

Serum Aromatase – Marker Enzyme for Uterine Fibroids

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ABSTRACT

Background: Uterine leiomyoma commonly called as uterine fibroids are the commonest smooth muscle tumors that originate from the uterine muscular tissue. Several factors are involved in the pathogenesis like the reproductive hormones-estrogen, testosterone and progesterone, age, positive family history, parity, race, environmental factors etc. Most common benign condition in women in the reproductive age group.

Aims and objectives: To analyze the biochemical parameters along with reproductive hormones- Estrogen, Testosterone, Progesterone and serum enzyme Aromatase in fibroid cases and to compare the same with healthy study subjects.

Materials and methods: 50 uterine fibroid cases and 40 age matched controls were studied for their biochemical parameters including lipid profile, liver function tests, Steroid reproductive hormones and enzyme aromatase or estrogen synthase which converts testosterone into estrogen.

Results: Uterine fibroid patients had earlier age of menarche and first child birth when compared to normal subjects. They were anemic with low hemoglobin Hb (gm %) 8.6 ± 2.12 and HDL-C against controls. Among liver function tests, alkaline phosphatase (IU/L) was significantly elevated 196.7 ± 31.4 IU/L when compared to the controls with mean value of 112.5 ± 22.6 IU/L. Serum reproductive hormones Estrogen and Testosterone were significantly elevated in fibroid patients. There was not much difference in the serum progesterone levels. But difference in enzyme Aromatase level among cases and controls was statistically significant.

Conclusion: Uterine fibroids are the most common benign condition in reproductive age group of females, which can develop in patients who attain early menarche and having earlier age of first child birth. Serum testosterone levels should be estimated routinely in uterine fibroids. Serum Enzyme Aromatase should be used as a marker enzyme for patients with leiomyoma..

Keywords:

Uterine fibroids, Estrogen, Progesterone and Testosterone, Enzyme Aromatase

1.Introduction

Uterine fibroid or Leiomyoma is the commonest smooth muscle tumor of the uterus. It is a benign condition with late onset in the third decade. More common in the age group between 30 - 50 years. It is made up extracellular matrix like collagen, fibronectin, hyaline, proteoglycans etc., It has been proved already that they are hormone dependent tumors mainly influenced by reproductive steroid hormones Estrogen, Progesterone and Testosterone^{1,2,3,4}. They undergo degenerative changes in the postpartum period with calcification, hyalination, red degeneration and sometime necrosis. Though may remain asymptomatic; uterine fibroids are known for their disabling symptoms posing a negative impact on the daily routine of affected individuals especially the employed population.

The typical presenting complaints in women with uterine fibroids are irregular heavy menstrual bleeding, dysmenorrhea, pelvic pressure symptoms like urinary incontinence, increased frequency of micturition, dyspareunia, bowel disturbances etc.^{6,7,8,9}.

It is also known to cause infertility; which is more common with cervical type of fibroids and with pregnancy it results in miscarriages, preterm labor, postpartum hemorrhage, precipitated labor etc., Those who underwent myomectomy may also experience secondary infertility¹⁰⁻¹⁷.

Experimental studies show that the myoma size increases with estrogen and progesterone hormones when given in stipulated doses. It is also evident by the fact that the tumor is more common in the reproductive age group and get regresses after attaining menopause. Malignant changes are very rare in this condition.

2. Aims And Objectives

The objectives of this study are to analyze the uterine steroid hormones estrogen, progesterone and testosterone in fibroid patients and to compare the same with healthy study subjects. Also, we aimed at evaluating the serum aromatase enzyme status in subjects with fibroid uterus.

3. Materials And Methods

This study was conducted in the department of Biochemistry Vinayaka Mission's Medical College & Hospital, Karaikal, from august 2019 to march 2020. Ethical committee approval was obtained for conducting this study. A total of hundred (90) study subjects which includes fifty (50) uterine fibroid cases within the age group of 30- 45 years and forty (40) age matched controls were investigated for their routine biochemical parameters along with liver function tests, serum aromatase enzyme, serum estrogen, progesterone and testosterone levels. Patients' demographic details were collected along with clinical history in a structured proforma which includes their weight, height, personal, menstrual, family and treatment history. Blood pressure and pulse rate was recorded for all the study subjects.

