Research Article

Profile of Estrogen Metabolism in Endometriosis Patients

Profil Metabolisme Estrogen pada Penderita Endometriosis

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Abstract

Objective: To assess the estrone (E1), estradiol (E2) and estriol (E3) blood level and its ratio (E2:E1, E2:E3 and E1:E3) between women with and without endometriosis.

Method: We performed an analytical cross sectional study with 27 women with endometriosis and 27 women without endometriosis who met the inclusion criteria. The samples were recruited in Dr. Cipto Mangunkusumo hospital and other satellite hospitals from October 2012 to April 2013. The blood level of estrogen metabolites was examined by enzyme-linked immunosorbent assay (ELISA). Comparison between the two groups was analyzed by using Mann-Whitney test.

Result: The level of estrone was found to be lower in endometriosis group compared to that in the control group (54.66 pg/ml vs 73.52 pg/ml, p=0.229). Similarly, the levels of estradiol and estriol were lower in endometriosis group (29 pg/ml vs 35 pg/ml, p=0.815 and 1.11 pg/ml vs 1.67 pg/ml, p=0.095, consecutively). The E2:E1 ratio was higher in endometriosis group (0.51 pg/ml vs 0.38 pg/ml, p=0.164), as well as E2:E3 ratio (26.53 pg/ml vs 21.11 pg/ml, p=0.223) and the E1:E3 ratio (58.55 pg/ml vs 50.28 pg/ml, p=0.684). However, all those differences were not statistically significant.

Conclusion: The estrone, estradiol and estriol levels in women with endometriosis were lower compared to those in women without endometriosis. The ratio of E2:E1, E2:E3 and E1:E3 were higher in endometriosis group. However, all those differences failed to reach statistical significance.

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Keywords: endometriosis, estradiol, estriol, estrogen, estrone

Abstrak

***Tujuan**: Menganalisis kadar estron (E1), estradiol (E2) dan estriol (E3) dalam darah dan rasio E2:E1, E2:E3 dan E1:E3 antara wanita dengan dan tanpa endometriosis.*

***Metode**: Dikerjakan penelitian dengan desain potong lintang analitik pada 27 wanita dengan endometriosis dan 27 wanita tanpa endometriosis yang memenuhi kriteria inklusi. Sampel didapatkan dari Rumah Sakit Dr. Cipto Mangunkusumo dan rumah sakit jejaring lainnya periode Oktober 2012-April 2013. Kadar metabolit estrogen dalam darah diperiksa dengan uji enzyme-linked immunosorbent (ELISA). Perbandingan data antara dua kelompok dianalisis dengan uji Mann-Whitney.*

***Hasil**: Kadar estron ditemukan lebih rendah pada kelompok endometriosis dibandingkan kontrol (54,66 pg/ml vs 73,52 pg/ml, p=0,229). Demikian pula, kadar estradiol dan estriol lebih rendah pada kelompok endometriosis (29 pg/ml vs 35 pg/ml, p=0,815 dan 1,11 pg/ml vs 1,67 pg/ml, p=0,095, berturut-turut). Rasio E2:E1 lebih tinggi pada kelompok endometriosis (0,51 pg/ml vs 0,38 pg/ml, p=0,164), demikian pula dengan rasio E2:E3 (26,53 pg/ml vs 21,11 pg/ml , p=0,223) dan rasio E1:E3 (58,55 pg/ml vs 50,28 pg/ml, p=0,684). Namun, semua perbedaan itu tidak signifikan secara statistik.*

***Kesimpulan**: Kadar estron, estradiol dan estriol pada wanita dengan endometriosis lebih rendah dibandingkan pada wanita tanpa endometriosis. Rasio E2:E1, E2:E3 dan E1:E3 lebih tinggi pada kelompok endometriosis. Namun, semua perbedaan itu gagal mencapai signifikansi secara statistik.*

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***Kata kunci**: endometriosis, estradiol, estriol, estrogen, estron*

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INTRODUCTION

Endometriosis is a disease in which endometrium that normally lines the uterus, grows outside the uterine cavity and is usually related to pelvic pain and infertility.[1] Endometriosis is an estrogen-dependent disease, therefore it affects not only women in their reproductive age, but also post-menopausal women who uses hormone replacement therapy. The prevalence of endometriosis during reproductive age ranged between 5-10%.[2]

