

A Study to Find Out the Association of Vitamin D Levels with Leiomyoma Uterus

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Abstract

Objective: To find out the the association of vitamin D levels with leiomyoma and to study its role as a risk factor for development of leiomyoma.

Materials & Methods: Total 50 patients, belonging to age group 18-50 years, attending gynaecology OPD of CSS Hospital, were included in this study. Those diagnosed with leiomyoma on ultrasound scan, were labeled as case group. Control group comprised of 50 women whose pelvic ultrasound was normal and there was no fibroid. Volume of each fibroid was measured by transvaginal sonography. The quantitative detection of serum vitamin D levels was performed using chemi-luminescence assay. Statistical analysis was performed using suitable tests of significance.

Results: The mean level of vitamin D in the case group was 12.9 ± 3.09 ng/ml while in the control group was 18.1 ± 4.72 ng/ml ($p = 0.001$). Out of 50 cases, 32 (44%) cases were deficient i.e.vitamin D levels were less than 10 ng/ml, while in controls, 15 (14%) were deficient with p value < 0.001 . In our study 24 cases (48%) were vitamin D insufficient i.e. vitamin D levels between 10-20 ng/ml while 16 (32%) controls were insufficient .

Conclusions: In this study low levels of vitamin D were associated with a higher risk of uterine fibroids. So vitamin D may be a potential protective factor against the development of leiomyoma. Cohort and Interventional studies are however pressingly needed to confirm a causal relationship and to investigate the potential therapeutic benefits of vitamin D supplementation. Vitamin D could be a potential safe, nonsurgical therapy for the treatment of uterine fibroids.

Keywords: *Vitamin D; Leiomyoma uterus; Cell proliferation; Extracellular matrix production; Antifibrotic factor*

Introduction

Uterine fibroid represents a localized proliferation of smooth muscle cells surrounded by a capsule of compressed muscle fibers. Vitamin D is known as the main regulator of calcium homeostasis. Functional effects of vitamin D include reduced cell proliferation, increased apoptosis, enhanced differentiation, and regulation of biological processes including

angiogenesis, extracellular matrix production, and immune response.¹ The pathogenesis of fibroids has been hypothesized to involve a positive feedback loop between extracellular matrix production and cell proliferation and vitamin D might act to block the positive feedback.² Vitamin D is an antifibrotic factor and inhibits growth as it is found to induce apoptosis in cultured human leiomyoma cells through the down-regulation of the genes PCNA, CDK1, and BCL-2 and suppression of catechol-o-methyltransferase expression and activity in human leiomyoma cells.³⁻⁵ Laboratory studies of fibroid tissue in culture that were treated with calcitriol, the active form of vitamin D, demonstrate reduction in cell proliferation and extracellular matrix

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production.⁶ Additionally, recent studies have demonstrated that vitamin D is a potent anti-tumor agent that effectively inhibits human uterine fibroid cells in vitro and shrinks fibroid lesions in preclinical animal studies.⁷ So study of vitamin D levels in women having leiomyoma was planned to establish their association.

Materials & Methods

The study was conducted in the Department of Obstetrics and Gynaecology at Chittaranjan Seva Sadan College of Obstetrics, Gynaecology and Child Health, Kolkata after ethical informed consent was taken from all the recruited patients.

Case group comprised of 50 women belonging to age group 18-50 years who were diagnosed with leiomyoma of more than 10 mm at transvaginal ultrasound with mean clearance from the Institutional Ethical Committee.

Control group comprised of 50 women belonging to age group 18-50 years whose pelvic ultrasound was normal and there was no fibroid.

Following patients were excluded from the study :

- Pregnant women, menopausal women, women on hormonal treatment (including oral contraceptive) during the last 3 months.
- Vitamin D supplementation in last 6 months.
- Patients currently lactating or lactating in last 6 months.
- Patients having history of prior myomectomy.
- Women diagnosed with Adenomyosis and other causes of abnormal uterine bleeding.
- Patients reporting chronic medical problems.
- Patients having malignancy, multiple sclerosis, autoimmune disorders.
- Women having leiomyoma less than 10mm on transvaginal sonography (TVS).

Detailed history of clothing, outdoor activity, and fish intake was taken followed by basic investigations like haemogram, blood sugar, LFT, KFT and routine urine examination were done. Three perpendicular diameters were measured for each myoma in cases, and

the volume of each of them was recorded on transvaginal sonography (TVS). Then the total fibroid volume was recorded by adding all the individual fibroids volume. All the selected women provided a venous blood sample for the quantitative detection of vitamin D levels. Serum concentration of vitamin D was measured. The quantitative detection of vitamin D levels was performed using a commercially available kit based on a chemiluminescence technology. Women were grouped into three different groups, according to World Health Organization recommendations: 25-hydroxyvitamin D3 deficiency (<10 ng/mL), insufficiency (10-19.9 ng/mL), and sufficiency (>20 ng/mL). Statistical analysis was done using suitable tests of significance.