Patients on hormonal treatment, diabetic, hypertensive patients, post-menopausal women, jaundiced patients and subjects on any other long-term treatment were excluded from the study. Fasting Blood glucose was done by the glucose oxidase peroxidase method. Blood Urea and Creatinine were estimated by enzymatic UV- kinetic Glutamate dehydrogenase and by modified Jaffe's kinetic method respectively Serum total cholesterol estimation was done by Cholesterol oxidase-peroxidase and serum triglycerides by glycerol 3 phosphate oxidase - peroxidase enzymatic method. HDL-C was done by direct method. Serum bilirubin was analyzed by modified Jendrassik & Grof's method. Liver enzymes SGOT, SGPT and ALP were estimated by standardized enzymatic kinetic kit methods. Serum total protein was measured by Biuret and Albumin by Bromocresol green end point calorimetric methods. Serum hormonal assay for estrogen, progesterone and testosterone was done by Chemiluminescent (CLIA) method in Maglumi 2000 fully automated analyzer and Serum Aromatase enzyme by ELISA method. Complete Blood Count (CBC) was done in Horiba ES 60 automated analyzer and blood grouping & typing by tulip kit slide agglutination method.

Calculated parameters include Body Mass Index, VLDL and LDL. LDL was calculated by Fried Wald's formula.

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.

4. Results

Table 1: Demographic parameters of the study subjects.

GENERAL PARAMETERS	CASES	CONTROLS
Age of menarche(years)	12.6 ± 0.8	14.2 ± 1.2
Age at first child birth(years)	19.8 ± 4.8	22 ± 3.9
Weight(kg)	66.7 ± 9.2	60.8 ± 9.5
BMI (kg/m ²)	27.6 ± 4.4	24.5 ± 3.6
Mean age (years)	40.8 ± 5.8	39.7 ± 4.9

Table 2: Biochemical parameters of the study subjects

PARAMETERS	CASES	CONTROLS
Hemoglobin (gm/dl)	8.6± 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)	40.5 ± 5.8(S)	52.8 ± 6.7
LDL-C(mg/dl)	107.8 ± 23.4	88.6 ± 22.7
VLDL-C(mg/dl)	30.5 ± 11.6	19.6 ± 8.8
SGOT(IU/L)	21.6 ± 6.8	19.8 ± 5.8
SGPT(IU/L)	22.6 ± 4.8	21.7 ± 4.3
ALP(IU/L)	196.7 ± 31.4(S)	112.5 ± 22.6
Protein (gm/dl)	6.3 ± 0.3	7.2 ± 1.3
Albumin(gm/dl)	3.7 ± 0.22	4.4 ± 0.3
Bilirubin (mg/dl)	0.64 ± 0.4	0.54 ± 0.3
Direct(mg/dl)	0.27 ± 0.01	0.23 ± 0.02

Table 3: Hormonal & Enzyme Assay In Study Subjects

PARAMETERS	CASES	CONTROLS
S. ESTROGEN (pg /ml)	88(S)	60
S. PROGESTERONE (ng/ml)	0.8	0.6
S. TESTOSTERONE (ng/dl)	50(S)	30
S. AROMATASE (ng/dl)	13(S)	6

5. Discussion

The mean age (years) of the uterine fibroid patients was 40.8 ± 5.8 and in control group it was 39.7 ± 4.9 . The chief presenting complaints were dysmenorrhea and menorrhagia. There were also complaints of vague lower abdominal pain, urinary retention, increased frequency of micturition, weakness, tiredness, dyspareunia and infertility.

The presenting complaints, diagnosis and treatment can vary from simple to complex procedure depending on patient's health status, economic condition, marital status, severity of symptoms and health care services availability in their locality.

On analyzing the menstrual history, patients with fibroids had earlier age of menarche and first child birth when compared to the control group. Early menarche and late menopause are important risk factors in the development of uterine fibroids due to prolonged estrogen exposure.¹⁸ Our study contradicted with the fact that nulliparity as a known risk factor for uterine fibroids because only two out of fifty patients (2/50) were nulliparous while rest of them (48/50) were multiparous with at least two live births. Body Mass Index (BMI) in kg/m^2 was 27.9 ± 4.4 in fibroid patients while in controls it was 24.5 ± 3.6 .

The mean hemoglobin (gm/dl) level in patients and control groups was 8.6 ± 2.12 and 11.3 ± 3.1 respectively. Patients as expected were found to be anemic due to heavy blood loss during each menstrual cycle which was reflected in their presenting complaints as general body weakness and tiredness. Fasting Lipid profile of the patients showed normal or low normal values in most of the cases. Serum High Density Lipoprotein Cholesterol (mg/dl) was found to be decreased significantly in cases with mean value of 40.5 ± 5.8 against control value of 52.8 ± 6.7 . Except for serum alkaline phosphatase enzyme and total protein, other liver function tests values including serum bilirubin levels, enzymes SGOT and SGPT were within the normal range. Serum enzyme alkaline phosphatase ALP(IU/L) was significantly elevated in fibroid patients with mean value of 196.7 ± 31.4 and 112.5 ± 22.6 in controls. Total protein and subsequently serum albumin were low in cases when compared to the control group. There are five isoenzymes forms of Alkaline phosphatase and the fifth isoenzyme is from placental origin. The enzyme level is usually elevated even in normal pregnancy and returns back to baseline levels once the gestation period is complete. Another reason for the elevated enzyme level in patients with uterine fibroids is proved by genotypic expression of the enzyme Catechol O methyl transferase (COMT) by the uterine tissues and hepatic cells.^{19, 20}

Angiogenesis in this benign tumorous condition results in dilatation of arterioles and venules in the myomatous uterus. Traumatic injury due to ischemia, myometrial hypoxia, cyclical contraction and healing by fibrosis results in oxidative stress due to free radical formation and further cellular damage.

