metalloproteins (MMP-2 and MMP-9) involved in ECM degradation, inhibits the growth of UFs [31], in addition, by reducing the activity of transforming the growth factor beta 3, increased fibronectin expression, collagen type 1, plasminogen activator inhibitor-1 proteins, and Smad2 phosphorylation, as well as Smad2 and Smad3 nuclear translocation [32].

Although Vitamin D deficiency is obviously due to geographical and cultural conditions, it could be also because of some factors associated to lifestyle, such as poor nutrition. This issue has not received much attention in national prevention and treatment programs. On the other hand, UFs are the most common cause of women being hospitalized and hysterectomy is causing high costs of treatment to the Ministry of Health. It should be taken into consideration, Vitamin D supplements, such as iron supplements, are compulsorily distributed in girls' schools and avoid expensive medical expenses at low cost.

Interestingly, the duration of sunlight exposure was found to be significantly lower in women with UF. Furthermore, the frequency and volume of UF was found to be significantly higher in these women than in the control subjects; this is in agreement with prior reports and confirms negative correlation between serum Vitamin D levels and UF prevalence and volume. Furthermore, there was a dose dependent between serum Vitamin D and magnitude of fibroid tumor [33].

As with any research, this study has several limitations that should be acknowledge. First was the nature of cross-sectional design and temporality between Vitamin D and UF. Hence, we cannot infer causal inference. Another issue is the lack of adjustment for potential confounders. Confounding is the most important challenge in observational studies. There may be several factors like other gynecological diseases that can confound the association between Vitamin D and UF which more studies are needed to seek the causal association between Vitamin D and UF.

Conclusion

For women with UFs, the serum level of 25-hydroxyvitamin D3 was significantly lower than in controls. Vitamin D3 deficiency is a potential risk factor for UFs to occur.

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