Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 5756 - 5762
Received 15 December 2020; Accepted 05 January 2021.

A Study on Association of Vitamin D, Calcium and Oxidative Stress as Factors Involved in the Pathogenesis of Uterine Fibroids

Shanmugapriya.V[1*],Vijayasamundeeswari.C.K[2],Karthikeyan.D[3]

[1]DeptofBiochemistry,VinayakaMission'sMedicalCollege&Hospital(VMRF-DU),Karaikal,Puducherry,India
[2]DeptofBiochemistry,VinayakaMissionkirupanandavariyarmedicalCollege&Hospital(VMRF-DU),Salem,India
[3]DeptofMicrobiology,VinayakaMission'sMedicalCollege&HospitalKaraikal(VMRF-DU),Puducherry,India
E-mail: [1]priyasri_1011@yahoo.com

ABSTRACT

Background: Uterineleiomyoma commonly called as uterine fibroids are the commonest smooth muscle tumors that originate from the uterine muscul artissue. Several factors are involved in the pathogenesis like the reproductive hormones-estrogen and progesterone, age, family history, parity, race etc. The prevalencerate is 20-50% in women in the reproductive age group.
Aims and objectives: To analyze the biochemical parameters, vitamin D, serum calcium and oxidative stress marker, Malondialdehyde (MDA) in fibroid cases and to compare the same with healthy study subjects.
Materials and methods: 60 uterine fibroid cases and 40 age matched controls were studied for their biochemical parameters including lipidpro file, liver function tests, vitamin D, serum calcium, phosphorus, complete blood count and Plasma MDA.
Results: Patients with uterine fibroids had earlier age of menarche and first child birth when compared to normal subjects. They were anaemic with low hemoglobin Hb (gm%) 8.8±2.12 against controls 11.3±3.1.Their HDL (mg/dl) was low with mean value 41.4±5.8 compared to control values (52.8±6.7). In liver function tests, alkaline phosphatase (IU/L) was significantly elevated 198.7±31.4 IU/L when compared to the controls with mean value of 112.5±22.6 IU/L. Vitamin D and calcium were significantly lower in the cases than the controls. Uterine fibroid patients have increased oxidative stress level than their counterparts.
Conclusion: Uterine fibroids are more common in the reproductive age groupe specially in women between 30-50 years of age. Most of the patients were anemic, hypocalcaemic with significant low vitamin D level and increased oxidative stress when compared to the controls.

Keywords
Uterine fibroids, Vitamin D, Calcium, Oxidative stress

INTRODUCTION

Uterine leiomyoma or uterine fibroids in common terms is the most common benign smooth muscle tumors of the uterus. They consist of extracellular matrix like proteoglycans, collagen, and fibronectin and sometimes get calcified, hyalinated while undergoing degeneration. Studies done so far in these benign tumors imply that they are under the influence of steroid hormones estrogen, progesterone and testosterone.[1,2,3,4] Even now they are the commonest cause for hospitalization for gynecological problems in women under the age group of 30 -50 years.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 5756 - 5762
Received 15 December 2020; Accepted 05 January 2021.

Uterine fibroids remain the first cause for hysterectomy in women in India as well as in western population. The prevalence rate is 20-50% or 1 in 20 women will develop fibroid during their fertility period.[2, 5]

Uterine fibroids are classified as sub serous, sub mucous, intramural, cervical, polypoidal and pedunculated fibroids depending on their location. They develop as a single larger or multiple smaller fibroids .very rarely there can be recurrence after surgical removal. Since fibroids are hormone dependent tumors, they rarely appear before menarche and usually regress after menopause. One in thousand fibroids rarely undergo malignant changes at later stages of life as age increases.

