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The aromatase expression in uterine mioma tissue

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SUMMARY: **The aromatase expression in uterine mioma tissue.**

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Aim. *The uterine mioma is located within the hyperestrogenic environment. The determination of aromatase expression is incredibly important to determine the pathogenesis and management.*

Method. *The Research is analytic (cross-sectional) observation to 24 block pyrroffin with immunohistochemical (IHC) examination to know the difference between aromatase expression of uterine and surround-ing uterine tissues.*

Result. *Statistically, it's significantly found the correlation between the aromatase expression towards the distance, the further the distance of miometrium from uterine myoma the lower the expression of aromatase enzyme (r = -0.563; p = 0.00).*

Conclusion. *the Expression of aromatase in miometrium is around myoma is lower than uterine myoma, because uterine myomas can synthesize the estrogen and it has the localized aromatization in tissues that have cell growth promoting effect.*

KEY WORDS:
Uterine myoma - Myometrium - Aromatase - Immunohistochemistry.

Introduction

The uterine myoma is a gynecologically benign tumor which is oftentimes found towards the women in the reproductive age ranging from 20-25%, and can continue to grow at the age of 10% of menopause. The incidence of uterine myomas is 60% asymptomatic and almost 50% are found by any chance. In the United States, uterine myomas are connected with many hysterectomies of 200,000 and myomectomy of 30,000/year (1). In Indonesia uterine myoma is in the position of second rank from all gynecological tumors. The incidence of uterine myomas ranges from 20-30% from all women in Indonesia (2).

The growth of uterine myoma is closely linked to steroid hormones such as estrogens. The uterine myoma is located in the hyperestrogenic environment, the increasing of estradiol concentrations increase, hence the estrogen receptor (ER) and progesterone (PR) estrogen receptors are commonly found in the expression of aromatase, due to the process of converting androgens to estrogen. Thus, the uterine myoma tissue becomes hypersensitive (3).

The activity of aromatase is not only found in uterine myoma but also in the eutopic endometrium but not in the normal endometrium, it indicates that the hyperestrogenic environment plays an important role in the development of uterine pathology (4). The smooth muscle of uterine myoma contains high levels of aromatase enzyme synthesizing estrogend compared to the surrounding myometrium (5, 6).

The average recurrence of 5 years post-myomectomy in a single uterine myoma is about 10%, while in the multiple cases 25%, with a hysterectomy risk in 1/3 of recurrent patients, but it has no studies that have linked about the aromatase enzyme activity towards the recurrence, the aromatase expression in uterine myoma cells depends on estrogen, so it is

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crucial in determining the pathogenesis and proper management direction (7).

Based on the above explanation we want to know how the aromatase expression of myometrial tissue in uterine myoma patients, in Haji Adam Malik General Hospital, Universitas Sumatera Utara (USU), Medan.

Method

It has been performed on 24 paraffin blocks of uterine myoma tissue and normal myometrium (2 cm and 4 cm from the margins of the uterine mioma) on the same uterus, it has obtained from hysterectomy and confirmed histopathology.

Inclusion criteria:

1. The paraffin block of the uterine mioma from hysterectomy is confirmed by histopathology. The taken tissue is the tissue of the uterine myoma and the normal myometrium from the periphery.
2. It has no history of gynecological abnormalities such as ovarian cysts, endometriosis and confirmed with histopathology.
3. It has no a history of taking hormonal medication for at least 3 months and confirmed with a medical record.
4. It has no history of previous pelvic surgery.

Exclusion criteria: The damaged block paraffin or IHC staining failed so that it cannot be read.

The paraffin block that fulfill the criteria is checked by IHC to determine the expression of aromatase. The Specimens is labeled with specific CYP19 A1 antibodies.

The IHC examination was performed by 2 Anatomical Pathologists. The Aromatase expression was used by Immunoreactive Score (IRS) / Remmele Score. Scoring system with multiplication of intensity score (IS) and proportion score (PS). Scoring scores are 0-12, score 0-1 (negative), 2-3 (weak positive), 4-8 (positive moderate), and 9-12 (strong positive). The hypothesis of this research is the correlation of aromatase expression in uterine myoma and the surrounding distance.

Statistic analysis

The correlation of aromatase expression in uterine myoma and the surrounding distance was tested by Pearson correlation test ($p < 0.05$) it was considered as meaningful. This study used a confidence level of 95%.

Result

It's found at the age of patients> 20 years with the average age in the 4th decade. The occurrence of uterine myoma is oftentimes found with IMT> 18.5kg / m^2, with the lowest IMT of 19.1 and the highest BMI of 35.4. The incidence of uterine myoma is often considerably found in multiparas (62.5%). The characteristics of the sample in this study can be seen in Table 1.

The most common type of uterine myoma which is mostly found is intramural (54.2%), followed by submucosa (29.2%) and subserosa (16.7%) as seen in Table 2.

Table 1 - CHARACTERISTICS OF THE STUDY SAMPLE.

