Giorn. It. Ost. Gin. Vol. XL - n. 4
Ottobre-Dicembre 2018

clinical case

Difference in expression of 17 beta-hydroxysteroid dehydrogenase type 1 in uterine fibroid and normal myometrium

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SUMMARY: **Difference in expression of 17 beta-hydroxysteroid dehydrogenase type 1 in uterine fibroid and normal myometrium.**

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Background. *Uterine fibroid is a gynecological problem that frequently occurs to women in reproductive age. Based on World Health Organization (WHO), in 2010, uterine fibroid was estimated occurred in approximately 20-25% women worldwide, while in Indonesia, the incidence was 2.39-11.7%. Estrogen is one of the things that takes a role in uterine fibroid development. 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) is a type of 17β-HSD enzyme that most often is associated in estrogen metabolism.*

Objective. *To determine the difference in expression of 17β-HSD1 enzyme in uterine fibroid and normal myometrium.*

Methods. *This study is a case control study that was conducted in each 20 tissues of uterine fibroid and normal myometrium of the same uterine that were randomly taken from September to November 2017 in Adam Malik General Hospital. The data were subsequently analyzed using Wilcoxon Signed Rank test.*

Results. *It was found that patients with obese body mass index, multiparity, and submucous uterine fibroid type were the largest groups in this study. Strongly positive expression was found highest in uterine fibroid group (75%). There is a difference in expression of 17β-HSD1 in uterine fibroid and normal myometrium with $p<0.05$.*

Conclusion. *The results of this study demonstrate a statistically significant difference between uterine fibroid and normal myometrium.*

KEY WORDS: 17β-HSD1 - Uterine fibroid - Normal myometrium.

Background

Uterine fibroid is a gynecological problem that often occurs in women of reproductive age. Uterine fibroid is a benign genital tract tumor that is often associated with sub-fertility and spontaneous abortion. According to the data from the World Health Organization (WHO) in 2010, uterine fibroid is estimated to be approximately 20-25% of women or reaching 235 million women which reflects 6.6% of the female population worldwide. The incidence of uterine fibroid begins at a young age, increases according to age until menopause, and reaches a peak at the age of 40 years with a prevalence of 5-21% in women of reproductive age and 70-80% in women who have reached the age of 50 years globally (1-3). In Indonesia, the incidence of uterine fibroid is 2.39-11.7% in all gynecological inpatients. In addition, according to annual report data from dr. Hasan Sadikin General Hospital Bandung, the incidence of uterine fibroid was around 6.34-12.46% (4, 5).

Most women with uterine fibroid are asymptomatic at first and become less noticed so that they become undiagnosed. Of the 70-80% of women who have uterine fibroid, only 25% of women show symptoms. Women with symptomatic uterine fibroids usually present with complaints of abdominopelvic mass with or without abnormal uterine bleeding, such as menorrhagia. In addition, other symptoms, such as fullness, pelvic pain,

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constipation, retention or urinary incontinence can also be complained of. These symptoms can adversely affect quality of life and productivity, especially in women who live in developing countries (1, 6, 7).

In the development of uterine fibroid, there are several things that play a role, one of which is estrogen. Estrogen in its metabolism is aided by 17β-hydroxysteroid dehydrogenase (17β-HSD), where 17β-HSD plays an important role in the regulation, synthesis, inactivation, and metabolism of the end steroid hormone. 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) is the type most commonly associated with estrogen metabolism. This is an enzyme that converts estrone to estradiol (the active form of estrogen) in estrogen synthesis (8-10).

Estradiol is an active form of estrogen that binds closely to estrogen receptors. Estradiol has many roles in the uterus, starting from fertility regulation to regulation of growth and proliferation of myometrial cells. This can increase uterine fibroid growth (6, 7, 11).

In a study conducted by Kasai et al., it was found that uterine fibroid tissue expressed 17β-HSD1 in large quantities when compared to the surrounding myometrial tissue. In other words, the expression of 17β-HSD1 plays an important role in the occurrence of uterine fibroid (12). Based on these reasons, this study aims to assess difference in the expression of 17β-HSD1 enzyme in uterine fibroid and normal myometrium.

Methods

Subject

The study was conducted in the Department of Obstetrics and Gynecology, H. Adam Malik General Hospital Medan from January to March 2018. The target population was the entire paraffin blocks of uterine fibroid patients in the Department of Anatomical Pathology, H. Adam Malik General Hospital Medan. Samples were taken randomly from paraffin blocks between September and November 2017 as many as 20 uterine fibroid tissues and 20 normal myometrial tissue from the same uterus.