Fibroids are known for their wide range of symptoms starting from dysmenorrhea, irregular, heavy bleeding called menorrhagia, dyspareunia, non cyclical pain, pelvic pressure symptoms like urinary frequency, urgency, stress incontinence, bladder and bowel disturbances.[6,7,8,9]

Pregnancy related complications include infertility, miscarriages, preterm labour, post partum hemorrhage etc.,[10-17] Secondary infertility is also a important consequence in women who underwent myomectomy.

Research in uterine fibroids wholly deserves because involves many molecular mechanisms like tumor biology, angiogenesis, fibrosis, hormone signaling, genetics, mutation, polymorphisms etc..,[18] More exploratory work is needed on behalf of millions of woman suffering from this disability called uterine fibroid disorder; especially working women who desires for their family in their late thirties.

Weight gain, stressful life, loss of physical activity, change in lifestyle pattern even in household women increases the body fat and adipose tissue; which forms the substrate for the formation of steroid hormones from cholesterol. Loss of exposure of sunlight makes them deficient in serum vitamin D content also.

AIMS AND OBJECTIVES:

With this background we aimed at studying the biochemical parameters involved in the pathogenesis of developing uterine fibroids and to compare the same with apparently healthy normal subjects from the general population. Also we wanted to evaluate the oxidative stress marker for lipid peroxidation in patients with uterine fibroids.

MATERIALS AND METHODS:

This study was conducted in the Biochemistry department in association with obstetrics and gynecology, Vinayaka Mission's Medical College & Hospital, Karaikal, from November 2018 to September 2019. Ethical committee approval was obtained for conducting this study. A total of hundred (100) study subjects which includes sixty (60) uterine fibroid cases within the age group of 30- 45 years and fourty (40) controls were investigated for their fasting blood sugar, Urea, Creatinine, fasting lipid profile, serum calcium ,phosphorus, liver function tests and serum vitamin D level. Complete Blood Count (CBC) was also done. Patients' details were collected in a structured proforma which includes their height weight, personal, menstrual, family and clinical history. Blood pressure and pulse rate was recorded for all the study subjects.

Diabetic, hypertensive patients, post menopausal and women on hormonal treatment, jaundiced patients were excluded from the study. Fasting Blood glucose was done by the glucose oxidase peroxidase method. Serum total cholesterol estimation was done by Cholesterol oxidase - peroxidase and serum triglycerides by glycerol 3 phosphate oxidase - peroxidase enzymatic

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 5756 - 5762
Received 15 December 2020; Accepted 05 January 2021.

method. HDL-C was done by direct method. Blood Urea and Creatinine were estimated by enzymatic UV- kinetic Glutamate dehydrogenase and by modified Jaffe's two point kinetic method respectively. Serum bilirubin was analyzed by modified Jendrassik&Grof's method. SGOT, SGPT and ALP were estimated by standardized enzymatic kinetic kit methods. Total Protein was measured by Biuret and Albumin by Bromocresol green end point calorimetric methods. Serum Calcium was done by Arsenazo III method and phosphorus by phosphomolybdate method. Serum level of Vitamin D was estimated by ELISA method. Plasma oxidative stress marker Malondialdehyde (MDA) was done by Esterbauer and Steinberg method.[19]

Calculated parameters include Body Mass Index, VLDL and LDL. LDL was calculated by Friedwald's formula. Complete Blood Count (CBC) was done by automated Horiba- ES 60 analyzer.

Statistical analysis: Statistical analysis was done by SPSS software. The results were given as Mean and Standard deviation.P value of less than 0.05 was taken as significant.