Parameter	
Age (year)	
Average ± SD	42 ± 7.96
Min.	26
Max.	62
Body Mass Index (BMI)	
Underweight (<18.5)	0 (0%)
Normoweight (18.5-24.9)	11 (45.8%)
Overweight (25-29.9)	9 (37.5%)
Obese (>30)	4 (16.7%)
Paritas	
Nullipara	5 (20.8%)
Primipara	4 (16.7%)
Multipara	15 (62.5%)

Table 2 - DISTRIBUTION OF UTERINE MYOMA TYPE BASED ON LOCATION.

Types of uterine myoma	N	%
Subserosa	4	16.7
Intramural	13	54.2
Submukosa	7	29.2
Total	24	100.0

The expression of aromatase in most uterine myomas is strongly positive (83.3%). While the 2 cm and 4 cm myometrial tissue from the margin of the uterine myoma of strong positive aromatase expression began to decrease respectively 66.7% and 33.3%. In contrast, moderate and low positive expressions in the myometrial tissue of 2 cm and 4 cm from the margin of the uterine myoma are increasing in number compared to uterine myomas. But the three are not found negative aromatase expression. Statistically there was a significant correlation of aromatase expression to tissue distance (p = 0.002), as seen in Table 3 below.

Statistically, there was a significant relationship between aromatase expression towards the myometrial tissue distance in uterine myoma, with moderate correlation relationship (negative, r=-0.563), p value <0.05, meaning the further distance of miometrium from uterine myoma expression of aromatase enzyme is lower. In addition, there was a significant relationship between aromatase expression to parity in uterine myoma, with correlation correlation unidirectional (positive, r=0.276), p value <0.05 means more parity then aromatase enzyme expression is stronger. While the expression of aromatase on age and BMI in uterine myoma we did not find a significant correlation (p> 0.05), as seen in Table 4.

Discussion

In this study we found the average age was 42 ± 7,967 years (average ± SD), the youngest age 26 years and age 62 years old. But we did not find a correlation relationship between aromatase expression of age in uterine myoma sufferers.

The incident of uterine myomas is mostly found in women of reproductive age 20-25%. Uterine myoma has never been found in women who have not menarche, but still can be found after menopause 10%. Based on prevalence study in 2009, there was an increasing of incidence of uterine myoma in age group> 40 years at 14.1%, with mean age 33.5-36.1 years. In the USA there are 60% cases of mioma uteri in African American women aged 50 years, 40% in caucasian women aged 35 years and 70% at age 50

Table 3 - THE DIFFERENCES OF AROMATASE EXPRESSION IN UTERINE MYOMA TISSUE, MYOMETRIAL TISSUE TAKEN 2 CM AND 4 CM FROM THE MARGIN OF THE UTERINE MYOMA.

Aromatase Expression	**Types of network-retrieval**			
	Myoma uteri	**Miometrium (2 cm)**	**Miometrium (4 cm)**	**P**
Strong positive (Score 9-12)	20 (83.3)	16 (66.7)	8 (33.3)	0.02*
Moderate positive (Score 4-8)	4 (16.7)	6 (25.0)	8 (33.3)	
Weak positive (Score 2-3)	0 (0)	2 (8.3)	8 (33.3)	
Negative (0-1)	0 (0)	0 (0)	0 (0)	

*Test Chi-square (p <0.05)

Table 4 - RELATIONSHIP OF AROMATASE EXPRESSION TOWARDS MYOMETRIAL TISSUE DISTANCE IN UTERINE MYOMA, AGE, PARITY, BMI.

	P	**R**
Expression of Aromatese-Distance of Myometrial Tissue to Urine	0.000*	-0.563
Myoma Aromatase-Age Expression	0.441	-0.092
Aromatase-Parity Expression	0.019*	0.276
Expression of aromatase-IMT	0.342	-0.114

* Test Pearson (P <0.05)

years. In Indonesia, there was the highest incident of uterine myoma at the age of 4th decade (56.7%), with mean age 44.47 ± 6,075 (mean ± SD) (2).

Age is a form of risk factor for uterine myoma, this is because the growth and development of uterine myoma is affected by the stimulation of estrogen produced by ovaries. The effect of estrogen exposure on uterine myoma events progresses gradually with increasing age (2). The increasing of estrogen production of extragonad is connected to the increasing of an aromatase activity at the age of menopause (13).

Several studies on the relationship between aromatase mRNA levels showed a positive trend between aromatase and age in patients. This is showing that aromatase expression locally may determine the availability of estrogen in uterine myoma tissue even if circulating estrogen levels decrease in perimenopausal and postmenopausal women. There is no correlation relationship between the level of aromatase expression on age, although there is a trend between age and aromatase expression (11).

In this study the average BMI was 25.86 ± 4.247 (mean ± SD), the lowest BMI was 19.1 and the highest BMI was 35.4. The occurrence of uterine myoma is common in patients with BMI> 18.5 kg/m^2. There was no correlation relationship of aromatase expression to body mass index in this study (19).

Patients with uterine myoma had a BMT cutoff point value of 20.44 kg/m^2, the risk for uterine mioma was greater in patients with BMI above the cutoff point compared with patients with BMI below the cut-off point. Changes in BMI affect the incidence of uterine myoma (14). Orogenity increases the risk of uterine mioma caused by increased estrogen levels (8). Obese people are at increased risk of uterine myomas 2.7 times (10).

