Original Article

Association between serum vitamin D level and uterine fibroid in premenopausal women in Indian population

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SUMMARY   We aim to evaluate the association between serum 25-hydroxyvitamin D_3 levels and total number, volume and location of uterine fibroids (UFs) in premenopausal women in North Indian population. This case control study was undertaken in 310 women between 18 years and 45 years of age. Cases comprised of 102 women with fibroid lesion and the control group included 208 women with normal uterine morphology on ultrasonography. Blood samples were taken for measuring 25-hydroxyvitamin D_3 levels. The mean serum 25-hydroxyvitamin D_3 level in the study and control group was 14.52 ± 7.89 ng/mL and 26.6 ± 14.36 ng/mL respectively ($p < 0.05$). There was significant inverse correlation between serum 25-hydroxyvitamin D_3 levels and total volume of fibroids ($p = 0.000$) while none between 25-hydroxyvitamin D_3 levels with location, number of fibroids. 25-hydroxyvitamin D_3 deficiency was more common in the study group (54.90%) compared to healthy controls (6.7%) while sufficiency was more common among controls (67.8% *vs.* 27.45) ($p < 0.05$). Women with deficient 25-hydroxyvitamin D_3 levels have an odds of 18.36 for developing uterine fibroid. Women with low parity, those belonging to higher socioeconomic status and having less than 1-hour sun exposure per day were independently found to have high risk for development of UFs. Vitamin D may have a role in growth of UFs. Women not able to get adequate sun exposure due to indoor working conditions may need evaluation and supplementation as prophylaxis for development of fibroid.

Keywords   Serum 25-hydroxyvitamin D_3 levels, uterine leiomyomas, pelvic imaging

1. Introduction

Uterine fibroids (UFs) are the most common benign pathology of female genital tract with 5-70% of women developing the tumor. Uterine fibroids may be associated with abnormal uterine bleeding, abdominal and pelvic pain, infertility and obstetric complications like miscarriage and premature labor, anemia, gastric disorders like bloating, constipation, and voiding symptoms (*1*). Conventional medical therapies provide only a short-term relief (*2*), and surgical intervention still remains the mainstay of treatment. Vitamin D, which is shown to have an antitumor activity, may play one of the major roles in UF biology and prophylaxis (*1*).

Vitamin D is a prohormone produced in the skin *via* a sunlight-initiated reaction and converted to the active metabolite 1, 25-dihydroxyvitamin D_3 mainly in the liver and kidneys (*3*). It exerts its effects *via* activation of its cellular receptor (vitamin D receptor), which in turn alters the transcription rates of target genes responsible for various biological responses (*4*). Vitamin D receptors are present in various human tissues like skin, colon, brain, monocytes, macrophages, and UF tissues of human uterus throughout the menstrual cycle. The active vitamin D has the ability to inhibit growth and promote differentiation of a variety of cell types (*5*). Besides, studies have shown that vitamin D is an antifibrotic factor that inhibits the growth of human fibroid cells in a dose-dependent fashion by reducing many of immediate effects of transforming growth factor beta 3 (TGF-β3) significantly. It has also been demonstrated to shrink UF legions in Eker rats and authentic preclinical animal model for UFs (*4*). Besides vitamin D has good safety profile, and only chronic overdosage in the presence of markedly impaired renal function may lead to toxicity (*6*).

Presently, there is paucity of Indian data on the association between vitamin D insufficiency and the development of UFs. Keeping this in view, this study was conducted to compare serum 25-hydroxyvitamin D_3 levels in women with and without UFs. It was also aimed to evaluate the association between serum

25-hydroxyvitamin D_3 levels and total number, volume and location of UFs.

2. Materials and Methods

2.1. Design

The cross-control study was conducted in the Department of Obstetrics and Gynaecology in a tertiary care centre in the northern India for a period of 1 year from 2014 to 2015 in collaboration with the Department of Biochemistry. Clearance was taken from ethical committee of the institution prior to starting the study.