The inclusion criteria for the case group in this study were uterine fibroid tissue obtained from hysterectomy and confirmed by histopathological examination. In addition, the inclusion criteria for the control group were normal myometrial tissue from uterine fibroid patients with a distance of Ø>2 cm from the location of uterine fibroid tissue. Exclusion criteria in this study were damaged paraffin blocks and failed immunohistochemical staining so that reading could not be carried out. This research was approved by the Ethics Commission for Health Research, Faculty of Medicine, University of Sumatera Utara/H. Adam Malik General Hospital Medan.

Demographics and clinical data

Demographic data were recorded in the form of age, parity, body mass index, and type of uterine fibroid. Parity is defined as the number of births both alive and dead and divided into nullipara, primipara, and multiparous groups.

Body mass index (BMI) is defined as the calculation of body weight in kilograms divided by squared height in meters grouped in underweight (<18.5 kg/m^2), normoweight (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2), and obese (≥30 kg/m^2). The type of uterine fibroid is determined based on the results of histopathological examination.

Determination of expression of 17β-HSD1

The selected paraffin blocks are stored in the freezer until they are cool enough, then thinly cut using a 4 μm thick microtome and affixed to coated object glasses. Then the paraffin block was stained with hematoxylin-eosin. Preparations that are ready to be applied then put into a full automatic Bondmax for 4 hours. The primary antibody used was Cytochrome P459 1A1 (human) polyclonal antibody with a 1:100 dilution. Furthermore, dehydration is done by sequentially dipping on 70, 80, 90%, and 98% ethanol liquids, each of which is 20 dips. Then, the preparation is put into xylol liquid for 3 minutes. After that, the paste is dripped and covered with a cover glass.

The next step, the results of immunohistochemical examination was read under a microscope by two observers that are two anatomical pathology specialists. The results of the examination are displayed in the form of a score called the Immunoreactive Score (Table 1). This score is the result of multiplication of the percentage score of the cell which is colored/Proportion Score (PS) and the color intensity/Intensity Score (IS) (Table 2) (13).

Table 1 - IMMUNOREACTIVE SCORE.

Total Score	Interpretation
0-1	Negative
2-3	Weak Positive
4-8	Moderate Positive
9-12	Strong Positive

Table 2 - ASSESSMENT OF PROPORTION SCORE (PS) AND INTENSITY SCORE (IS).

PS Observed	PS or IS	IS Observed
none is stained	0	none is stained
less than 1% stained cells	1	weak color intensity
1 – 10% stained cells	2	moderate color intensity
11 – 33% stained cells	3	strong color intensity
34 – 66% stained cells	4	
67 – 100% stained cells	5	

Statistic analysis

Data is collected and analyzed with SPSS 20.0 for Windows. Numerical variables are displayed in the mean ± standard deviation. Categorical variables are displayed in frequency and percentage. The Kolmogorov-Smirnov test is used to assess the normal distribution of numerical variables. Kappa test is used to see the agreement between the two observers. Differences in expression of 17β-HSD1 in uterine fibroid and normal myometrium were analyzed with Wilcoxon signed rank test. The value of $p < 0.05$ was considered as significant.

Results

In this study, the results of Kappa test were 87.5% which showed that there was agreement/equality between the two observers from the Department of Anatomical Pathology, H. Adam Malik General Hospital Medan so that the data from one observer could be used to perform statistical analysis. Frequency distribution of research subjects is shown in Table 3.

In this study, the same number was found in the group of patients with age ≤40 years and >40 years which is 10 people. The highest BMI was in the obese group which was 10 people (50%), followed by normoweight and overweight groups as many as 5 people (25%), where the lowest BMI was 19.1 kg/m^2 and the highest was 35.4 kg/m^2. The highest number was also found in the multiparous parity group (55%) and the lowest was in the primipara group (20%). The submucosal group was the most common type of uterine fibroid in this study (55%).

The expression of 17β-HSD1 in both tissue groups is shown in Table 4, where the strong positive expression was the most result in both groups and no negative results were found. There was statistically significant difference between the two groups (p=0.003).

Discussion

Looking at the age distribution, in this study there were similar proportions between the two age groups of patients (≤ 40 years and >40 years). During the reproductive period, the risk of developing fibroid increases with age. Fibroids do not occur before puberty and their frequency decreases with menopause (14). Uterine fibroids are diagnosed in 20-25% of women of reproductive age, and 30-40% of women older than 40 years (15).

In a retrospective study of ultrasound records of fibroid patients in Israel, those aged 41-50 or 51-60 years had a ten times greater risk of suffering from fibroid than those aged 21-30 years (16). However, in the postmenopausal age group, that is more than 60 years, the risk of fibroid decreases. A similar retrospective review of USG recordings in the United Kingdom found that women over the age of

TABLE 3 - FREQUENCY DISTRIBUTION OF SUBJECTS.