The process of aromatic dilation is related to body weight. The increasing of aromatic dilation is higher in obese women. Women who enter the menopause show a decline in ovarian function, resulting in estrogen and progesterone production also decreased. Circulating levels of estradiol in postmenopausal women ranged from 10-20 pg/ml, while estrone levels ranged from 30-70 pg/ml (20).

Besides, this research found the most of uterine myoma incidence in multipara (62.5%), while the incidence of myoma in nullipara and primipara almost the same. Statistically the significant correlation was found between aromatase expression to parity (r = 0.276; p <0.05), the more parity the aromatase enzyme expression grew stronger. The Risk of uterine myomas was compared with parity (8). Women with parity ≥1 were more at risk 2,254 times exposed to uterine myoma compared to nullipara (10). Parity is defined by decreased menstrual cycle and pregnancy, causing changes in ovarian hormone, growth factor, estrogen receptor level and changes in uterine tissue (7).

In this study, it's found the the most common type of uterine myoma was intramural (54.2%), followed by submucosa (29.2%) and subserosa (16.7%). The uterine myoma has a variety of sizes and amounts, it's found the various parts of the uterus including the cervix. The uterine myoma may be embedded in the myometrial wall projected into the uterine cavity, or grows outward towards the pelvic cavity (15).

The subserous uterine myoma can grow to a considerable size. Cytogenetic abnormalities are more common in large uterine myomas, it's showing that cytogenetic abnormalities may influence tumor growth potential (16). In contrast, submucous uterine myomas, are more susceptible to hormonal influences and are present with fewer clonal cytogenic abnormalities than subserosa (17).

In this study, the most aromatase expression is mostly found in uterine myomas was strong positive (83.3%). While on the myometrial tissue taken 2 cm and 4 cm strong positive aromatase expression began to decline from 66.7 to 33.3%. But the three tissues are not found the negative aromatase expression. Statistically, it's found the meaningful correlation between aromatase expression towards myometrial tissue distance (r=-0.563; p<0.05), the further distance of miometrium from uterine myoma expression of aromatase enzyme is lower.

Aromatase expression was found as much as 75% in myometrial tissue biopsied 2 cm from the margins of uterine mioma (9). The changes in healthy tissue bounded with primary tumors, showing a neoclassical focal development in the myometrium. This is proven by the presence of telomerase like active tumors in normal myometrial 4 cm tissue is taken from the uterine mioma border. Tumor cells derived from aromatization in *in situ* pathological tissues act as local mitogenic factors that facilitate indirect tumor growth (12, 18).

In the normal myometrium where we can find estrogen receptors, aromatase expression but the amount numbers is not as many as in uterine myomas. In uterine myomas, it occurs the excessive proliferation of myometrial tissue from genetic mu-

tations so that it causes the suppression of tissue apoptosis, due to uncontrolled regulation of estrogen and progesterone. Due to uncontrolled estrogen and progesterone regulation, an abnormal proliferation of fibroblasts in uterine myoma can cause the more aromatase expression in the uterine myoma tissue than in the normal myometrium (1).

Analogue GnRH can be given to uterine myoma sufferers as it can relieve the bleeding that occurs and may improve ovarian function and maintain fertility (21).

The size of the myoma may shrink according to the age, but the amount of estrogen produced does not decrease until the production of androgens in the suprarenal decreases. The expression of aromatase in uterine myoma is not known for certain. Some genes exhibit overexpression in uterine myomas, compared to the surrounding myometrium. Some genes are stimulated by estrogen and manifest in normal expression in the myometrium of hypoestrogen condition which is induced by agonist releasing hormone gonadotropin (GnRH). The activity of mitogen which is being activated by MAPK protein kinase, stimulating extracellular signals including growth factors causes overexpression of aromatase in uterine myomas. Compared with myometrium, MAPK expression and the increasing activity in myoma tissue, causing different expression of extracellular dimatriks between tissues (1). The expression of aromatase may also be in the endometrium of uterine myomas, because aromatase expression in uterine myomas is higher than the surrounding myometrial and endometrial (22).

From the data that we obtained, the hypothesis in the study that it was found the differences in aromatase expression in uterine myoma tissue and myometrium which is taken 2 cm and 4 cm from the margin of the uterine mioma edge are already answered, the further distance of the myometrium of the uterine myoma expression of aromatase enzyme is lower.

The weakness within this study, we examined the expression of aromatase from the network that is available in patients who undergo hysterectomy. Until now the researchers have not obtained any evidence of research whether it can be detected in blood or urine as a comparison of expression in the tissues. This research has not been able to explain the relationship of expression distance towards recurrence, this research is expected to be the basis for further research.

Conclusion

The uterine myoma can synthesize the estrogen and it has localized aromatization in the tissues that has cell growth promoting effects. The observation of aromatase expression can be used as evidence of uterine myomas in the myometrium. Although the aromatase expression in myometrium around myomas is lower than uterine myoma, but its expression is higher than the normal myometrium.

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