RESULTS:

Table 1: Demographic parameters of the study subjects

GENERAL PARAMETERS	CASES	CONTROLS
Age of menarche(yrs)	**12.8 ± 0.8**	**14.2 ± 1.2**
Age at first child birth(yrs)	**19.5 ± 4.8**	**22 ± 3.9**
Height (cm)	157.2 ± 5.5	160.7 ± 7.8
Weight(kg)	65.8 ± 10.2	60.8 ± 9.5
BMI (kg/m^2)	**27.9 ± 4.7**	**24.6 ± 3.8**
Mean age (yrs)	40.4 ± 6.3	39.7 ± 5.7

Table 2: Biochemical parameters of the study subjects

PARAMETERS	CASES	CONTROLS
Hemoglobin (gm/dl)	**8.8 ± 2.12(S)**	**11.3 ± 3.1**
Glucose(mg/dl)	88.5 ± 7.9	80.8 ±5.8
Urea(mg/dl)	23.5 ± 4.9	23.7 ± 6.5
Creatinine(mg/dl)	0.78 ± 0.14	0.9 ± 0.3
T.Cholesterol(mg/dl)	182.8 ± 25.8	165.7 ± 23.9
Triglycerides(mg/dl)	170.2 ± 32.6	148.7 ± 25.5
HDL-C(mg/dl)	**41.4 ± 5.8(S)**	**52.8 ± 6.7**
LDL-C(mg/dl)	108.8 ± 23.4	88.6 ± 22.7
VLDL-C(mg/dl)	30.5 ± 11.6	19.6 ± 8.8
SGOT(IU/L)	22.6 ± 6.8	19.8 ± 5.8
SGPT(IU/L)	24.6 ± 4.8	21.7 ± 4.3
ALP(IU/L)	**198.7 ± 31.4(S)**	**112.5 ± 22.6**
T.Protein(gm/dl)	6.4 ± 0.3	7.2 ± 1.3
Albumin(gm/dl)	3.6 ± 0.22	4.4 ± 0.3
T.Bilirubin(mg/dl)	0.64 ± 0.4	0.54 ± 0.3
Direct(mg/dl)	0.27 ± 0.01	0.23 ± 0.02

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 5756 - 5762
Received 15 December 2020; Accepted 05 January 2021.

TABLE 3: BIOCHEMICAL PROFILE IN STUDY SUBJECTS

PARAMETERS	CASES	CONTROLS
S. CALCIUM (mg/dl)	**8.6 ± 1.2(S)**	10.2 ± 2.2
S. PHOSPHORUS (mg/dl)	3.7 ± 0.8	4.3 ± 1.1
S. VITAMIN D (ng/ml)	**23.6 ± 5.9(S)**	45.4 ± 7.8
PLASMA MDA (µmol/L	**6.16 ± 1.8(S)**	3.28 ± 0.9

DISCUSSION:

Patients with uterine fibroid disorder presented with irregular and heavy menstrual bleeding with dysmenorrhea as their major complaints while other symptoms include lower abdominal pain, urinary retention, stress incontinence, dyspareunia and fertility complications.

The mean age in years of attaining menarche among patients with fibroid was 12.8± 0.9 while it was 14.2±1.3 among the controls, as attaining early menarche and late menopause are considered as important factors in the etiology of developing fibroid uterus due to prolonged estrogen exposure.[18] Patients also had earlier age of their first delivery with mean in years of 19.5 ± 4.7 against controls with mean of 22 ± 3.4. Nulliparity is a known risk factor for uterine fibroids. But only three out of sixty patients were nulliparous while rests of them are multiparous with minimum of at least two live births. Body Mass Index (BMI) in kg/m^2 was elevated in fibroid patients with mean value of 27.9 ± 4.4 while in controls it was 24.5 ± 3.6. The mean age in years among cases were 40.4 ± 6.2 and in controls 39.7 ± 5.4.

