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Association between mRNA Expression of Aromatase, 17β-HSD2, Level of TGF-β1 and Stage of Endometriosis

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Abstract

Aim: To identify the correlation between mRNA expression of aromatase, 17β-HSD2 level of TGF- β1 and stage of endometriosis. Methods: Case control study was done on 80 patients divided by 40 endometriosis patients and 40 patients as control. mRNA expression of aromatase, mRNA 17β- SD2, was checked using reverse-transcriptase polymerase chain reaction (RT-PCR) and TGF-β1 serum and peritoneal fluid was checked using ELISA. Result: Level of mRNA aromatase from ectopic and eutopic endometrium increased significantly compared to control group ($p < 0.001$). mRNA 17β-HSD2 expression is significantly lower compared to control ($p < 0.001$). Level of TGF- β1 on endometriosis group was significantly higher compared to control group ($p < 0.05$). Level of peritoneal fluid TGF-β1 on endometriosis group was higher than control group ($p < 0.001$). There are no correlations between mRNA aromatase, type 2 17β-HSD, TGF-β1 serum level to endometriosis stage. There are significant correlations between peritoneal fluid TGF-β1 levels to endometriosis stage.

Keywords

Aromatase, 17βHSD2, TGFβ1, Stage Endometriosis

1. Introduction

Endometriosis is a disease caused by abnormal tissue growth mimicking endometrial tissue which adheres and lives outside the uterus. It's incidence ranged around 5% - 10% of reproductive age [1].

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Etiopathogenesis of endometriosis has not been fully understood. Many factors are proposed such as genetics and hormonal cause. Risk of acquiring endometriosis increases 7 folds if one of the families acquired endometriosis [2] [3].

Estrogen is hormone which is known to influence the growth of endometriosis. The strongest estrogenic influence is 17β-estradiol which originates from C_{19}steroid catalyzed by P450 aromatase enzyme. In advance, 17β-estradiol production located in ovarium, but latest study shows that endometriosis tissue also produces it [4]. [5] mRNA expression level found highly in extra ovarium endometriosis and endometriosis cysts (endometrioma). Estrogenic effect on normal endometrium was lower than endometriosis tissue. It is because of type 2 17β-HSD (17β-HSD2) enzyme that converts 17β-estradiol into estron with weak estrogenic effect [6]-[9].

mRNA expression or 17β-HSD2 protein almost did not find on endometriosis, but its' expression was highly found in eutopic endometrium in patient without endometriosis on secretion phase. It is concluded that endometriosis didn't have mechanism to lower estradiol level [10].

TGF-β1 is a cytokine that is responsible for endometriosis cycle because it regulates cell cycle and stimulates endometrium stroma cell [11]. Immune system suppresses through TGF-β1 secretion by endometrium cell mimicked metastase process [12] and stimulates inflammation in peritoneum [13].

During this study, correlation between mRNA aromatase, 17β-HSD2 level, TGF-β1 level to stage of endometriosis will be discussed further.

2. Methods

Observational analytic and case control study was done to know the correlation between mRNA aromatase, 17β-HSD2 level, TGF-β1 level to stage of endometriosis.

Samples are taken from endometriosis patients and normal endometrium as control. All of the samples are included in inclusion criteria.

Results of the study was inputted in forms for editting, verification and input for further analysis. Data analysis was done using SPSS 13th on Windows. Statistical analysis started with normality test on parametric data (TGF-β1 level, aromatase and type-2 17β-hydroxysteroid dehidrogenase).

Mann-Whitney test was done to compare mRNA aromatase expression, 17βHSD2, andTGF-β if data spreading is abnormal.t-test was done to compare the difference between mRNA aromatase expression, 17βHSD2, and TGF-β if data spreading is normal. Multiple logistic regression was done to manage bias variable.

3. Results

This study was done in Hasan Sadikin General Hospital during April 2011 until September 2013. 80 cases were divided into 40 control and 40 sample. Analysis on endometriosis tissue, serum and peritoneal fluid was done to analyse aromatase expression, 17β-HSD2, and TGF-β1 level. Specifically on endometriosis group, aromatase and17β-HSD2 was analyzed on ectopic and eutopic tissue, and stage of endometriosis.