In this present study, Serum Estrogen and Testosterone are significantly elevated in uterine fibroid patients when compared to the healthy controls. Serum Progesterone levels is increased but it is not statically significant. The mean value of Serum enzyme Aromatase is 13.6 ng/dl in cases against control value of 6 ng/dl which was again statistically significant as the value is doubled in patients. So, it is noted that increased serum aromatase causes increased rate of conversion of serum testosterone to estrogen; increasing the circulating levels serum estrogen which is a known risk factor for initiating fibroid growth.

Progesterone, steroid hormone with 21 carbons is mainly produced by the ovaries, placenta, adrenal glands and brain tissues. Its otherwise called as Pregnancy Hormone because progesterone supplements were prescribed till 34 weeks of gestation and observed to prevent pregnancy loss. It is also involved in maintaining menstrual cycle and embryogenesis in

mammalian species. Other functions include regulation of estrogen levels, uterine growth promotion, suppression of myometrial contraction and regulation of immune and inflammatory response^{21,22}.

Adiposity and prolonged estrogen exposure are known risk factors for uterine fibroids as it is most common in the menstruating women in reproductive age group. The biologically active form of estrogen – 17β estradiol increases the proliferation and growth of fibroids through progesterone receptors mediated gene expression. The enzyme Aromatase which converts androgens, especially testosterone to estrogen is overexpressed in the myomatous tissues compared to the normal myometrium²³.

Aromatase is a naturally occurring enzyme located in multiple tissues body like the brain, muscles, testicles, ovaries, placenta and lining of the uterus. Higher the amount of aromatase in circulation, more the testosterone being converted into estrogen. Aromatase activity appears to be enhanced in certain estrogen-dependent local tissue next to breast tissue like endometriumin endometriosis and uterine fibroids.

Aromatase activity increases with age, in line with the testosterone that decreases with age. It has also been found that it increases with the amount of fatty tissue present. Poor nutrition, weight gain, high stress and lack of exercise also cause increased aromatase levels. Sufficient circulating levels of estrone can also be produced from extra glandular aromatization of androstenedione²⁴.

Subsequently it undergoes oxidation reduction reaction to become the biologically active estradiol in peripheral tissues, to cause uterine bleeding and endometrial hyperplasia in obese anovulatory or postmenopausal women.

Studies are there proving therapeutic doses of aromatase inhibitors reducing the size and growth of uterine fibroids.²⁴

Androgens and androgen receptors are over expressed in uterine fibroid tissue. So also, the overexpressed aromatase in fibroid tissue suggests that androgen might play an important role in growth and development of fibroid. Exogenous and endogenous estrogen are established factors in the development of uterine fibroids. Endogenous E_2 levels dramatically decrease with age as nearing menopause so also the risk of fibroids compared to pre-menopausal women²⁵.

Estrogen is a known pro-oxidant. Estrogen mediated free radicles induce cell proliferation, migration, transformation and genomic instability by its influence on transcription factors that are redox sensitive. Estrogen forms catechol estrogen by oxidation reaction, which are the precursors of quinones. Quinones further turns into semiquinones and reactive oxygen species by a reversible oxidation-reduction reaction, which are potent pro-oxidants causing DNA and protein damage resulting in tumourogenesis.²⁶ Uterine fibroid patients are usually anemic due to excessive blood loss which again makes them more prone to oxidative stress and its consequences.

6. 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 causing mental, social and economic burden resulting in work loss and productivity. Still hysterectomy remains the first line surgical management of uterine fibroids. Serum Aromatase can be used as a marker enzyme for patients with uterine fibroids. Serum testosterone should be recommended as a routine investigation along with other two hormones estrogen and progesterone while evaluating patients with uterine fibroids. Family history, age of menarche and first child birth are indicative factors that can be followed up for developing leiomyoma in near future. Multidisciplinary Research in Uterine fibroids is needed to propose models for planning and developing minimally invasive, less expensive and medical treatment strategies. So that it

will reduce the health care expenditure, hospital stay and most importantly will have a positive impact on outcomes and quality of life for all women suffering from uterine fibroids worldwide.

7. Limitations:

Smaller sample size.

Need to be conducted further in a larger group.

Age factor need to be considered for hormonal analysis.

Follow up of patients for serum aromatase levels after surgery need to be done.

Conflicts of interest:

There is no conflict of interest for this study.

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