

How to Cite:

Pradhan, S., Dash, S., Nanda, S. S., Pradhan, S., & Kar, T. (2022). Prospective study on association of 25-hydroxy vitamin D3 and uterine fibroid. *International Journal of Health Sciences*, 6(S4), 5219–5230. <https://doi.org/10.53730/ijhs.v6nS4.10066>

Prospective study on association of 25-hydroxy vitamin D3 and uterine fibroid

Shobhana Pradhan

Senior Resident, Dept. Of O&G, PRM Medical College, Baripada, Odisha, India ,
(Ex PG , SCB Medical College, Cuttack)

Subhalaxmi Dash

Asst. Professor, Dept. Of O&G, MKCG Medical College, Berhampur, Odisha, India

Sudhanshu Sekhara Nanda

Asst. Professor, Dept of O&G, PRM Medical College, Baripada, Odisha, India
Corresponding author email: sudhansu81@gmail.com

Subrat Pradhan

Senior Resident, Dept. of Biochemistry, SCB Medical College, Cuttack, Odisha, India

Tushar Kar

Professor & HOD, Dept of O&G, SCB Medical College, Cuttack, Odisha, India

Abstract---Objective: To find the association of 25-hydroxy Vitamin D3 and uterine fibroid. Study Design: This is a prospective case control study. Setting: The study was conducted at the PG Department of Obstetrics and Gynaecology , S.C.B. Medical College, Cuttack, Odisha, India. Material & Method: One hundred diagnosed cases of uterine fibroid were included as cases. The control group comprised of age matched hundred healthy volunteers. After obtaining the written consent of cases and controls, they were subjected for routine haematological tests and for Serum total 25-hydroxy vitamin D by chemiluminescent immunoassay (CLIA) method. Ultrasound evaluations were performed by TVS and TAS as per on all consenting subjects. All the data collected were entered into the excel spread sheet and the statistical analysis was carried out by using the graph pad prism 8.0. The quantitative data were expressed as mean \pm SD. P value < 0.05 was considered to be statistically significant and P value < 0.01 was considered to be highly significant. Conclusion: The mean vitamin D level in cases was 8.6 ± 2.6 ng/ml while in controls it was 19.23 ± 4.3 ng/ml. The levels of vitamin D were significantly less in fibroid patients than in healthy controls ($p < 0.001$). Vitamin D levels was significantly lower in patients having two and three fibroids in

comparison to those with single fibroid with p value=0.003 and p value= 0.001 respectively. Patients with lower Vitamin D levels were observed to have larger volume of fibroids.

Keywords---uterine fibroid, vitamin D3, patients.

Inroduction

Uterine fibroids are the most common benign tumors of women of reproductive age. Many fibroid patients suffer menorrhagia, dysmenorrhea, dyspareunia, pressure-related symptoms, miscarriage, and subfertility. There are a range of current management options: hysterectomy, myomectomy, uterine artery embolization, MRI-guided focused ultrasound thermal therapy, and in mild cases, reassurance and observation[1]. Currently, novel noninvasive treatment options for Uterine fibroids – such as localized gene therapy, oral green tea extracts, and selective progesterone receptor modulators – are being explored. A safe and effective oral treatment option for Uterine fibroids would be a major advance in the field and it would have an immense impact on women's health worldwide[2]. Vitamin D (Vit D) is a prohormone produced in the skin via a sunlight-initiated reaction and metabolically converted to the active metabolite 1,25-dihydroxyvitamin D3 [1,25(OH)2D3], mainly in the liver and kidneys. Vit D exerts its effects via activation of its cellular receptor (vitamin D receptor [VDR]), which in turn alters the transcription rates of target genes responsible for various biological responses[3]. Diverse functions for Vit D have been confirmed by the presence of VDR in a wide range of human tissues, including skin, colon, brain, pancreas, and breast, as well as activated T and B lymphocytes, monocytes, and macrophages[4]. VDR expression have recently been demonstrated in both the myometrium and the endometrium of the human uterus, throughout the menstrual cycle, in addition to uterine fibroid tissue[5]. The ability of 1,25(OH)2D3 to inhibit growth and promote differentiation of a variety of cell types has suggested diverse functions in preventing cancers, modulating the immune system, and controlling various endocrine systems. This effect is mediated predominantly through a G1/S (gap 1/synthesis) phase block of the cell cycle. The 1,25(OH)2D3 regulates many of the cell cycle regulatory genes and modulates activities of cyclin-dependent kinases, leading to a decreased number of cells in the S phase and an accumulation of cells in the G0–G1 phase[6].