First, Kolmogorov-Smirnov test was done to test data normality. When the result is $p < 0.05$, nonparametric analysis will be used. If the result is $p \geq 0.05$, parametric analysis will be used. On normal numerical data distribution, mean, standard deviation, and range will be used. As for abnormal distribution data, only median and ranged will be used. Mann Whitney test was done to independent group and Wilcoxon test will be done to pair groups (**Table 1**).

Based on age, most of endometriosis patients are in 35 - 39 years old (n = 21). There are no significant differences between endometriosis group compared to control group ($p > 0.05$).

Table 2 shows that aromatase expression level in endometriosis group are higher (8.09) than control group (2.86) and statistically significant ($p < 0.001$). Eutopic aromatase expression was higher (4.90) than control group (2.86) and statistically significant ($p < 0.05$).

Table 3 shows that 17β-HSD2 expression in ectopic group is lower compared to control group (0.21:3.07) and statistically significant ($p < 0.001$). It also shows that 17β-HSD2 expression in eutopic endometriosis is lower compared to control group and statistically significant (1.96:3.07).

Table 4 shows that TGF-β1 serum level in endometriosis is higher than control group significantly ($p < 0.05$).

Table 5 shows that peritoneal fluid TGF-β1 level in endometriosis group are higher than control group significantly ($p < 0.001$).

Table 6 shows that aromatase expression level in ectopic endometrium tissue is higher than eutopic endome-

Table 1. Subjects characteristic.

Age	Group		p value
	Endometriosis (n = 40)	Control (n = 40)	
			0.581
20 - 34	3 (7.5%)	4 (10%)	
35 - 39	21 (52.5%)	23 (57.5%)	
40 - 44	16 (40%)	13 (32.5%)	
Standard(SD)	38.35 (3.98)	37.92 (3.99)	
Deviation	27 - 44	27 - 44	

Table 2. Comparison of mRNA aromatase expression in endometriosis and control group.

Variable	Group		p value
	Endometriosis	Control	
1. Ectopic mRNA *Aromatase* expression	n = 40	n = 40	**<0.001**
(SD)	8.09 (1.55)	2.86 (1.55)	
2. Eutopic mRNA *Aromatase* expression	n = 25	n = 40	**<0.001**
(SD)	4.90 (1.21)	2.86 (1.55)	

Note: Based on unpair T test.

Table 3. Comparison of mRNA 17β-HSD2 expression in endometriosis and control group.

Variable	Group		p Value
	Endometriosis	Control	
1. Ectopic mRNA 17β-HSD2 expression	n = 40	n = 40	**<0.001**
Median	0.21	3.07	
Range	0.01 - 0.84	1.07 - 10.13	
2. Eutopic mRNA 17β-HSD2 expression	n = 25	n = 40	**<0.001**
Median	1.96	3.07	
Range	0.49 - 4.13	1.07 - 10.13	

Note: Based on Mann Whitney Test.

Table 4. Comparison of TGF-β1 serum level in endometriosis and control group.

TGF-β1serum level (pg/mL)	Group		P value
	Endometriosis	Control	
	n = 40	n = 40	**0.019**[*)]
Standard Deviaton (SD)	32.300 (8340.50)	28.000 (6052.58)	

Note: Based on unpaired T Test.

Table 5. Comparison of peritoneal fluid TGF-β1 level in endometriosis and control group.

Peritoneal Fluid TGF-β1 level (pg/mL)	Group		p value
	Endometriosis	Control	
	n = 40	n = 40	**<0.001**
Median	574.66	288.18	
Renting	154.14 - 1415.97	26.16 - 988.84	

Note: Based on Mann Whitney Test.

Table 6. Comparison of mRNA aromatase expression and dan 17β-HSD2 in ectopic and eutopic endometriosis patients.