Results

The mean age of the 50 cases enrolled in the study was 35.79+7.97 years while mean age of controls was 37.60+6.78 years. The mean age of menarche in cases was 13.26+1.06 years and in controls was 13.12+1.08 (p=0.820)

The median parity of our cases and controls was 2 and hence there was no difference in the parity of both groups. Family history of fibroid was positive in 5.7% of cases and 4.3% of controls (p = 0.719). The mean BMI in cases was 25.2+4.38 while in controls it was 23.08+12.6 (p 0.145). There was no statistical difference between the incidence of confounding high risk factor of age, parity, age at menarche, family history of fibroids and BMI in cases and controls.

In 50 cases of leiomyoma and in 50 controls the values of serum vitamin D were estimated. The mean level of vitamin D in cases was 12.58±4.09 ng/ml while in controls it was 18.99±5.72 ng/ml. The levels of vitamin D were significantly less in cases than in controls (p < 0.001). Thus there was statistically significant difference in cases and controls.

On using the WHO criterion, out of our 50 cases 22 (44%) were deficient i.e. vitamin D levels less than 10ng/ml while in controls they were 7 (11%) with p value <0.001 and odds ratio of 13.5 (95% CI). Thus, the risk of having a fibroid in vitamin D deficient individuals was 13.5 times more than individuals having Vitamin D sufficient levels.

In our study 24 cases (48%) were vitamin D insufficient i.e. vitamin D levels between 10-20 ng/ml while 16 (32%) controls were insufficient with p value 0.001 and OR 13.5 (95%CI). Thus, the risk of having a fibroid in vitamin insufficient individuals was 13.5 times more than individuals having sufficient vitamin D levels.

Table 1. Distribution of Vitamin D Levels in Case and Control Group

Vit D levels (ng/ml)	Cases		Controls		P Value
	Frequency	%	Frequency	%	
5 – 10	22	44%	7	14%	<0.001
10 - 15	17	34%	1	2%	
15 – 20	7	14%	15	30%	
20 – 25	3	6%	23	46%	
25 – 30	1	2%	4	8%	
Total	50	100%	50	100%	

Table 2. Distribution of vitamin D status in case and control groups according to WHO criterion

WHO classification	Cases (n=50)		Controls (n=50)		P value	OR
	Frequency	%	Frequency	%		
Deficient	22	44%	7	14%	<0.001	13.5
Insufficient	24	48%	16	32%		
Sufficient	4	8%	27	54%		

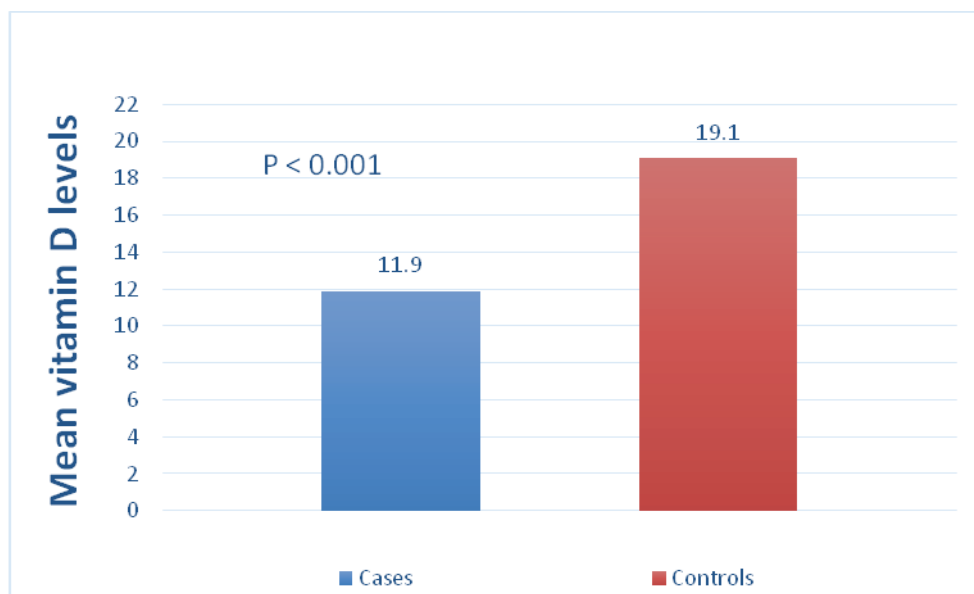


Figure 1. Mean vitamin D level (ng/ml)

Discussion

Vitamin D has emerged to be an important regulator of uterine leiomyoma development. The mean value of vitamin D levels in cases was 12.58 ± 4.09 ng/ml while in controls it was 18.99 ± 5.72 ng/ml with p value < 0.001 . Thus, it was statistically significantly lower in cases than in controls. Vitamin D levels were divided according to WHO i.e, vitamin D deficient (< 10 ng/ml), vitamin D insufficient ($10-20$ ng/ml) and vitamin D sufficient (> 20 ng/ml). It was observed that 44% of our cases were vitamin D deficient, while 48% of cases were vitamin D insufficient. However, 54% of our controls were vitamin D sufficient, 32% of controls were vitamin D insufficient and only 14% of controls were vitamin D deficient.