Various researches have recently shown that Vit D inhibits extracellular signal-regulated kinase activation and downregulates the expression of anti-apoptotic (BCL-2, BCL-w), cell cycle-regulating (cyclin-dependent kinase 1), and cell-proliferating (proliferating cell nuclear antigen) genes in human fibroid cells[7]. Vit D is an antifibrotic factor that inhibits the growth of human fibroid cells in a dose-dependent fashion by significantly reducing many of the transforming growth factor beta 3 (TGF-β3)–mediated effects such as the TGF-β3 induction of fibronectin and collagen type 1 protein expression, the induction of protein expression of plasminogen activator inhibitor-1, and the phosphorylation of Smad2 as well as nuclear translocation of Smad2 and Smad3.[8] Studies have recently demonstrated the ability of Vit D to safely shrink Uterine fibroid lesions in Eker rats, an authentic orthotopic animal model . Taken together, these

findings suggest that Vit D may be a potentially useful therapeutic agent for the nonsurgical management of Uterine fibroids[9]. Hypovitaminosis including vitamin D deficiency is very common in Indian women. However, there is no such study in Indian population to find out this potential factor as aetiology in the development of uterine fibroid. Hence, we took up this study to assess serum levels of vitamin D3 in women with fibroids in our population taking healthy women as control.

Vitamin D (Calcitriol)

The Endocrine Society recommends measuring the serum circulating 25(OH) Vit D level, to evaluate vitamin D status in patients. Serum Vitamin D levels is defined as

Normal: >30 ng/ml ,	Insufficiency: 21-29 ng/ml
Deficiency: <20 ng/ml ,	Severe deficiency: < 10 ng/ml

The vitamin D receptor and mechanism of action

The active form of vitamin D binds to intracellular receptors that then function as transcription factors to modulate gene expression. Like the receptors for other steroid hormones and thyroid hormones, the vitamin D receptor has hormone-binding and DNA-binding domains. The vitamin D receptor forms a complex with another intracellular receptor, the retinoid-X receptor, and that heterodimer is what binds to DNA. In most cases studied, the effect is to activate transcription, but situations are also known in which vitamin D suppresses transcription. The vitamin D receptor binds several forms of cholecalciferol. Its affinity for 1,25-dihydroxycholecalciferol is roughly 1000 times that for 25 hydroxycholecalciferol, which explains their relative biological potencies.

Vitamin D3 and sex steroid receptor

1,25-Dihydroxyvitamin D3 [1,25(OH)2D3] is a member of the steroid hormone family and serves as the major regulator of calcium and phosphate homeostasis in the body system. 1,25(OH)2D3 can induce growth arrest, differentiation, and apoptosis in a wide variety of cancer cells.[10] Halder SK et al have demonstrated that 1,25(OH)2D3 or its noncalcemic analog, paricalcitol, inhibits fibroid tumor growth in vivo and can inhibit proliferation of human Uterine fibroid cells in vitro. 1,25(OH)2D3 can inhibit the expression and activities of matrix metalloproteinases and reduce the expression of extracellular matrix proteins in cultured Uterine fibroid cells. UFs with missense mutations in the Med12 gene also showed an overexpression of IGF-2 as compared with UFs that have no mutations, indicating the functional role of these mutations in fibroid pathogenesis.[11] Mittal P et al had demonstrated that the conditional expression of a common Med12 somatic variant in the uterus promotes UF formation and genomic instability in a murine model.[12]