Parameter	**Subjects**	
	n	**%**
Age (years)		
≤ 40	10	50.0
> 40	10	50.0
Body Mass Index (kg/m^2)		
Normoweight (18.5-22.9)	5	25.0
Overweight (23-24.9)	5	25.0
Obese (≥ 25)	10	50.0
Parity		
Nullipara	5	25.0
Primipara	4	20.0
Multiparous	11	55.0
Fibroid Type		
Subserous	6	30.0
Intramural	3	15.0
Submucous	11	55.0

TABLE 4 - DIFFERENCE IN EXPRESSION OF17β-HSD1.

17β-HSD1Expression	**Uterine Fibroid**		**Normal Myometrium**		
	n	**%**	**n**	**%**	***p***
Strong Positive	15	75.0	8	40.0	
Moderate Positive	5	25.0	7	35.0	0.003
Weak Positive	0	0	5	25.0	

40 had a four times greater risk of developing fibroid than those under 40 years (17).

Patients with obese body mass index dominated the percentage in this study sample. Significantly high body mass index was reported in cohort studies in Finland (18). He et al. also found an increased risk of fibroid in premenopausal Asian women with high BMI (19). This may be due to an increase in the amount of circulating estrogen produced by aromatization of androgens from peripheral fat tissue in obese women (20).

Based on the amount of parity, multiparous was obtained as the most research sample group. The inverse relationship between uterine fibroid risk and the amount of parity has long been known and an increase in the number of long-term pregnancies reduces the risk of fibroid (21). Hormonal and non-hormonal mechanisms can explain the mechanism of this relationship. Parity means a decrease in the menstrual cycle and aterm pregnancy causing changes in ovarian hormones, growth factors and estrogen receptor levels, and changes in uterine tissue (22).

Based on the type of fibroid, submucous fibroid is the most common type of fibroid in this study. A cross-sectional study of 986 first premenopausal patients aged 35-49 years, reported subserous fibroid as the most common type of fibroid. There is no

significant amount in the profile of the factors in all three types of fibroid (23).

In this study, it was found that there was a significant difference between the expression of 17β-HSD1 with uterine fibroid and normal myometrium. This is in line with research conducted by Kasai et al. who found that uterine fibroid tissue revealed a large amount of 17β-HSD1 when compared to the surrounding myometrial tissue (12). It was found that stronger expression was associated with a higher risk of uterine fibroid. 17β-HSD catalyzes the interconversion between androstenedione and testosterone, and also between estrone and estradiol so it plays an important role (in combination with aromatase activity) in determining tissue estradiol concentration. 17β-HSD activity has been detected in normal uterine and myometrial fibroid tissue (24). Eiletz et al. found 17β-HSD oxidative activity that converts estradiol to low estrone in uterine fibroids compared to normal myometrium during the menstrual cycle. The difference in activity level becomes prominent, when the total myometrial activity increases more than a few times compared to the increase seen in uterine fibroid (25). Only one study compared reductive 17β-HSD1, being part of estrone to estradiol, between uterine fibroid and normal myometrium, where it was found that uterine fibroid had a level of reductive activity similar to normal myometrium (26).

Conclusion

In conclusion, this study found a significant difference in expression of 17β-HSD1 enzyme between uterine fibroid and normal myometrium tissue.