Variable	Location		p value
	Ectopic (n = 40)	Eutopic (n = 25)	
1. mRNA *Aromatase* expression			**<0.001**[*)]
Standard Deviation (SD)	7.58 (1.11)	4.90 (1.21)	
2. mRNA 17β-HSD2 expression			**<0.001**[**)]
Median	0.21	1.96	
Range	0.04 - 0.52	0.49 - 4.13	

Note: [*)]Based on paired t test; [**)]Based on Wilcoxon test.

triosis significantly ($p < 0.001$). 17β-HSD2expression level is also higher in eutopic endometriosis compared to ectopic endometriosis significantly ($p < 0.001$).

Table 7 shows that aromatase expression in severe endometriosis is not statistically significant compared to mild endometriosis ($p > 0.05$).17β-HSD2 expression level in mild endometriosis compared to severe endometriosis is also not statistically significant ($p > 0.05$). TGF-β1 level in severe endometriosis is higher compared to mild endometriosis but not significant ($p > 0.05$). Peritoneal fluid TGF-β1 serum level in severe endometriosis is higher compared to mild endometriosis significantly ($p < 0.001$).

Table 8 shows that TGF-β1 peritoneal fluid level is the most significantly correlated with beta coefficient of 0.623 and $p < 0.001$. Because other factors don't have significant correlation to stage of endometriosis, therefore biserial coefficient statistical analysis was done.

Table 9 shows the correlation between cut off point of each variable to incidence of endometriosis. Odd ratio forTGF-β1serum level shows that subjects with high level ofTGF-β1 (>33352.71 pg/mL) increase the risk of endometriosis 13.632 times higher than subjects withTGF-β1 level less than 33352.71 pg/mL. Based on peritoneal fluidTGF-β1 level, high level of peritoneal fluidTGF-β1 level (>336.05 pg/mL) will increase risk of endometriosis 11.67 higher that subjects with peritoneal fluid TGF-β1 level less than 336.05 pg/mL.

4. Discussion

It was said that endometriosis was derived from endometrium tissue that grow outside the uterus. Growth and development of endometriosis lesion are estrogen-dependent. Estrogen metabolism like aromatase expression and 17β-HSD2 expression have pattern changes either in eutopic or ectopic endometrium in endometriosis patien [14] [15]-[17].

Result of the study shows that aromatase expression in ectopic endometriosis is higher than control group. It shows that endometriosis tissue has the ability to synthesize local estrogen to support lesion development [6].

On control group endometrium tissue, we found that aromatase expression is very low. It means that endometrium can't synthesize local estradiol alone. Proliferation on endometrium was thought influenced by peripherall estradiol which mainly comes from ovary and done synergically with ovulatory cycle [14].

Aromatase expression in eutopic endometrium also found higher than control. It may be caused by endometrium lession that altered peritoneal fluid status [18].

Table 7. Aromatase, 17β-HSD2, and TGF-β1 (serum dan fluid) expression level based on stage of endometriosis.

Variable	Stage		p value*)
	Mild (n = 10)	Severe (n = 30)	
1. Ectopic mRNA *Aromatase* expression			**0.548**
Median	7.56	7.77	
Range	6.77 - 11.36	5.20 - 11.60	
2. Ectopic mRNA 17β-HSD2 expression			**0.794**
Median	0.19	0.24	
Range	0.12 - 0.51	0.01 - 0.84	
3. TGF-β1serum level (pg/mL)			**0.792**
Standard Deviation (SD)	31,720.76 (7986.10)	32,537.76 (8578.21)	
Range	16,559.26 - 40110.11	17,957.35 - 51171.01	
4. Peritoneal fluid TGF-β1 level (pg/mL)			**< 0.001**
Standard Deviation (SD)	302.869 (81.891)	780.083 (301.169)	
Range	154.14 - 387.46	394.80 - 1415.97	

Notes: *)Based on Mann-Whitney test (aromatase and17β-HSD2) and t test (TGF-β1 serum and peritoneal fluid).

Table 8. Correlation between mRNA aromatase expression, 17β-HSD2, TGF-β1 (serum and fluid) level with stage of endometriosis.