The Mediator is a large complex of 30 subunits that regulate eukaryotic transcription and thereby controls organismal development and homeostasis. The Mediator is conserved in all eukaryotic organisms and is required for the transcription of almost all genes[13]. The Mediator interacts directly with a

numbers of transcription factors to facilitate RNA polymerase II recruitment to target genes[14]. Med12 has been linked to general functions of the complex and to specific interactions with transcription factors. Med12 is a subunit of the Cdk8 kinase module that can function as a transducer of Wnt/ β -catenin signalling[15]. Zhou H et al using a gene knockdown approach, showed that Med12 is essential for early mouse embryogenesis and for canonical Wnt and Wnt/PCP signalling pathways[16].

β -catenin physically and functionally targets the Med12 subunit to activate transcription and that the Med12 gene is essential for the transactivation of Wnt/ β -catenin signalling. Med12 is functionally linked to the modulation of hedgehog signalling. Moreover, Med12 can regulate TGF receptor signalling and estrogen receptor- signalling in human breast cancer cells[17].

Kim S et al demonstrated that Med12 expression is up-regulated in pancreatic cancer, and silencing Med12 by knockdown inhibits the cell-cycle progression in pancreatic cancer cells[18]. Al Hedy et al in a study in Georgia found that vitamin D3 has the potential to reduce the expression of β -catenin in HuLM cells. β -catenin is localized in both the cytosol and nucleus of HuLM cells, whereas vitamin D3 treatment considerably reduced its expression at physiological concentrations, suggesting that vitamin D3 possess the ability to reduce β -catenin expression in Uterine fibroid cells[19]. Vitamin D3 can reduce Wnt4 in a concentration-dependent manner in HuLM cells, which indicates the inhibitory role of vitamin D3 on the activation of Wnt4/ β -catenin signalling and that the inhibitory function of vitamin D3 may ultimately reduce the pathogenesis of human Uterine fibroids[19].

Hypovitaminosis and DNA damage

Studies have identified somatic mutations in the transcriptional mediator complex subunit 12 (MED12) as a dominant driver of Uterine fibroids accounting for ~85% of tumors[20,21]. Notably, MED12-mutant Uterine fibroids, but not the adjacent myometrium, have been characterized by significant chromosomal loss and rearrangement, suggesting genomic instability as a driving force in tumor progression[22]. Although the cause of these specific fibroid-causing mutations remains unknown, many tissues with defects in DNA repair, indicative of a compromised DNA damage response (DDR), are more susceptible to somatic tumor-forming mutations[23]. Uterine fibroids express reduced levels of vitamin D receptor when compared with adjacent myometrium; therefore, the loss of vitamin D functions, due to reduced levels of serum vitamin D3 and/or reduced expression of VDR, might be a pivotal step for the development of uterine fibroids[24]. Sabry M et al was the first to demonstrate an association between lower serum vitamin D levels and an increased risk of uterine leiomyoma in 2013 in a cohort of black and white females from North Africa[25].

Objective

The aim of the study were

- 1) To find the association of 25-hydroxy VitaminD3 and uterine fibroid.

- 2) To detect the relationship between level of 25-hydroxy VitaminD3 and number of fibroids.
- 3) To detect the relationship between level of 25-hydroxy VitaminD3 and volume of fibroids.
- 4) To detect the relationship between level of 25-hydroxy VitaminD3 and type of fibroids.
- 5) To detect the relationship between level of 25-hydroxy VitaminD3 and site of fibroids.

Materials

This was a Prospective Case control study. Hundred diagnosed cases of uterine fibroid attending the Department of Obstetrics and Gynaecology, S.C.B. Medical College, Cuttack, Odisha, were included. The control group comprised of age matched hundred healthy volunteers.

