



ASSOCIATION OF VITAMIN D, CALCIUM AND PHOSPHATE WITH UTERINE FIBROID IN PREMENOPAUSAL WOMEN OF COASTAL ODISHA.

Biochemistry

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ABSTRACT

BACKGROUND: In tropical countries, the extent of sun exposure, skin pigmentation play an important role altering the vitamin D levels and hence the causation of uterine fibroid in Indian women.

AIM AND OBJECTIVE: To determine association between serum vitamin D, calcium and Phosphate levels with uterine fibroid in premenopausal women.

MATERIALS AND METHODS: Forty women with uterine fibroid within the age group of 25 to 45yrs were enrolled as cases with forty healthy control. All participants were evaluated on the basis of a questionnaire, serum 25 hydroxyl vitamin D, calcium and phosphate.

RESULTS: Most of the cases were home makers with sun exposure less than 1 hr/day. Fibroid uterus was commonly associated with multi-parity without history of oral contraceptive pills. There was a significant fall in serum Vitamin D as well as calcium levels ($p < 0.05$) in cases with significant negative association between vitamin D and the size of fibroid.

CONCLUSION: Vitamin D and Calcium have a strong association with uterine fibroid hence could be treated as risk factors for developing advanced stage symptomatic fibroid uterus.

KEYWORDS

Vitamin D, Calcium, Phosphate, Uterine fibroid.

INTRODUCTION:

Uterine fibroid(s) (leiomyoma) are the most common benign neoplasm in the female genital tract that develop in the uterine muscle of premenopausal women^[1, 2]. It is known that 20-50% of women in reproductive age group suffer from fibroid uterus^[3]. Fibroid uterus is the leading causes for the hysterectomy with a significant health and economic burden on women in child bearing age group^[4, 5]. There is marked racial/ethnic disparity^[6]. The incidence was observed to be lower in Caucasians women in USA and among Swedish women in Italy^[7]. Blacks have earlier onset, higher incidence, more severe symptoms, larger tumor as compared to whites, could be attributed to insufficient synthesis of vitamin D under UV radiation^[8, 9]. Serum vitamin D contributes to racial/ethnic disparities^[10]. Uterine fibroid are most frequent in Afro-American women than in Caucasian and Hispanic population^[11-13]. Few studies have found similar association among blacks and whites correlating fibroid uterus with exposure to the sunlight. Recent studies found 32% lower odds of fibroid in patients with sufficient vitamin D levels as compared with those with vitamin D insufficiency^[14].

In tropical countries like India, the extent of sun exposure, skin pigmentation that affects the absorptions of ultraviolet rays from the sun, may play an important role altering the vitamin D levels and hence the causation of uterine fibroid in Indian women. Vitamin D plays an integral role in metabolism of calcium and phosphate^[15] and there exists a complex interrelationship between these three parameters. Though preliminary research suggests the role of Vitamin D in fibroid uterus, the interplay of calcium and phosphate levels along with vitamin D in these patients is not evaluated. Hence the current levels of data are insufficient to establish the risk association of vitamin D, calcium and phosphate levels with uterine fibroid in Indian women. So this study was conducted with an objective to determine association between serum vitamin D, calcium and Phosphate levels with uterine fibroid in premenopausal women.

MATERIAL AND METHODS:

Study design: This was a Case-Control study Conducted in the Department of Biochemistry, All India Institute of Medical Sciences, Bhubaneswar.

Study Subjects: Forty clinically diagnosed women with uterine Fibroid(s) within the age group of 25 to 45yrs, irrespective of their parity, attending the OPD of Obstetrics and Gynaecology department,

satisfying the inclusion and exclusion criteria, were enrolled in the study.

Inclusion Criteria:-

- Premenopausal women within the age group of 25-45yrs.
- Uterine fibroid of size 2-7 cms

Exclusion criteria:-

- Post menopausal women.
- Women with associated liver and Kidney disorders, cardiovascular diseases, auto Immune condition, known diabetic or hypertensive were excluded from this study.

Forty apparently healthy, age, ethnicity, location matched women were enrolled as Control group. Control participants were selected from nursing staff, office staff, and patient attendants as well as women undergoing general health examinations at AIIMS, BBSR. This study was conducted from March 2018 to November 2018. Total sample size was 80. Forty cases and 40 controls were evaluated on the basis of a Questionnaire prepared pertaining to the study i.e. Parity (no of childbirth), age of menarche, Last menstrual period, history of intake of oral contraceptive pills, miscarriage and duration of exposed to sunlight i.e. less than one hour / day, 1 hr. /day and > 1 hr. /day. The blood Pressure was recorded. Height and weight were measured to calculate Body Mass Index (BMI).