Correlation of Stage to	Pbi Coefficient	p value
1. EctopicmRNA Aromatase expression	0.040	0.807
2. EutopicmRNA Aromatase expression	−0.142	0.499
3. EctopicmRNA 17β-HSD2 expression	0.041	0.80
4. EutopicmRNA 17β-HSD2 expression	0.121	0.564
5. TGF-β1 serum level	0.043	0.792
6. TGF-β1 peritoneal fluid level	0.623	<0.001

Note: Pbi coefficient = biserial point coefficient correlation.

Table 9. Correlation of cut off point based on ROC to incidence of endometriosis.

Variable (*Cut off point*)	Group		p value	OR
	Endometriosis (n = 40)	Control (n = 40)		(CI 95%)
1. TGF-β1serum level (pg/mL)			**<0.001**	**13.632**
>33,352.71	21	3		**(3.60 - 51.55)**
≤33,352.71	19	37		
2. Peritoneal fluid TGF-β1 level (pg/mL)			**<0.001**	**11.667**
>336.05	35	15		**(3.75 - 36.29)**
≤336.05	5	25		

Notes: OR: Odd Ratio, CI: Confidence interval.

Literature said that endometriosis lession can alter peritoenal fluid status. Local estradiol produced by endometriosis tissue that secretes out to peritoneal cavity will cause higher estradiol level in peritoneal fluid on endometriosis patients [19].

Peritoneal fluid could also enter into uterine cavity which causes exposure to endometrium. Because of that, eutopic endometrium could express higher aromatase level compared to control.

Some theories said that normal endometrium cannot express aromatase [20] [21]. But, in this study, we found that aromatase expression in control group. Matsuzaki also find similar findings in his study [4] that shows mRNA aromatase expression that detected in 4 women with normal pelvic cavity. One of the causes could be because of microscopic endometriosis or adenomyosis [22].

Aromatase expression in eutopic and ectopic endometrium on endometriosis patient shows differences. Study shows that the difference could be caused by regulation of mRNA aromatase expression such as SF-1. SF-1 expression was found in endometriosis but not in normal endometrium. It is concluded that SF-1 expression was responsible to estrogen production from PGE2 pathway through aromatase promotor gene activation [18].

Growth and development of endometriosis are estrogen dependent. 17βHSD2 is one of the enzymes predicted to influence estrogen metabolism. 17βHSD2 expression was found higher in control group compared to endometriosis lession. It is concluded that alteration in 17βHSD2 happened endometriosis tissue [23].

Result of the study shows that difference of TGFβ-1 serum level in endometriosis patients is higher than control. It means that there are potential influence of immunology disturbance and inflammation in endometriosis patients.

Comparison of aromatase expression in mild and severe doesn't show any difference. It means that there are no correlation between aromatase expression and stage of endometriosis. Same result is applied to correlation of 17βHSD2 expression to stage of endometriosis [3].

TGFβ-1 level in endometriosis peritoneal fluid is found higher in severe endometriosis compared to mild endometriosis. It shows that there is correlation between peritoneal fluid TGFβ-1 level and stage of endometriosis [24].

5. Study Limitation

Study limitation in this study is the difficulties to obtain good tissue either eutopic endometrium or control tissue.

Sample collecting for this study requires a long time to complete. It is related to inclusion and exclusion criteria for selection. Most of the patients came to Hasan Sadikin General Hospital diagnosed as severe endometriosis, only few of them diagnosed as mild endometriosis.

6. Conclusion

Lower mRNA 17β-HSD2 expression in eutopic and ectopic endometrium compared to control group. TGF-β1 serum level found higher in endometriosis group compared to control group. Peritoneal fluid TGF-β1 level found higher in endometriosis group compared to control group. mRNA aromatase expression level, 17β-HSD2 and TGF-β1 level didn't have correlation to stage of endometriosis, but peritoneal fluid TGF-β1 was correlated to stage of endometriosis.

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Conflict of Interest

None declared.

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