Inclusion criteria

1. Women between 18 and 50 years of age with uterine fibroid size $\geq 2\text{cm}^3$ (through TVS or TAS)

Exclusion criteria

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

Method

After obtaining the written consent of patients and controls, 5 ml of venous blood was collected, of which 2 ml was kept in EDTA vacutainer for routine haematological tests and 2 ml in plain vacutainer for biochemical and immunological tests.

Ultrasonography

Ultrasound evaluations were performed by TVU on all consenting subjects, while transabdominal ultrasonography was performed as needed for some subjects. One physician (a certified sonologist) who was not aware of the study objectives or group assignments performed all of the ultrasound scans with the following parameters

1. Uterine size, as measured in three perpendicular planes
2. Number of fibroids.
3. Volume of all fibroid lesions, 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.
4. Position/location of each fibroid lesion within the uterus (uterine fundus, lower uterine segment, cervix, extrauterine) charted on a standardized anatomical sketch of the uterus and numbered to ease identification of each fibroid lesion over the course of the study.
5. Unusual characteristics (echogenicity, presence of calcifications, presence of central necrosis, etc.) of each fibroid lesion.

Serum total 25-hydroxy vitamin D3 was measured by chemiluminescent immunoassay (CLIA) method.

Results

Comparison of vitamin D among study groups

Table 1 compares the serum Vitamin D levels in study population. p value <0.05 was significant. Serum Vitamin D was measured in 100 fibroid patients and 100 healthy controls. Their levels were analysed as per Vitamin D deficiency classification by independent t-test. The mean vitamin D level in cases was 8.6 ± 2.6 ng/ml while in controls it was 19.23 ± 4.3 ng/ml. The levels of vitamin D were significantly less in fibroid patients than in healthy controls ($p < 0.001$). According to WHO criterion, majority of the fibroid patients (70%) were severely Vitamin D deficient (<10 ng/ml) in comparison to 4% in controls and 25% of cases and 58% of controls were Vitamin D deficient (10- 20 ng/ml) with p value <0.001 and OR=11.65. The risk of having a fibroid in vitamin D deficient woman was 11.65 times more than individual normal vitamin D levels.

Comparison of vitamin D with number of fibroids

Table 2 compares the serum Vitamin D level with number of fibroids in cases. Group 1 comprises patients with single fibroid, group 2 with 2 fibroids and group 3 with ≥ 3 fibroids. p value <0.05 was considered significant. Majority of the patients (49%) had single fibroid, 24 % had multiple fibroids. Patients having single fibroid had mean Vitamin D level of 10.35 ± 2.9 ng/ml. Vitamin D level in patients having two number of fibroids was 8.37 ± 2.3 ng/ml and 7.18 ± 2.0 ng/ml in patients having three number of fibroids. Vitamin D levels was significantly lower in patients having two and three fibroids in comparison to those with single fibroid with $p = 0.003$ and $p = 0.001$ respectively.

Comparison of serum vitamin D with volume of fibroids

Figure 1 demonstrates the correlation between serum Vitamin D and volume of fibroids in cases. p value <0.05 was considered significant. A statistically significant inverse correlation ($r = -0.78$; $p < 0.001$) was observed between serum 25-(OH) Vit D levels and total volume of fibroids within the case cohort. Patients with lower Vitamin D levels were observed to have larger volume of fibroids and vice versa.

Comparison of serum vitamin D with site of fibroids

Table 3 compares the distribution and serum Vitamin D levels with site of fibroids in cases. p value <0.05 was considered significant. Majority of the fibroids (62%) in our study was located in the posterior surface of uterus followed by anterior and fundus of the uterus. Few fibroids were located in cervix. The distribution of Vitamin D levels in patients when analysed according to the site of fibroid was not significant (p = 0.63).