Hence, it was observed in our study that vitamin D deficiency was more common in the case group and it was statistically significant. Thus, vitamin D deficiency is a risk factor for development of leiomyoma.

In vitamin deficient cases odds ratio was 13.5 (95% CI). Thus the risk of having a fibroid in vitamin deficient individuals was 13.5 times more than in individuals having sufficient vitamin D levels.

Bläuer et al, were the first few to determine the effect of $1,25(\text{OH})_2\text{D}_3$ and $25(\text{OH})\text{D}_3$ vitamin D derivatives on the growth of leiomyoma and myometrial cells in vitro in 2009 and observed that their growth in vitro was effectively inhibited by $1,25(\text{OH})_2\text{D}_3$ hence concluded that vitamin D may play a role in the growth of uterine leiomyomas.⁸

Our findings were similar to a study by Alessio Paffoni in 2013 who conducted a case-control study of women referring to two infertility units in Italy, in which all the 128 cases of fibroid had mean value of vitamin D levels of 18 ± 7.7 ng/ml and 256 controls had mean value of vitamin D levels of 20.8 ± 11.1 ng/ml with p value of 0.010 which was statistically significant.⁹

Similar study was done by Mohamed Sabry in 2013 in which the mean level of serum vitamin D, was found to be significantly lower in 104 cases with leiomyoma of value 19.7 ± 11.8 ng/mL than in healthy 50 controls of value 22.3 ± 6.5 ng/mL with p value of 0.01 which was statistically significant.¹⁰ A total of 154 premenopausal women were recruited for this cross sectional study. The control group comprised 50 subjects with a normal,

fibroid-free uterine structure, confirmed by transvaginal ultrasonography. The 104 case subjects had at least one fibroid lesion that was 2 cm^3 in volume or larger, confirmed by transvaginal ultrasonography. For each case subject, total uterine volume and total volume of all existing fibroids were measured in three perpendicular planes, with volume determined according to the prolate ellipse formula ($a \times b \times c \times 0.523$), where a is height, b is width, and c is depth. Serum Vitamin D levels were measured by radioimmunoassay. The independent t-test was used to compare serum Vitamin D levels across groups. Correlations were assessed by Spearman's rank correlation test. Lower serum 25-(OH) Vit D levels were significantly associated with the occurrence of fibroid ($P = 0.01$). A statistically significant inverse correlation was also observed between serum Vitamin D levels and total uterine fibroids volume ($r = -0.31$; $P = 0.002$) within the case cohort. Subjects with larger fibroid volumes had lower serum Vit D levels and vice versa. Thus, vitamin D deficiency was observed to be a possible risk factor for the occurrence of fibroid.¹⁰

Micha Ciebierain 2016, enrolled 188 women, including patients admitted for uterine fibroid surgery ($n=105$) as the study group and healthy women of similar age ($n=83$) as controls. Comparative analysis demonstrated that the mean vitamin D serum concentration was statistically significantly different in both groups (Student's t-test with the grouping variable $t=3.15$, $df=186$; $P=0.0019$). The mean vitamin D serum concentration in patients with fibroid was 21.91 ± 8.87 ng/mL, which was statistically significantly lower than the mean vitamin D serum concentration in the control group. In the latter group, the mean concentration of vitamin D was 26.68 ± 11.89 ng/mL. An increase in serum vitamin D concentration by one unit decreases the risk for fibroid by 1.04 (OR 0.96; 95% CI, 0.93-0.99).¹¹

Oskovi Kaplan in 2017 enrolled sixty-eight women with at least one uterine leiomyoma of 10mm and 56 healthy controls. Serum vitamin D levels were measured by electro-chemiluminescence immunoassay. The groups were similar in terms of age, BMI, gravidity and parity numbers. In his study documented the mean level of vitamin D were 7.28 ± 4.94 ng/ml and 78% of patients ($n=97$) had severe vitamin D deficiency (< 10 ng/mL). The mean level of vitamin D in cases were 6.54 ± 4.66 ng/ml and in controls were 8.18 ± 5.16 ng/ml.

It was significantly lower in the study group compared to control group (p 0.009).¹²

These studies were in agreement with our results that vitamin D deficiency is a common finding in patients with fibroid and vitamin D deficiency has emerged as one of the major risk factors.

Conclusion

Low levels of vitamin D were associated with a higher risk of uterine fibroids in our study. Hence, vitamin D may be a potential protective factor against the development of leiomyoma. Vitamin D is an emerging regulator of uterine leiomyoma development. Cohort and RCT studies are however needed to confirm a causal relationship and to investigate the potential therapeutic benefits of vitamin D supplementation. Vitamin D could be a potential safe, nonsurgical therapy for the treatment of uterine fibroids. Because fibroids constitute a considerable burden on health budgets of all countries, the solution for the future may be to identify high-risk patients before they develop leiomyoma.

Vitamin D could become a new generation drug that is cheap, safe, and with additional effects in prevention and treatment of leiomyoma.

Conflict of Interest: There was no conflict of interest. Ethics approval and consent to participate was taken from all the patients recruited in this study. This study was approved by Institutional Ethical Committee.

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