The role of estrogen in the pathophysiology of endometriosis has been well known for a long time. Recent medical treatment is aimed to put the patient in a hypoestrogen state. Various studies found that there were sources of estrogen outside the gonads (extra-gonad). The adipose tissue and the skin were found to be able to convert androstenedione into estrone. Lately, endometriosis lesions itself is suspected of having the ability to convert androgens to estrogens independently. Accordingly, endometriosis tissue expresses complete steroido-

genic genes, including aromatase. This finding could change the endometriosis treatment approaches in the future, because recent treatment has only aimed the hypothalamus-pituitary-ovarian axis to cause a hypoestrogen state in patients. Along with the discovery of estrogen sources outside the gonads, current treatment has been developing with the application of aromatase inhibitor drugs to suppress estrogen production outside the gonad.[3,4]

Similar to endometriosis, breast cancer is also known to be estrogen-dependent. Estrogen has an important role in promoting cancer cells growth. Likewise in endometriosis, breast cancer itself also produces estrogens through aromatase and steroid sulfatase pathways. The enzyme 17 -hydroxysteroid dehydrogenase (17 -HSD) must also play a role in the regulation of estradiol and estrone. A study found that a low ratio between 17 -HSD type 2 and 1 gave a higher incidence of recurrence in post-menopausal breast cancer patients treated with tamoxifen. Patients with high level of 17 -HSD type 1 have a higher risk for relapse. The expression of the enzyme 17 -HSD type 1 and 2 in breast cancer were different to that in normal mammary glands, giving a clear prognosis in breast cancer cases.[5,6]

Mechanisms of estradiol metabolism in the body is by converting estradiol (E2) into estrone (E1). Furthermore, the estrone is converted into metabolites ring A and ring D (estriol/E3). Biologically, estradiol is the most active form of estrogen. Comparison of these three hormone levels is E2:E1:E3 = 10:5:1.[7] Similar to breast cancer, the enzyme 17 -HSD plays an important role in estrogen metabolism in endometriosis. In a recent study, it was found that the ratio between 17 -HSD type 1 and type 2 in endometriosis lesions is higher compared to normal endometrial tissue. This study was conducted in patients with endometriosis and normal subjects. In other words, it indicates a high synthesis of estradiol in the lesion.[8] However, no study has observed the ratio of estradiol, estrone, and estriol in patients with endometriosis and normal women without endometriosis. The role of estrogen metabolites derived from the metabolism of estrogen is not yet widely known. While the metabolites was initially considered to have no biological activity, recent research showed estrogenic effects from the hormone metabolites.[5] The finding raises the thought whether the metabolism of estrogen metabolites also have a role in the pathophysiology of endometriosis.

This study is particularly aimed to assess estrone (E1), estradiol (E2) and estriol (E3) level and its ratio (E2:E1, E2:E3 and E1:E3) between women with and without endometriosis. Thus, it is hoped to give a better understanding on the role of estrogen metabolites on the pathogenesis of endometriosis to help develop new strategies of endometriosis treatment approach in the future and to guide further research on drugs influencing estrogen metabolites.

METHODS

This study is an analytical cross sectional study on 27 women with endometriosis and 27 normal women without endometriosis who met the inclusion criteria. The subjects were recruited in Dr. Cipto Mangunkusumo hospital and other satellite hospitals from October 2012 to April 2013.

The inclusion criteria in endometriosis group were women diagnosed with endometriosis through laparoscopy or laparotomy procedure, having regular menstrual cycle, aged 20-45 years old, body mass index (BMI) 19-29 kg/m^2, and willing to participate in the study. The inclusion criteria in control group were diagnosed as free of endometriosis through laparoscopy or laparotomy or underwent caesarean section and being declared as free of endometriosis; having regular menstrual cycle and willing to participate in the study. The exclusion criteria applied to both groups were use of hormonal therapy in the last three months, presence of other estrogen-dependent gynecologic conditions such as uterine myoma, uterine hyperplasia or endometrial carcinoma, presence of breast carcinoma, or refused to participate in the study.