Comparison of serum vitamin D with type of fibroids

Table 4 compares the distribution and serum Vitamin D levels with types of fibroid in cases. p value <0.05 was considered significant. Majority of the fibroids in our study were intramural followed by submucosal and subserosal. Few fibroids were located in cervix. The distribution of Vitamin D levels in patients when analysed according to the site of fibroid was not significant (p = 0.48)

Discussion

Uterine fibroid represents a localized proliferation of smooth muscle cells surrounded by a capsule of compressed muscle fibers. Although the initiator or initiators of fibroids are unknown, several predisposing factors have been identified, including age (late reproductive years), African-American ethnicity, nulliparity and obesity. Nonrandom cytogenetic abnormalities have been found in about 40% of tumors. Estrogen and progesterone are recognized as promoters of tumor growth. Growth factors with mitogenic activity, such as transforming growth factor- β , basic fibroblast growth factor, epidermal growth factor and insulin-like growth factor-I are elevated in fibroids and may be the effectors of estrogen and progesterone promotion. 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. 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. Vitamin D is an antifibrotic factor and inhibits growth as it is found to induce apoptosis in cultured human leiomyoma cells through the downregulation of the genes PCNA, CDK1, and BCL-2 and suppression of catechol-o-methyltransferase expression and activity in human leiomyoma cells[26,27]7.

Serum Vitamin D

It was observed that 70% of our cases were severely deficient vitamin D (<10 ng/ml), while 25% of cases were vitamin D deficient (10-20 ng/ml). However, only 4% of our controls were severely vitamin D deficient with 58% of controls were among vitamin D deficient category. Both our case and control groups were far lagging behind to reach a normal Vitamin D level. In fact, only 4% of healthy volunteers had normal Vit D level (>30 ng/ml) whereas none of the patients with fibroid had normal Vitamin D level. Among Vitamin D insufficient category (21-30 ng/ml) mostly were healthy volunteers 34% vs. 4% of cases.

The serum Vitamin D level analysis indicated that women with low serum vitamin D level were about 11.65 times more likely to have fibroid compared to ones with normal Vitamin D levels. This finding connotes a possible inverse correlation between serum vitamin D3 and uterine fibroid in the present study population which corroborates the results of the studies conducted on different populations outside India. A study by Paffoni et al. 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, $p = 0.010$). 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% CI 1.1–4.3, $p = 0.022$)[25]. In a study conducted in Jharkhand by Singh V et al in 2018 demonstrated significantly lower serum vitamin D3 level in women with fibroid as compared to control population (10.81 ± 6.18 vs. 22.91 ± 16.18 , p value <0.0001). Furthermore, the relative odd of the presence of fibroid in a woman with vitamin D3 level <10 ng/dl was 4.64 (95% CI: 2.28–9.44, $p = 0.0001$)[28]

Association of Vitamin D with number of fibroid

Majority of the patients (49%) had single fibroid, 24 % had multiple fibroids. Patients having single fibroid had mean Vitamin D level of 10.35 ± 2.9 ng/ml. Vitamin D level in patients having two numbers of fibroids was 8.37 ± 2.3 ng/ml. Among the women with three or more number of fibroids had a significantly lower mean Vitamin D of 7.18 ± 2.0 ng/ml. Patients with two to three fibroids tend to have decrease Vitamin D in comparison to those with single fibroid ($p=0.003$ and p value= 0.001 respectively), suggesting a rise in number of fibroids with decreased serum Vitamin D levels. Study by Singh et al stated in their study that in women with number of fibroids more than three, the serum vitamin D3 level was lower in comparison with women with fibroids less than three. However, the result was not statistically significant (9.36 ± 5.77 vs. 11.24 ± 6.22 ; $p = 0.26$)[28].