2.2. Study population

Premenopausal women 18-45 years of age who attended gynaecological outpatient department (GOPD) with different complaints for the period of 1 year and matched for age and BMI were recruited to the study. Those patients specially coming with complaints of abnormal uterine bleeding and infertility were approached to participate. There were a few patients who were diagnosed with UFs elsewhere and had attended GOPD with ultrasound scan report were too included. All of them were posted for an ultrasound scan. Those patients with at least one fibroid lesion with a mean volume of ≥ 2 cm^3 were taken as cases. The rest of the patients who had normal uterine morphology on ultrasonography were approached to participate as controls. Following women were excluded from our study: current pregnancy or pregnancy within the last 6 months, currently lactating or lactating within the last 6 months, history of abortion within 6 months prior to start of study, history of myomectomy, women currently using vitamin D supplements or hormonal treatment (including oral contraceptives) or H/O use within the last 6 months.

2.3. Study procedure

Blood samples from patients consenting to participate as cases and controls were taken for measuring 25-hydroxyvitamin D_3 levels. Written informed consent was taken from all the selected women in a language understood by them. A detailed history regarding their diet and history of calcium intake was taken. The selected women were specifically asked for hours of exposure to sunlight between 10:00 AM and 3:00 PM. A thorough general and gynaecological examination was carried out. Age and BMI (Body Mass Index) were matched for all cases and controls. Ultrasonography was performed through both transabdominal and transvaginal routes on all consenting subjects.

The ultrasonography assessment was performed by the same ultrasonologist using ultrasound machine fitted with endovaginal probe 4-8 MHz for transvaginal sonography and 2-5 MHz convex probe for transabdominal sonography. The number of fibroid lesions, volume, and location of all fibroid lesions were accurately noted. The volume of each fibroid was determined according to the ellipsoid formula ($a \times b \times c \times 0.523$), where a is the height, b is the width, and c is the depth (in cm). The total volume of fibroid in a patient was calculated by adding the volumes of all lesions detected. The location of each fibroid lesion within the uterus was classified as submucosal, intramural, or subserosal. A myoma with > 50% of its diameter bulging out of the uterine contour line was defined as subserous. Intramural fibroids were those mostly within the uterine shape. Myomas distorting the cavity line were defined as submucosal.

The estimation of serum 25-hydroxy vitamin D levels in both the study and control groups was carried out at Biochemistry Department of the same hospital by ELISA technique. The intra-assay coefficient of variation was based on 40 measurements for each serum, and the interassay coefficient of variation on the four measurements was performed in six different test runs. Vitamin D_3 status of the study and control population was categorized into 3 groups: deficient (levels < 10 ng/mL), insufficient (levels between 10 ng/mL and 19.9 ng/mL), and sufficient (≥ 20 ng/mL).

2.4. Statistical analysis

The association between 25-hydroxy vitamin D levels and the presence of fibroid and total fibroid volume (TFV) was analysed using means, frequencies, standard deviations, and percentages. The independent t test was used to compare serum 25-hydroxy vitamin D levels across groups. Correlations were assessed by Spearmen's rank correlation test. p value of < 0.05 was considered significant.

2.5. Sample size calculation

Paffoni A. *et al.* (*7*) found in controls a mean serum concentration of 25-hydroxyvitamin D_3 to be 20.8 $\pm$ 11.1 ng/mL. Presuming a 20% difference in 25-hydroxyvitamin D_3 level as clinically significant at 5% type 1 error with 80% power and assuming 10 % drop out rate when taking controls twice as cases, sample size was estimated to be 95 cases and 190 controls.

3. Results

3.1. Recruitment of participants

A total of 392 women with complaints of abnormal uterine bleeding or coming with ultrasound diagnosis of UF elsewhere were recruited and posted for ultrasonography. Uterine fibroid was diagnosed in 166

patients, and other uterine/ovarian pathology was found in 7 patients. A total of 123 patients with UF consented to participate in the study. In total, 102 patients were ultimately recruited who fulfilled eligibility criterion. A total of 219 patients who had normal uterine morphology on ultrasonography were approached to participate as controls. Ultimately, 208 patients were found to be eligible to participate in the study as controls. Recruitment of patients in study and control groups is shown in Figure 1.

3.2. Serum vitamin D level

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 study group (54.90%) as compared to healthy controls (6.7%) while

sufficiency was more common among controls (67.8% *vs.* 27.45%), the difference being statistically significant ($p < 0.05$).