Ethical Approval: Taken from the institutional ethics committee, AIIMS Bhubaneswar. Written informed consent taken from the participants, confidentiality is maintained.

METHODOLOGY:

After obtaining informed written consent, 3 ml of blood sample was collected from all Participants (cases and control) and were processed to separate out serum. The serum samples were stored at -80 °C until used for Biochemical evaluation. Vitamin D status was Analyzed by estimating 25 hydroxyl vitamin D levels (Circulating active metabolite) in the Serum by enzyme linked Immune Sorbent Assay (ELISA) method. Fasting blood sugar, Serum Calcium and Phosphate levels were evaluated by fully automated chemistry Analyzer, Beckman coulter (AU 5800) by using system compatible packs. The clinical Findings as well as biochemical reports were recorded in the case study form of individual Participant.

Data analysis: Data obtained from questionnaire as well as from Biochemical evaluation were analyzed statistically using SPSS software. The data were analysed and expressed as mean and standard deviation. We compared the parameters among the cases and control. The p value less than 0.05 was considered as significant. We also tried to find out the strength of association between the variables and the size and number of uterine fibroid.

RESULTS:

General characteristics (table-1) of the study population showed that

TABLE-1 General Characteristics Of The Study Population

Sl. No	Parameters	Cases (n=40)	Control(n=40)
1	Age	39.97±4.83	36.48±6.60
2	Number of fibroid	1.11±0.39	-
3	Parity	1.97±1.1	2.10±1.1
4	Age of menarche	12.27±0.45	12.02±0.59
5	BMI	25.35±2.10	24.62±1.95
6	Type of fibroid uterus	<ul style="list-style-type: none"> Intramural = 37/40 Sub mucous= 3/40 	--
7	Occupation	<ul style="list-style-type: none"> Home maker =32/40 Business = 5/40 Others =3/40 	<ul style="list-style-type: none"> Home maker=30/40 Professionals=4/40 Skilled laborers=6/40
8	Exposure to sunlight	<ul style="list-style-type: none"> Low=18/40 Medium =16/40 Adequate=6/40 	<ul style="list-style-type: none"> Low=7/40 Medium =13/40 Adequate=20/40
9	H/O miscarriage	<ul style="list-style-type: none"> Yes =13/40 No =27/40 	<ul style="list-style-type: none"> Yes=4/40 No=36/40

Table-2 showed a significant low level of haemoglobin among the cases as compared to the control group revealing a state of anaemia in uterine fibroid patients. Serum calcium and phosphate levels were found to be low among the cases and this difference was statistically significant (p< 0.05) .There was a significantly low levels of serum vitamin D levels in cases as compared to control group which along with the observation of less exposure to sunlight(table-1) points towards the role of access to sunlight, skin production of vitamin D and its association with occurrence of uterine fibroids in premenopausal women.

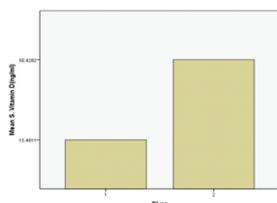
Table-2 Biochemical Parameters Of The Study Population

Sl. No.	Parameters	Cases	control	P value
1	Hemoglobin (%)	9.98± 1.30	*12.51±0.54	0.007
2	FBS (mg/dl)	96.83±6.68	99.21±4.42	0.74
3	Serum Calcium(mmol/l)	1.05±0.07	**1.15±0.13	0.000
4	Serum phosphate(mg/dl)	3.17± 0.68	*3.48±0.67	0.047
5	Serum vitamin D(ng/ml)	15.48 ± 4.96	**50.42±9.04	0.000

Note: * = significant (p<0.05); ** = highly significant (p<0.001)

Figure 1 bar graph represents the mean serum vitamin D levels of cases (15.48±4.96) which is very low when compared to the controls(50.42±9.04) gives association between the uterine fibroid occurrence and serum vitamin D levels.

Figure 1bar Graph Representation Of Mean Serum Vitamin D Levels [y Axis-mean S. Vitamin D; X Axis- 40cases (1), 40controls (2)]



Attempts were made to find the correlation between the size and number of uterine fibroid(s) with the serum calcium, serum phosphate and vitamin D levels(Table-3) there was a negative association with vitamin D levels (r value=-0.008, -0.02 respectively).Correlation between Serum calcium with size and number of uterine fibroid(s) found was not significant (r value= -0.13, -0.22 respectively). Serum phosphate had a positive association with size of uterine fibroid(s) (r

most of the uterine fibroid cases (37/40) are of intramural type and only (3/40) cases were sub mucosal in nature. There is no significant difference in the age of menarche parity H/o miscarriage and BMI among the cases and control group ruling out there confounding role. Most of the cases and controls 32/40 and 30/40 respectively are home makers but the extent to sun exposure is adequate in only 6/40

cases and 20/40 controls suggesting the association between exposure of sunlight and occurrence of uterine fibroid.

value=0.003).