Association of Vitamin D with volume/size of fibroid

Volume of fibroid >2 cm³ were included in study group. The minimum volume of fibroid was 3.12 cm³ and maximum 131 cm³. On estimation of vitamin D3, we observed that low vitamin D was found in patient with larger fibroids ($r = -0.78$; $p <0.001$). The study by Sarkar et al demonstrated 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[29].

Association of Vitamin D with type of fibroid

In our study most of the patient had intramural fibroid (63%), followed by submucosal and sub serosal fibroid. The serum vitamin D level among different groups did not show much difference. Vit D level among the few patients with

cervical fibroid was less than any other group. However, there was no statistically significant association between vit D level and type of fibroid. There are no such studies conducted till now that demonstrates the correlation among type of fibroid and vitamin d level.

Association of Vitamin D with site of fibroid

While detecting the variables of fibroid we found posterior myometrial fibroid was most common among women with either single or multiple fibroids, around . Anterior and fundal fibroid were present along with posterior myometrial, but was not prominent among study group. The serum vitamin D level among the groups were assessed, however no significant association could be gained. (p value <0.68). No studies to find association between site and vitamin d level have been conducted till now. The best way to confirm a definite causal association would have been to identify women with vitamin D deficiency and follow them regularly to see how many of them actually develop uterine fibroid. This type of exhaustive analytical study may take many years and difficult to achieve.

Conclusion

This study on association of 25-hydroxy vitamin D3 and uterine fibroid suggests the role of Vitamin D in uterine fibroids.

1. Women with low serum Vitamin D are at increased risk for developing uterine fibroids.
2. Fibroids in Vitamin D deficient women are more in number and larger volume in comparison to patients with normal Vitamin D.

Multicenteric studies with larger sample size will lend validity to the observations.

Funding: The study was self-funded.

Conflict of interest: None.

Ethical approval: The study was approved by the institutional ethics committee.

TABLE:1
Serum 25 Hydroxy Vitamin D3 level

Vitamin D(ng/ml)	Case(n=100)	Control(n=100)	P Value
Severe Deficiency (<10)	70	4	<0.001
Deficiency (10-20)	25	58	
Insufficient (21-30)	5	34	
Normal (>30)	0	4	
Mean±SD	8.6±2.6	19.23±4.3	

TABLE:2
Comparison of number of fibroid with Vitamin D

Number of fibroid	Vitamin D(ng/ml)	P value
1(n=49)	10.35±2.9	0.003(1 vs 2)
2(n=27)	8.37±2.3	0.121(2 vs 3)
2(n=24)	7.18±2.0	0.001(1 vs 3)

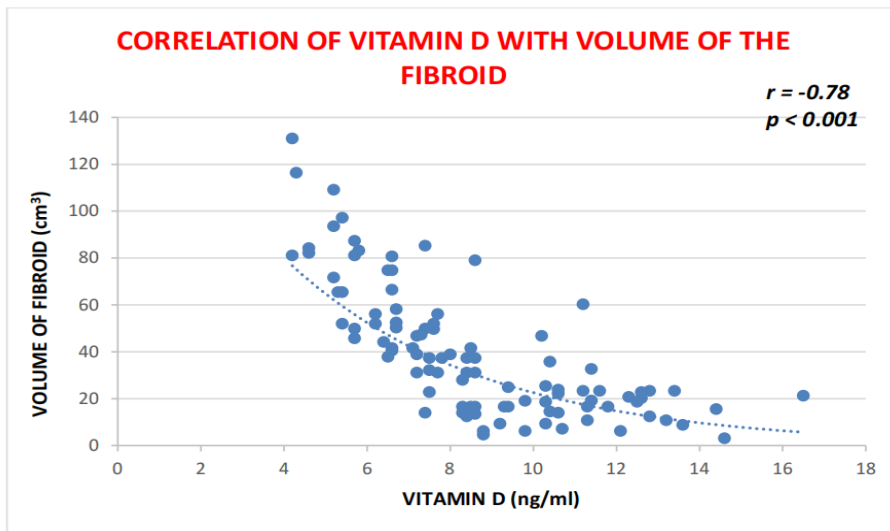
TABLE:3
Comparison of Vitamin D with site of fibroid