The mean hemoglobin (gm/dl) level in patients and control groups was 8.8 ± 2.1 and 11.3 ±3.1 respectively. Patients were found to be anemic as expected due to continuous heavy blood loss during every menstrual cycle. Serum fasting blood glucose, urea and creatinine were found to be within normal limits. Fasting Lipid profile showed normal or low normal values in most of the patients. Serum HDL cholesterol (mg/dl) was found to be decreased in cases with mean value of 41.4 ±5.8 and was in normal range in controls with mean of values 52.8 ± 6.7 which was of statistical significance. Among Liver function tests, serum bilirubin levels including total and direct, enzymes SGOT and SGPT were within the normal range. But serum enzyme alkaline phosphatase in IU/L (ALP) was significantly elevated in fibroid patients with mean value of 198.7±31.4 against control value of 112.5±22.6. Serum total protein (gm/dl) level was low in patient group with mean 6.4 ± 0.3 when compared to control group and so also serum albumin (gm/dl) level with mean 3.6 ± 0.2. Alkaline phosphatase enzyme has five isoenzyme forms and the fifth one is of placental origin. The enzyme level is elevated usually in pregnancy and returns back to normal level once pregnancy get terminated.[20] Another reason for elevated enzyme level in patients with uterine fibroids is proved by genotypic expression of Catechol O methyl transferase (COMT) enzyme.[21, 22]

There was significant difference in the serum calcium (mg/dl) levels among cases and controls with mean value of 8.6 ± 1.2 and 10.2 ± 2.2 respectively. So also there was difference in the serum phosphorus (mg/dl) levels but it was not significant. The mean values are 3.7 ± 0.8 and 4.3± 1.1 in cases and controls. Regarding vitamin D (ng/ml) level, there was drastic difference in the serum values between cases and controls with statistical significance. The mean values are 23.6 ± 5.9 and 45.4 ±7.8 respectively. Patients with uterine fibroids are oxidatively stressed with nearly double the values of lipid peroxidation marker plasma MDA with mean of 6.16 ± 1.8 and 3.28 ± 0.9 in cases and controls.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 5756 - 5762
Received 15 December 2020; Accepted 05 January 2021.

Vitamin D is the main regulator of calcium homeostasis in our body. It is strong anti fibrotic factor. Experimental in vitro animal studies proved that it a potent antitumour agent decreasing leiomyoma by causing shrinkage of fibroid tissues. Vitamin D acts through its receptor called vitamin D receptor (VDR) which is a cell membrane as well as nuclear receptor. VDR is a nuclear transcription factor causing modulation of gene expression resulting in growth arrest, induction of apoptosis, differentiation and cell signaling. Vitamin D_3 prevents malignant cell proliferation with induction of differentiation and apoptosis. Experimental models of vitamin D_3 analogs when injected in murine squamous cell carcinomas showed strong antitumour activity.[23]
Inverse dose dependent response was also recorded regarding severity in fibroid tumour tissues. Increased doses resulted in reduction in the size and number of the fibroid mass. It is also proved that vitamin D decreased the expression of cell proliferation marker PCNA in eker rats.
As antifibrotic agent it acts by reducing the expression of connective tissues like collagen, fibronectin and other tumour growth factor dependent key profibrotic factors in a dose dependent manner. It also reduces regulatory proteins of cell cycle like CDK_1, CDK_2 and CDK_4 in fibroid tissues.[23]
Vitamin D production in our body is influenced by various factors including environmental factors, climatic conditions in tropical countries, dressing pattern and using cosmetics.[24] Inadequate sunlight exposure in working women as well as in home makers and using sunscreens when happened to get exposed to natural light makes most of the females to become deficient in serum vitamin D level even in affluent societies. Dark skinned people need 5 – 10 times more exposure to natural sunlight in order to get sufficient vitamin source when compared to white population.[24, 25]
In this study, a low vitamin D level is again reflected in the low serum calcium levels in blood as well as increased serum alkaline phosphatase enzyme levels.
Oxidative stress is a condition where there is imbalance between the antioxidants and free radicals due to excessive production reactive oxygen species and deficient defense mechanisms. Oxidative stress is known to occur in many inflammatory and metabolic conditions like arthritis, hypothyroidism, diabetes, and hypertension and so also in uterine leiomyoma. In malignant conditions, the intra cellular oxidative status controls the cellular proliferation and also promotes metastasis of tumor cells.[26]
A myomatous uterus accommodates large number of enlarged arterioles and venules due to angiogenesis. Cyclical contraction, traumatic injury due to ischemia, myometrial hypoxia, healing by fibrosis causes oxidative stress due to free radical formation; resulting in further cellular damage.
Estrogen is a known pro oxidant. Estrogen mediated reactive oxygen species induce cell proliferation, transformation, migration and genomic instability by its influence on transcription factors that are redox sensitive.[27] Estrogen undergoes oxidation to form catecholestrogen, which are the precursors of quinones. Quinones in turn by a reversible oxidation- reduction reaction forms semiquinones and reactive oxygen species, which are potent pro oxidants causing DNA and protein damage resulting in tumourogenesis.[28]
Uterine fibroid patients are usually anemic due to excessive blood loss which again makes them more prone to oxidative stress and its consequences. In this study, patients with fibroids had significant increase in the level of oxidative stress marker MDA when compared to the control group indicating that they under free radical cellular damage by an estrogen mediated mechanism.