Table-3 Correlation Between The Size And Number Of Fibroid With The Biochemical Parameters (correlation Coefficient “r” Value)

	Size of the fibroid	Number of the Fibroid
Serum Calcium(mmol/l)	-0.13	-0.22
Serum phosphate(mg/dl)	0.003	0.15
Serum vitamin D (ng/ml)	-0.008	-0.02

DISCUSSION:

Uterine fibroid (leiomyoma) represents localised proliferation of smooth muscle cells in the uterus. This condition is rarely associated with symptoms and is detected accidentally. The symptomatic women typically suffer from menstrual disorders affecting the quality of life. The identification of risk factors and timely management with medical treatment could preserve the uterus and doesn't affect the fertility in reproductive women. Early menarche, nulliparity were some of the known risk factors [12, 16]. In our study we found no significant difference in the obstetric history among the cases and controls. Body mass index (BMI) was more or less equal in both groups which were in accordance with earlier study conducted by Andrea C et al [17]. Duration of exposure to sunlight was adequate among most of the control with sufficient vitamin D levels, suggesting the fact that though humans can get vitamin D in their diet, the main source of vitamin D is still the synthesis in the skin with the exposure of sunlight. The cases had less exposure to sunlight with insufficient vitamin D levels which could be due to inadequate UV mediated transformation of 7-dehydro cholesterol to vitamin D through non enzymatic thermal isomerisation found in skin [18].

Uterine fibroid cases have significant low levels of vitamin D as compared to control group. Maintenance of sufficient vitamin D levels has been associated with reduced risk of colon cancer . Vitamin D is known to reduce cell proliferation, differentiation, increase apoptosis, regulate angiogenesis and extracellular matrix production [19], thereby it might possible that low vitamin D could increase the susceptibility to uterine fibroids. Earlier studies have postulated that vitamin D reduces the effect of transforming growth factor β3 (TGF β3) induced protein expression of collagen type-1, fibronectin and plasminogen activator inhibitor-1 proteins, and the phosphorylation of Smad2 and nuclear translocation of Smad2 and Smad3 [20]. Halder et al. demonstrated that 1, 25 dihydroxy vitamin D3 was able to reduce uterine fibroid growth by modulating the expression and activity of metalloproteinase (MMP-2, MMP-9) that degrade the extracellular

matrix [21]. In other study by the same group the, size of fibroid was found to be inversely associated with vitamin D levels that could be due to the role of vitamin D regulating the cellular signalling pathways associated with cell growth and proliferation i.e. PCNA, CDK1, CDK2, CDK4, apoptosis i.e. BCL2, BCL2L1, BAD, caspase 3 as well as steroid hormone receptors (PGR-A, PGR-B). Insufficient vitamin D could lead to dysregulation between cell proliferation and apoptosis, resulting in uncontrolled growth prevailing in uterine fibroid patients [22].

We found significant low levels of serum calcium in fibroid patients. This could be due to the key regulatory role of vitamin D in calcium homeostasis. In the kidneys, 1α -hydroxylase activity is strictly controlled by parathyroid hormone that is released under the influence of hypocalcaemia, 1α -hydroxylase is known to convert the 25 hydroxyl vitamin D to 1, 25- dihydroxyl vitamin D i.e. the active vitamin D that stimulate the osteoclasts to release calcium from bone, enhance intestinal calcium absorption and reduce the renal calcium excretion, thereby maintaining the calcium levels. Hence the fall in calcium levels could be attributed to insufficient vitamin D levels prevailing in fibroid cases. In this study though we found normal levels of serum phosphate in both cases and controls, the fall in phosphate levels in cases was significant as compared to control group pointing towards the complex interplay of calcium and phosphate metabolism with vitamin D levels.

CONCLUSION:

Uterine fibroid cases are associated with low serum levels of haemoglobin, calcium and phosphate. Inadequate exposure to sunlight and significant deficiency in vitamin D levels was found in fibroid cases. There was a negative association between vitamin D and Calcium with the size and number of fibroid in the patient group suggesting the regulatory role of vitamin D in growth of uterine muscle cells.

Conflict of Interest: Nil

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Future extended study with patients of different ethnicity, skin colour (dark and fair) with larger sample size with a study design to conduct clinical trial with vitamin D supplementation would be useful to establish the beneficial role of vitamin D as a non-surgical, therapeutic avenue in asymptomatic fibroid uterus patients.

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