References

1. Ekine AA, Lawani LO, Iyoke CA, Jeremiah I, Ibrahim IA. Review of the clinical presentation of uterine fibroid and the effect of therapeutic intervention on fertility. Am J Clin Med Res. 2015;3(1):9-13.
2. Vilos GA, Allaire C, Laberge PY, Leyland N. The management of uterine leiomyomas. J Obstet Gynaecol Can. 2015;37(2):157-178.
3. Laughlin SK, Stewart EA. Uterine leiomyomas: Individualizing the approach to a heterogeneous condition. Obstet Gynecol. 2011;117(2):396-403.
4. Siregar MFG. Association between menarche age and menstrual disorder with the incidence of uterine fibroid in Medan, Indonesia: based on hospital data. Int J Reprod Contracept Obstet Gynecol. 2015;4(4):1025-1028.
5. Ilma N, Tjahyadi D, Judistiani TD. The relationship of age, parity, and body mass index as risk factors to the incidence of uterine myoma in dr. Hasan Sadikin general hospital. Althea Med J. 2015;2(3):409-413.
6. Georgieva B, Milev I, Minkov I, Dimitrova I, Bradford AP, Baev V. Characterization of the uterine leiomyoma microRNAome by deep sequencing. Genomic. 2012;99(5):275-281.
7. Zimmermann A, Bernuit D, Gerlinger C, Schaefers M, Geppert K. 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.
8. Mu XY, Du X, Yao K, Zhao JT, Bian C, Wang Q, et al. Association between HSD17B1 rs605059 polymorphisms and the risk of uterine diseases: a systematic review and meta-analysis. Int J Clin Exp Pathol. 2015;8(6):6012-6018.
9. Spadaro A, Frotscher M, Hartmann RW. Optimization of hydroxybenzothiazoles as novel potent and selective inhibitors of 17β-HSD1. J Med Chem. 2012;55:2469-2473.
10. Marchais-Oberwinkler S, Henn C, Moller G, Klein T, Negri M, Oster A, et al. 17β-hydroxysteroid dehydrogenases (17β-HSDs) as therapeutic targets: Protein structures, functions, and recent progress in inhibitor development. J Steroid Biochem Mol Biol. 2011;125:66-82.
11. Cohen S, Sewell C. Uterine leiomyomas. In: Hurt KJ, Guile MW, Bienstock JL, Fox HE, Wallach EE. The John Hopkins Manual of Gynecology and Obstetrics. 4th eds. Philadelphia: Lippincott Williams & Wilkins. 2011. pp. 448-453.
12. Kasai T, Shouzu M, Murakami K, Segawa T, Shinohara K, Nomura K, et al. Increased expression of type I 17β-hydroxysteroid dehydrogenase enchances in situ production of estradiol in uterine leiomyoma. J Clin Endocrinol Metab. 2004;89(11):5661-5668.
13. Fedchenko N, Reinfenrath J. Different approaches for interpretation and reporting of immunohistochemistry analysis results in the bone tissue – a review. Diagnostic Pathol. 2014;9:221.
14. Othman EE, Al-Hendy A. Molecular genetics and racial disparities of uterine leiomyomas. Best Pract Res Clin Obstet Gynaecol. 2008;22(4):589-601.
15. Duhan N. Current and emerging treatments of uterine myoma−An update. Int J Womens Health. 2011;3:231-241.
16. Lurie S, Piper I, Woliovitch I, Glezerman M. Age related prevalence of sonographicaly confirmed uterine myomas. J Obstet Gynaecol. 2005;25:42-44.
17. Selo-Ojeme D, Lawal O, Shah J, Mandal R, Pathak S, Selo-Ojeme U, et al. The incidence of uterine leiomyoma and other pelvic ultrasonographic findings in 2,034 consecutive women in a north London hospital. J Obstet Gynaecol. 2008;28:421-423.
18. Luoto R, Kaprio J, Rutanen EM, Taipale P, Perola M, Koskenvuo M. Heritabilty and risk factors of uterine fibroids: the Finnish twin cohort study. Maturitas. 2000;37(1):15-26.
19. He Y, Zeng Q, Li X, Liu B, Wang P. The association between subclinical atherosclerosis and uterine fibroids. PLoS One. 2013;8(2):e57089.
20. He Y, Zeng Q, Dong S, Qin L, Li G, Wang P. Associations between uterine fibroids and lifestyles including diet, physical activity and stress: A case control study in China. Asia Pac J Clin

Nutr. 2013;22(1):109-117.
21. Wise LA, Laughlin-Tommaso SK. Uterine leiomyomata. In: Goldman MB, Troisi R, Rexrode KM, eds. Women and Health. San Diego: Academic Press;2013. pp.285-306.
22. Wise LA, Palmer JR, Harlow BL, Spiegelman D, Stewart EA, Adams-Campbell LL, et al. Reproductive factors, hormonal contraception and risk of uterine leiomyomata in African-American women: A prospective study. Am J Epidemiol. 2004;159 (2):113-123.
23. Dragomir AD, Schroeder JC, Connolly A, Kupper LL, Hill MC, Olshan AF, et al. Potential risk factors associated with subtypes of uterine leiomyomata. Reprod Sci. 2010;17(11):1029-1035.
24. Borahay MA, Asoglu MR, Mas A, Adam S, Kilic GS, Al-Hendy A. Estrogen receptors and signaling in fibroids: Role in pathobiology and therapeutic implications. Reprod Sci. 2017;24 (9):1235-1244.
25. Eiletz J, Genz T, Pollow K, Schmidt-Gollwitzer M. Sex steroid levels in serum, myometrium, and fibromyomata in correlation with cytoplasmic receptors and 17β-HSD activity in different age-groups and phases of the menstrual cycle. Arch Gynecol Obstet. 1980:229(1):13-28.
26. Newton CJ, James VHT. 17β-Hydroxysteroid dehydrogenase activity in leiomyoma and myometrium and its relationship to concentrations of oestrone, oestradiol and progesterone throughout the menstrual cycle. J Steroid Biochem Mol Biol. 1985;22 (4):487-493.