Site of fibroid	Vitamin D(ng/ml) Mean ±SD	P value
Anterior (n=49)	8.47±2.9	0.63
Posterior (n=62)	8.78±2.5	
Fundal (n=25)	8.49±2.4	
Cervical (n=5)	7.26±2.4	

TABLE: 4

Type of fibroid	Vitamin D(ng/ml) Mean ±SD	P Value
Intramural(n=80)	8.62±2.6	0.48
Submucosal(n=39)	9.26±2.7	
Subserosal(n=18)	8.74±2.9	

Figure 1:
Correlation of Vitamin D with volume of fibroid



References

1. Shen SH, Fennessy F, McDannold N, Jolesz F, Tempany C. Image-guided thermal therapy of uterine fibroids. *Semin Ultrasound CT MR*. 2009;30(2):91–104.
2. Al-Hendy A, Salama S. Gene therapy and uterine leiomyoma: a review. *Hum Reprod Update*. 2006;12(4):385–400.
3. Halder SK, Goodwin JS, Al-Hendy A. 1,25-Dihydroxyvitamin D3 reduces TGF-3-induced fibrosis-related gene expression in human uterine leiomyoma cells. *J Clin Endocrinol Metab*. 2011;96(4): E754 –E762.
4. Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. *Am J Physiol Renal Physiol*. 2005;289(1):F8–F28
5. McDonnell DP, Mangelsdorf DJ, Pike JW, Haussler MR, O'Malley BW. Molecular cloning of complementary DNA encoding the avian receptor for vitamin Science. 1987;235(4793):1214–1217.
6. Wang QM, Jones JB, Studzinski GP. Cyclin-dependent kinase inhibitor p27 as a mediator of the G1-S phase block induced by 1,25-dihydroxyvitamin D3 in HL60 cells. *Cancer Res*. 1996;56(2):264–267
7. Sharan C, Halder SK, Thota T, Nair S, Jaleel T, Al-Hendy A 2011 Vitamin D inhibits proliferation of human uterine leiomyoma cells via catechol-O-methyltransferase. *Fertil Steril* 95:247–253
8. Halder SK, Goodwin JS, Al-Hendy A. 1,25-Dihydroxyvitamin D3 reduces TGF-3-induced fibrosis-related gene expression in human uterine leiomyoma cells. *J Clin Endocrinol Metab*. 2011;96(4): E754 –E762.
9. Halder SK, Sharan C, Al-Hendy A. Vitamin D treatment induces dramatic shrinkage of uterine leiomyomas growth in the Eker rat model. *Biol Reprod*. doi:10.1095/biolreprod.111.098145.. In press 2012; BOR papers in press. Online Feb 2012.
10. CampbellMJ, Reddy GS, Koeffler HP. Vitamin D3 analogs and their 24-oxo metabolites equally inhibit clonal proliferation of a variety of cancer cells but have differing molecular effects. *J Cell Biochem*. 1997;66(3):413– 425.
11. Di Tommaso S, Tinelli A, Malvasi A, Massari S. Missense mutations in exon 2 of the MED12 gene are involved in IGF-2 overexpression in uterine leiomyoma. *Mol Hum Reprod*. 2014;20(10):1009 –1015.
12. Mittal P, Shin YH, Yatsenko SA, Castro CA, Surti U, Rajkovic A. Med12 gain-of-function mutation causes leiomyomas and genomic instability. *J Clin Invest* 2015;125(08):3280–3284
13. Bourbon HM, Aguilera A, Ansari AZ, et al. A unified nomenclature for protein subunits of mediator complexes linking transcriptional regulators to RNA polymerase II. *Mol Cell*. 2004;14(5):553–557.
14. Malik S, Roeder RG. The metazoan Mediator co-activator complex as an integrative hub for transcriptional regulation. *Nat Rev Genet*. 2010;11(11):761–772. 28.
15. Clark AD, Oldenbroek M, Boyer TG. Mediator kinase module and human tumorigenesis. *Crit Rev BiochemMol Biol*. 2015;50(5):393– 426.
16. Zhou H, Kim S, Ishii S, Boyer TG. Mediator modulates Gli3-dependent Sonic hedgehog signaling. *Mol Cell Biol*. 2006;26(23): 8667– 8682.