3.3. Association between serum vitamin D level and uterine fibroid

Women with deficient vitamin D levels have an odds of 18.36 for the development of UFs (Table 1). There was an association found between multiple fibroids with deficient vitamin D levels. The majority of women with two (18/27, 66.67%) and three fibroid lesions (6/7, 85.71%) were vitamin D deficient. However, no statistically significant association was observed between vitamin D levels and number of UFs ($p = 0.105$). The most common fibroid lesion found in our study was intramural (60/102, 58.82%) followed by subserosal (42/102, 41.17%). No association was found between vitamin D levels and location of the fibroid lesion ($p = 0.760$). The total fibroid volume (TFV) in the study group ranged from a minimum of 2 cm^3 to a maximum of 248.6 cm^3. Among the 56 women with deficient vitamin D levels (< 10 ng/mL), 44 women (44/56, 78.57%) had TFV between 50 cm^3 and 100 cm^3, and 12 women (12/56, 21.42%) had TFV above 100 cm^3. Among the women with insufficient levels, the majority (14/18, 77.8%) had TFV between 10 cm^3 and 50 cm^3, whereas among women with sufficient vitamin D$_3$ levels, most (13/28, 46.42%) had TFV of < 10 cm^3. There was a significant inverse correlation between serum vitamin D levels and total volume of UFs ($p < 0.001$) (Table 2).

3.4. Secondary analysis of demographic factors

Demographic factors of recruited women as cases and controls are listed in Table 3. The age and BMI of the women were matched among cases and controls. The mean age of women in cases and controls were 34.50 ± 5.38 and 34.45 ± 5.37, respectively. The control group had a higher proportion of women who were housewives (90.9%) and belonging to lower socioeconomic status (86.53%) associating lower odds of UFs with these demographic variables. Parity was also found to offer protection for development of UFs with higher proportion (85.1%) of multiparous women in control group.
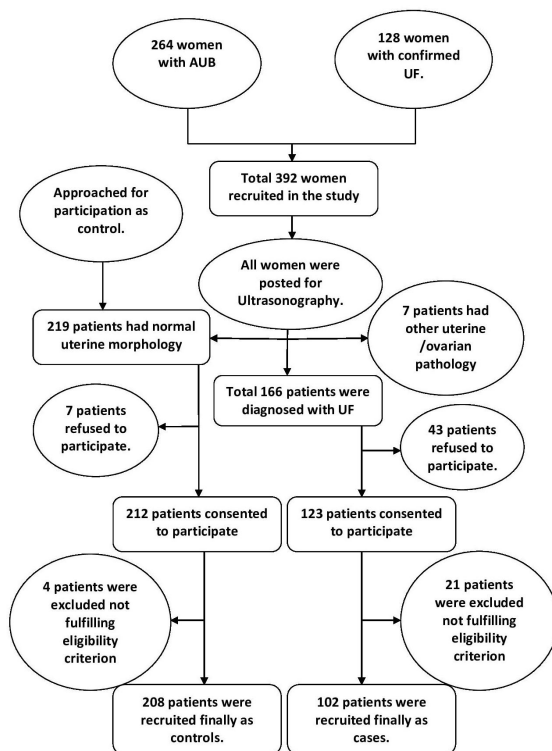

Figure 1. Flowchart showing recruitment of patients in study and control group. AUB: Abnormal uterine bleeding, UF: Uterine fibroid.

Table 1. Distribution of women among cases and controls according to serum vitamin D levels

	Study, n (%). total: 102	Control, n (%), total: 208	OR	p value
Vitamin D status				
Deficiency	56 (54.90)	14 (6.7)	18.36	< 0.05[a]
Insufficient	18 (17.64)	53 (25.5)	1.49	
Sufficient	28 (27.45)	141 (67.8)	1	
Mean vitamin D ± SD (ng/mL)	14.52 ± 7.89	26.6 ± 14.36		< 0.05[b]

[a]Chi-square test. [b]Independent *t* test.