CONCLUSION:

Uterine fibroids are the most common disabling factor for women in the reproductive age group. Symptomatic women typically suffer from menstrual disorders affecting their quality of life; also imposing mental, social and economic burden resulting in work loss and productivity. Identifying the risk factors at the earliest and timely medical management including Vitamin D supplements and anti-oxidants could preserve the uterus so that it does not affect the fertility in reproductive women.

LIMITATIONS:

Smaller sample size.
Need to be conducted further in a larger group.
Follow up with Vitamin D supplementation can be done to establish the beneficial effect.

CONFLICTS OF INTEREST:

There is no conflict of interest for this study.

FUNDING:

It's a self-funded research work. There is no funding agency involved in this study.

ACKNOWLEDGEMENT:

We humbly acknowledge all our study subjects for their kind cooperation in this research work.

REFERENCES

[1] 1.Gomes MT, Castro Rde ASilvla ID. The progesterone receptor gene polymorphism, progin, may be a factor related to the development of uterine fibroids. E pub. 2007; Jan16 87(5):1116- 21.
[2] J. Julie Kim, Elizabeth C. Sefton. The role of progesterone signaling in the pathogenesis of uterine leiomyoma. Mol cell endocrinol. 2012; july 25 358: [2] 223- 231.
[3] S. Govindana, S.N. Ahmada,d, Vedicherlaa Association of progesterone receptor gene polymorphism with endometriosis, uterine fibroids and breast cancer. Cancer biomarkers. 2007 ;(3): 73-78.
[4]
[5] Aamir t khan, ManjeetShehmar, and Janesh. Uterine fibroids; current perspectives. Int J women health. Jan 2014; 6: 95 -114.
[6] William H. Catherino,Heba M. Eltoukhi, and Ayman Al-Hendy.Racial and Ethnic Differences in the Pathogenesis and Clinical Manifestations of Uterine Leiomyoma: seminreprod med .2013; sep31 [5]: 370-379.
[7] Stewart EA. Uterine fibroids. Lancet 2001; 357: 293–8.
[8] Stewart, CL Cookson, RA Gandolfini, R SchultzeRath . Epidemiology of uterine fibroids: a systematic review: BJOG. 2017; 124(10):
[9] Zimmermann A, Bernuit D, Gerlinger C. Prevalence, symptoms and management of uterine fibroids: an international internet-based survey of 21,746 women. BMC Women's Health 2012; 12:6:1-11.
[10] Lippman SA, Warner M, Samuels S et al.., Uterine fibroids and gynecologic pain symptoms in a population based study.FertilSteril 2003;80:1488–94.
[11] Coronado GD, Marshall LM, Schwartz SM. Complications in pregnancy, labor, and delivery with uterine leiomyomas: a population-based study. ObstetGynecol .2000; 95(5):764–9.
[12] Benson CB, Chow JS, Chang-Lee W. Outcome of pregnancies in women with uterine leiomyomas identified by sonography in the first trimester.JClin Ultrasound .2001; 29:261–4.