17. Prenzel T, Kramer F, Bedi U, Nagarajan S, Beissbarth T, Johnsen SA. Cohesin is required for expression of the estrogen receptor- (ESR1) gene. *Epigenet Chromatin*. 2012;5(1):13.
18. Shaikhibrahim Z, Offermann A, Braun M, et al. MED12 overexpression is a frequent event in castration-resistant prostate cancer. *Endocr Relat Cancer*. 2014;21(4):663– 675.
19. Gonzalez EA, Sachdeva A, Oliver DA, Martin KJ: Vitamin D insufficiency and deficiency in chronic kidney disease: A single center observational study. *Am J Nephrol* 24: 503–510, 2004
20. Al-Hendy A, Laknaur A, Diamond MP, Ismail N, Boyer TG, Halder SK. Silencing Med12 gene reduces proliferation of human leiomyoma cells mediated via Wnt/beta-catenin signaling pathway. *Endocrinology*. 2017;158:592–603.
21. McGuire MM, Yatsenko A, Hoffner L, Jones M, Surti U, Rajkovic A. Whole exome sequencing in a random sample of North American women with leiomyomas identifies MED12 mutations in majority of uterine leiomyomas. *PLoS One*. 2012;7:e33251
22. Morton CC. Genetic approaches to the study of uterine leiomyomata. *Environ Health Perspect*. 2000;108(Suppl 5):775–8.
23. Dietlein F, Thelen L, Reinhardt HC. Cancer-specific defects in DNA repair pathways as targets for personalized therapeutic approaches. *Trends Genet*. 2014;30:326–39.
24. Sabry M, Halder SK, Allah AS, Roshdy E, Rajaratnam V, Al-Hendy A. Serum vitamin D3 level inversely correlates with uterine fibroid volume in different ethnic groups: a cross-sectional observational study. *Int J Womens Health* 2013;5:93–100.
25. Paffoni A, Somigliana E, Vigano P, et al. Vitamin D status in women with uterine leiomyomas. *J Clin Endocrinol Metab* 2013;98(8):E1374e8
26. Salama SA, Ho SL, Wang HQ, Tenhunen J, Tilgmann C, Al-Hendy A 2006 Hormonal regulation of catechol-O-methyl transferase activity in women with uterine leiomyomas. *Fertil Steril* 86:259 –262
27. Massague´ J 1990 The transforming growth factor- family. *Annu Rev Cell Biol* 6:597– 641
28. Singh V, Barik A, Imam N. Vitamin D3 level in women with uterine fibroid: an observational study in eastern India population. *The journal of obstetrics & gynecology of India*. 2019 69(2) 161-165.
29. Sarkar M K, Halder A, Chowdhury S. A study to find out the association of vitamin D levels with leiomyoma uterus. *Indian journal of public health research development*. 2020;11(9), 59-63.
30. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Get vaccinated when it is your turn and follow the local guidelines. *International Journal of Health Sciences*, 5(3), x-xv. <https://doi.org/10.53730/ijhs.v5n3.2938>
31. Wido, A., Bajamal, A. H., Apriawan, T., Parenrengi, M. A., & Al Fauzi, A. (2022). Deep vein thrombosis prophylaxis use in traumatic brain injury patients in tropical climate. *International Journal of Health & Medical Sciences*, 5(1), 67-74. <https://doi.org/10.21744/ijhms.v5n1.1840>