Table 2. Distribution of women based on number, location and volume of fibroid lesions among cases with relation to vitamin D status

	Number of uterine fibroids			
Vitamin D status	1, *n*	2, *n*	3, *n*	*p* value
Deficiency (*n* = 56)	32	18	6	0.105[a]
Insufficient (*n* = 18)	13	5	0	
Sufficient (*n* = 28)	23	4	1	
Total	68	27	7	

	Location of fibroid lesion			
Vitamin D status	Intramural (*n*)	Submucosal (*n*)	Subserosal (*n*)	*p* value
Deficiency (*n* = 56)	33	0	23	0.760[a]
Insufficient (*n* = 18)	11	0	7	
Sufficient (*n* = 28)	16	0	12	
Total	60		42	

	Volume of uterine fibroids						
Vitamin D status	< 10	10-50	50-100	100-150	150-200	200-250	*p* value
Deficient (*n* = 56)	0	0	44	3	4	5	0.000[a]
Insufficient (*n* = 18)	2	14	1	1	0	0	
Sufficient (*n* = 28)	13	12	3	0	0	0	

[a]Chi-square test.

Table 3. Demographic characteristics of study population

Parameters	Cases (total 102)	Controls (total 208)	*p* value
Age (Mean age ± SD)	34.50 ± 5.38	34.45 ± 5.37	0.997[b]
BMI (Mean ± SD)	24.95 ± 3.01	24.36 ± 3.01	0.11[b]
Education illiterate, *n* (%)	33 (32.35)	80 (38.46)	0.294[a]
Occupation			0.002[a]
House wife, *n* (%)	80 (78.4)	189 (90.9)	
Working, *n* (%)	22 (21.6)	19 (9.1)	
Parity			0.041[a]
0, *n* (%)	5 (4.9)	4 (1.9)	
1, *n* (%)	22 (21.6)	27 (13)	
≥ 2, *n* (%)	75 (73.5)	177 (85.1)	
SEC Status (PCMI)*			0.000[a]
< 938 (Lower), *n* (%)	63 (61.8)	180 (86.53)	
938-6253 (Middle), *n* (%)	39 (38.2)	28 (13.5)	
≥ 6254 (Upper), *n* (%)	0	0	
Sun exposure (hours/day)			0.000[a]
Low (< 1 hr), *n* (%)	44 (43.13)	19 (9.13)	
Medium (1 hr), *n* (%)	25 (24.50)	56 (26.92)	
High (> 1 hr), *n* (%)	33 (32.35)	133 (64)	

*Modified BG prasad scale, 2017 (*16*). SEC Status (PCMI): Socioeconomic status (Per Capita Monthly Income). [a]Chi-square test. [b]Independent *t* test.

3.5. Sun exposure and uterine fibroid

Of the women in control group, 64% had more than 1-hour sun exposure/day in contrast to 32.35% in cases, the difference being statistically different ($p < 0.05$). The cases with sun exposure less than 1 hour/day have an odds of 9.08 (95% CI 4.53-18.21) for developing UF, while the risk reduces to 1.43 (95% CI 0.744-2.77) when exposure is 1 hour (Table 4).

4. Discussion

The present cross-sectional study showed that vitamin D levels were significantly low in patients with UF when compared with those having normal uterine morphology similar to other studies (*7-10*). The total volume of fibroids had a significant inverse correlation with vitamin D levels identical to a recent study (*9*). There was a positive correlation of the number with serum vitamin D levels similar to most other studies (*9,10*) with no statistically significant difference. Our study observed that sun exposure ≥ 1 hour/day (weather permitting) provided protection against UFs. This was in tune with two other studies (*7,10*).

Oskovi Kaplan *et al.* (*10*) reported no relation of fibroid volume with vitamin D level. However, they found

Table 4. Univariate and multivariate analysis of factors associated with development of uterine fibroids

	OR	*p* value	Adjusted OR	*p* value
Parity				
0	2.95	0.114	2.008	0.399
1	1.92	0.040	2.055	0.05
≥ 2	1		1	
SECS				
Low	0.251	0.000	0.196	0.000
Middle	1		1	
Occupation				
Working	2.736	0.002	1.932	0.101
Housewife	1		1	
Sun exposure				
Low	8.41	0.000	9.085	0.000
Middle	1.58	0.139	1.436	0.281
High	1		1	

very low vitamin D levels in both cases and controls (6.54 ± 4.66 ng/mL *vs.* 8.18 ± 5.16 ng/mL, respectively). The apparent effect of vitamin D on the growth of UFs in the study without a vast margin of difference may have missed a causal relationship between fibroid volume with vitamin D level.