For all the included paricipants, the data regarding menstrual cycle, any obstetric diagnosis, surgery date and pathology examination result were collected. For measuring the blood hormone level, a five cc blood was drawn via venipuncture (day 7-15 in menstrual cycle or following surgery schedule for endometriosis group). Furthemore, the level of estradiol, estriol and estrone were examined by using enzyme-linked immunosorbent assay (ELISA) in Makmal Terpadu Laboratory Faculty of Medicine University of Indonesia.

The statistical analysis was conducted by using SPSS program 20th version. Since the data distribution was not normal, the data was presented in median value instead of mean value and the compari-

son of estradiol, estrone and estriol levels and its ratio between two groups was analyzed by using Mann-Whitney test.

RESULTS

Characteristics of Research Subjects

Fifty-four patients were recruited in the study, in which 27 patients diagnosed with endometriosis were included in the study group. The other 27 women who did not have endometriosis were included as control group. There were statistically significant differences regarding age and parity characteristic between endometriosis and control group. In contrast, there was no difference about BMI between those two groups.

Comparison of Estrone, Estradiol and Estriol Level between Women with and without Endometriosis

Because the data distribution was not normal, further analysis was carried out by Mann-Whitney test. Table 2 showed that the level of estrone (E1) hormone was found to be lower in endometriosis group compared to that in the control group (54.66 pg/ml vs 73.52 pg/ml, p=0.229). The similar results were found for estradiol (E2) and estriol (E3) hormone levels. Compared to control group, the level of estradiol and estriol were lower in endometriosis group (29 pg/ml vs 35 pg/ml, p=0.815 and 1.11 pg/ml vs 1.67 pg/ml, p=0.095, consecutively). However, the differences in estrogen level were not statistically significant since p>0.05.

Table 2. Comparison of Estrone, Estradiol and Estriol Level between Women with and without Endometriosis.

Hormone	Median (Mean Rank) (pg/ml)		p
	Endometriosis	Control	
Estrone	54.66 (24.93)	73.52 (30.07)	0.229
Estradiol	29.00 (27.00)	35.00 (28.00)	0.815
Estriol	1.11 (23.93)	1.67 (31.07)	0.095

Comparison of Ratio between Estradiol and Estrone, Estradiol and Estriol, Estrone and Estriol in Women with and without Endometriosis

Table 3 showed that the estradiol-estrone ratio (E2:E1 ratio) was higher in endometriosis group (0.51 pg/ml vs 0.38 pg/ml, p=0.164), as well as the estradiol-estrone ratio (E2:E3 ratio), which is higher in endometriosis group (26.53 pg/ml vs 21.11 pg/ml, p=0.223). However, both differences failed to reach statistical significance. In the meanwhile, the difference of the estrone-estriol ratio (E1:E3) was also not statistically significant between two groups, although the ratio is found to be lower in control group (58.55 pg/ml vs 50.28 pg/ml in control group, p=0.684).

Table 3. Comparison of Ratio between Estradiol and Estrone, Estradiol and Estriol, Estrone and Estriol in Women with and without Endometriosis.

Hormone Ratio	Median (Mean Rank) (pg/ml)		p
	Endometriosis	Control	
E2:E1	0.51 (30.48)	0.38 (24.52)	0.164
E2:E3	26.53 (30.11)	21.11 (24.89)	0.223
E1:E3	58.55 (28.37)	50.28 (26.63)	0.684

Table 1. Characteristics of Research Subjects.

Variable	Endometriosis Group (n=27)	Control Group (n=27)	p
Age			
20-40 years	19 (43.2%)	25 (56.8%)	0.036
>40 years	8 (80.0%)	2 (20.0%)	
Parity			
Parity 0	17 (73.0%)	0 (0,0%)	0.000
Parity 1	10 (27%)	27 (100%)	
Body Mass Index (BMI)			
BMI 19-24	21 (47.7%)	23 (52.3%)	0.484
BMI 25-29	6 (60.0%)	4 (40.0%)	

DISCUSSION

Endometriosis is a well-known estrogen-dependent disease. However, no study has analyzed the systemic estrogen metabolism in endometriosis compared to that in women without endometriosis. Recent studies have identified the process of local estrogen metabolism by endometriosis lesion.[3,4] Thus, this study aimed to analyze whether systemic estrogen metabolism is in accordance with its local metabolism in endometriosis. In this study, the blood serum estrogen levels were examined by ELISA test. The ratio between estrogen metabolites, comprising of estradiol to estrone, estradiol to estriol, and estrone to estriol, in women with and without endometriosis were compared.