[13] Sheiner E, Bashiri A, Levy a, Hershkovitz R, Katz M, Mazor M. Obstetric characteristics and perinatal outcome of pregnancies with uterine leiomyomas. J Reprod Med .2004; 49: 182–6.
[14] Qidwai GI, Caughey AB, Jacoby AF. Obstetric outcomes in women with sonographically identified uterine leiomyomata. ObstetGyneco.l 2006; 107:376–82.
[15] Ciavattini A, Clemente N, Delli CG, Number and size of uterine fibroids and obstetric outcomes. J Matern Fetal Neonatal Med .2015; 28:484–8.
[16] Shavell VI, Thakur M, Sawant A, Kruger ML, Jones TB, Singh M, et al. Adverse obstetric outcomes associated with sonographically identified large uterine fibroids. FertilSteril. 2012; 97:107–10.
[17] Klatsky PC, Tran ND, Caughey AB, Fujimoto VY. Fibroids and reproductive outcomes: a systematic literature review from conception to delivery. A M J ObstetGynecol .2008; 198:357–66.
[18] Cook H, Ezzati M, Segars JH, McCarthy K. The impact of uterine leiomyomas on reproductive outcomes. Minerva Ginecol. 2010; 62:225–36.
[19] Prakashtrivedi. Infertility in Indian Women. Int J of women health. 2014; 118- 221.
[20] Suryawanshi, N.P., Bhutey, A.K., Nagdeote, A.N. *et al.* Study of lipid peroxide and lipid profile in diabetes mellitus.
[21] *IndianJClinBiochem* **21,** 126(2006). https://doi.org/10.1007/BF02913080
[22] Svjetlanalozo, Atabeygi and Michael Healey. Extreme Elevation of Alkaline Phosphatase in a Pregnancy Complicated by Gestational diabetes and Infant with Neonatal Alloimmune Thrombocytopenia: Case Reports in Obstetrics and Gynecology. 2016; 1-3
[23] De Oliveira E, de Aquino Castro R, Gomes MT . The catechol-O-methyltransferase (COMT) gene polymorphism and prevalence of uterine fibroids. 2008; aug 60 [3-4]:235 - 238.
[24] O.C.Ojo and A.O.Oyeyemi . Evaluation of marker enzymes in fibroid patients: journal of endocrinology, toxicology and food technology. jul-aug 2013; 5(3): 29-31.
[25] Ayman Al-Hendy and MarwaBadr. Can Vitamin D reduce the risk of uterine fibroids? Women's Health; 2014 ; 10(4): 353-358
[26] MichałCiebiera, Marta Włodarczyk, et.al. Vitamin D and Uterine Fibroids—Review of the Literature and Novel Concepts; Int. J. Mol. Sci. **2018**, 19, 2051;Doi:10.3390/ijms19072051
[27] Suchitra Kumari, BajiDheerajBabu, SwetaSingh.Association of Vitamin D, Calcium and Phosphate with Uterine fibroid in Premenopausal women of coastal odisha;International Journal of Scientific Research:Volume-8 Issue-2 February-2019
[28] Nicole M. Fletcher, Mohammed S. Abusamaan et al.., Oxidative stress: a key regulator of Leiomyoma cell survival; Fertility and Sterility: Vol. 107, No. 6, June 2017 0015-0282
[29] Pietro Santulli1, Bruno Borghese1, HerveLemaréchal et al.Increased Serum Oxidative Stress Markers in Women with Uterine Leiomyoma; PLOS ONE | www.plosone.org: August 2013 Volume 8 , Issue 8 e72069.
[30] L.Nathan, G.Chaudhuri. Antioxidant and prooxidant actions of estrogens: potential physiological and clinical implications; SeminReprodEndocrinol: 1998;16(4):309-14.doi: 10.1055/s-2007-1016289**.**