Paffoni *et al.* (*7*) however discovered a relationship between lower vitamin D levels and higher number of leiomyomas (≥ 3) with no statistical significance (*p* = 0.08) but none with volume and location of lesions. Hence, they suggested the role of vitamin D in the development and none on growth. But we observed a significant relation between deficiency and dimension of lesions like Ciavattini *et al.* (*9*). Volume of fibroid (< 50 mL, > 50 mL) and vitamin D level (deficient < 20, sufficient > 20) were assumed as dichotomous variables for regression analysis. Univariate analysis showed that the odds of developing a fibroid with > 50 mL volume is 2.51 times higher in women with deficient vitamin D levels (< 20 ng/mL).

Baird *et al.* (*8*) observed population with medium and high sun exposure have the same reduction in adjusted odds of fibroids relative to low sun exposure (40% reduction in adjusted odds of fibroids relative to low sun exposure). Oskovi Kaplan *et al.* (*10*) reported significantly higher prevalence of severe 25-hydroxy vitamin D deficiency (< 10 ng/mL) in women with covered clothing style (*p* = 0.002). Our findings are in tune with these studies as we observed that the patients with less than 1 hour of sun exposure/day were having an odds of 9.08 of developing UFs after adjusting for parity, socioeconomic and occupation status (Table 4). Under sun light exposure, vitamin D synthesis in the skin takes place in a two-stage process. When the skin is exposed to solar UV-B (ultraviolet type B) radiation, 7-dehydrocholesterol is photolyzed to previtamin D_3. This previtamin D_3 then undergoes isomerization in the skin to vitamin D_3. Latitude, altitude, season, time of the day, ozone amount, cloud amount, aerosol, and reflectivity of the earth's surface are the factors that control the number of UV-B photons that reach the earth's surface (*11*).

There was significant difference between the groups with and without fibroids with respect to occupation, parity, socioeconomic status and duration of sun-exposure. Working women were having an odds of 2.73 (*p* = 0.002) to develop uterine fibroids. However, the odds decreased to 1.93 after adjusting for parity, socioeconomic status and sun exposure (Table 4). It is however pertinent to note that there is significant difference of parity among working women and housewives in our study. 81.8% of working women had low parity (parity 0 or 1) while only 17% women among housewives had parity 0 or 1. The overlap of optimum reproductive age and fulfilment of higher education and career ambitions among women in the past few decades has deferred the events of marriage and conception to late thirties. A systematic review to evaluate the relative strengths of various risk factors in UF epidemiology opined parity as the factor exerting greatest protective effect for the development of UFs (*12*). A monocentric analysis in Japan discovered fivefold reduction in the risk of UFs in women giving births for three or more times when compared to nulliparous women (*13*). The hypothesis for such finding is due to effects of pregnancy hormones along with cessation of menstruation and extensive remodelling in the immediate puerperium which can induce elimination of early as well as large leiomyoma lesions (*12,14*). We however discovered a twofold protective effect of parity on UFs presumably failing to consider the genetic and racial influence on its occurrence. The discovery of an increased incidence of UF in working women in our study is probably explained by the finding of lower parity in high numbers among them.

With only 40% of fibroids exhibiting genomic instability (*15*), a comprehensive analysis of multifactorial influence on UF development and growth is necessitated. However, the cross-sectional design of our study limits the precise understanding of the role of vitamin D in the causation of UFs. More robust prospective cohort studies with monitoring of vitamin D status and serial ultrasound imaging for uterine morphology at predetermined time spaced intervals can shed light on the role of vitamin D in development vs growth of UFs or both. Being a hospital based study, it may not be representative of the true burden of the problem with respect to demographic variables in the community.

Hence, we suggest vitamin D deficiency may have a role in the growth of UFs. Women working indoor for maximum hours of the day may undergo serum vitamin D estimation and subsequently opt for supplementation, which could be simple, non-invasive, and cheap means of preventing the most common benign pathology in women.

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