The diagnosis of endometriosis and non-endometriosis in the study was confirmed by laparoscopy or laparotomy. Therefore, it improves the reliability and validity of this study because it reduces the risk of false positives and false negatives during sample selection.

In patient characteristics, there was no difference regarding BMI between two groups, in which the percentage of normal and overweight-classified BMI was relatively similar. Nevertheless, significant differences were seen in age and parity characteristics. In women with endometriosis, the data showed that approximately 80% of the patients were older than 40 years of age. All infertile patients with nulliparity were identified as endometriosis group (100%). These significantly different results may be due to the relatively small sample size. This is attributable to the limited time and cost during study completion.

The relatively small sample size also affected the data distribution, where the data distribution is not normal based on normality test. Thus, either median or mean rank instead of data mean, were used to analyze the difference between the two study groups.

The estradiol level obtained in women with endometriosis is lower in comparison to women without endometriosis. This result is not in agreement with the study conducted by Bulun et al who used Nothern blot analysis. Bulun et al described a transcription process of 17 -HSD type 1 catalyzing estrone to estradiol-17 in ectopic endometrium and endometriosis. Estradiol levels in the tissue was found to be very high due to excessive production by aromatase activity, transcription enzyme 17 -HSD type 1 and accumulation of estradiol which was failed to be metabolized due to deficient 17 -HSD type 2.[4] However, the study strictly measured local estrogen activity in the endometriosis lesions, not the systemic estrogen activity. Similar to this study, estrone and estriol levels in the endometriosis group were found to be lower compared to those in women without endometriosis.

During growth of endometriosis, there are two intrinsic molecular abnormalities playing an important role and is related to estrogen. These include the high levels of aromatase enzyme, cytokines and tissue metalloproteinases, as well as deficiency of 17 -HSD type 2 as described earlier. The high production of aromatase enzyme caused by high inflammation process, further increasing the production of PGE2 and aromatase. Increased activity of aromatase leads to high local estrogen production. Deficiency of 17 -HSD type 2 also inhibits the metabolism of estradiol into estrone, thus causing estradiol to accumulate in the tissue.[4] Consequently, the ratio E2:E1 and E2:E3 was supposed to be increased in women with endometriosis. To verify that hypothesis, the ratio of each of these estrogen hormones was assessed as the main core of this research. The ratio of E2:E1 revealed that women with endometriosis have a higher ratio of E2:E1 than women without endometriosis. Similarly, the ratio of E2:E3 was found to be higher in women with endometriosis. These results were consistent with the hypothesis of this study. However, both differences were not statistically significant.

In addition, similar to the ratio of hormones E2:E3 and E2:E1, the ratio of E1:E3 was found to be higher in endometriosis group when compared to the control group. This result explains that in endometriosis, there is an increased activity of the enzyme 17 -HSD type 1 converting estrone to estradiol, so that estradiol level increases in the tissue,[8] however, estrone itself is unsuccessfully metabolized into its weaker form, estriol.

No statistical siginificant results in this study show that not enough evidence is available to support the hypothesis that systemic estrogen metabolism cannot convey the high local estrogen metabolism in endometriosis lesion. Regardless of the insignificant result, the ratio of E2:E3; E2:E1 and E1:E3 is higher in women with endometriosis. This may imply that the metabolism of estrogen has an

important role in the development of endometriosis. However, estrogen level is not the single absolute factor, because there are many other factors that play a role in the development of endometriosis, such as the environment, enzyme activity, immune defects, and genetics. In general, this study decribes that women with high levels of estrogen metabolism are at higher risk for developing an estrogen-dependent disease.

CONCLUSION

The estrone, estradiol and estriol level in women with endometriosis group was lower compared to those in women without endometriosis group, however the differences were not statiscally significant. The ratio E2:E1, E2:E3 and E1:E3 was higher in endometriosis group compared to those in non-endometriosis group, however, the differences also failed to reach statistical significance.

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