eISSN (Online): 2598-0580

Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

The Association between Aromatase Gene Polymorphism Cyp19 Val 80 and Endometriosis Risk

Syifa Alkaf[1]*, Ziske Maritska[2], Venny Patricia[3]

[1.] Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia
[2.] Department of Biology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia
[3.] Department of Histology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

ARTICLE INFO

Keywords:
Polymorphism
Aromatase gene
CYP19 Val80
Endometriosis

***Corresponding author:**
Syifa Alkaf

E-mail address:
dear.syifa@gmail.com

All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.32539/bsm.v5i5.230

ABSTRACT

Background: Endometriosis implant has been known to express aromatase enzyme, Cytochrome p450 that catalyzes androgen into estrogen. It causes local estrogen production, leading to increased estrogen level, and subsequently triggers endometriosis lesion. CYP19 gene resided at chromosome 15q21.1 is the biggest component of aromatase coding cytochrome p450 enzyme. **Objective:** To identify relationship between aromatase gene polymorphism CYP19 Val 80 and the risk of endometriosis. **Methods:** This is an observational case-control study using frozen DNA sample from women with endometriosis and/ or adenomyosis who had undergone laparotomy/ laparoscopy at Obstetrics and Gynecology Department Dr. Mohammad Hoesin General Hospital Palembang January-November 2013. Samples were amplified and cut by PCR-FRLP using Rsa1 restriction enzyme. Results were divided into A/A genotype (homozygote mutant), G/A (heterozygote mutant), and G/G (homozygote wild type). Data were analyzed by SPSS 21.0 version. **Results:** PCR-RFLP results for A/A genotype were 20 (21.3%) in endometriosis group and 8 (8.5%) in control group. G/A genotype were 18 (19.1%) in endometriosis group and 22 (23.4%) in control group. G/G genotype were 9 (9.6%) and 17 (18.1%) in endometriosis group and control group, respectively. There was significant increase risk of endometriosis in women carrying genotype A/A to those with genotype G/G with OR 4.722 ($p<0.05$). **Conclusion**: Polymorphism on aromatase gene CYP19 Val 80 A/A increases risk of endometriosis.

1. Introduction

Endometriosis is a gynecological disorder characterized by a similar tissue of glands and endometrium stromal outside the uterine cavity with general symptoms of chronic pelvic pain and infertility.[1] Endometriosis is one of the most common diseases, affecting 15-20% of women in reproductive age and over than 30% of infertile women. The exact prevalence of this disease varies from about 4% of asymptomatic endometriosis found accidentally in women undergoing tubal ligation to about 50% in women with severe dysmenorrhea.[2,3,4,5]

Estrogen and progesterone receptors are expressed in endometrial lesions, and the biological nature of endometriosis is also affected by estrogen exposure. Therefore antiestrogen therapy for endometriosis is widely used, such as danazol, progestin, and GnRH analog. Later, the role of aromatase inhibitors has been proposed as a new option for endometriosis therapy given the enormous role of aromatase enzymes in estrogen synthesis.[6]

Many aspects of human reproductive function are influenced by genetic factors, and there are many studies conducted to look for target genes associated with abnormalities that affect female fertility such as endometriosis. The incidence of endometriosis in first

degree relatives was increased compared to control (6.9% versus 0.9%). Genetic involvement in the pathophysiology of endometriosis was first introduced by Frey (1957) who found familial aggregation in a population-based study of twin individuals. This genetic predisposition in endometriosis has also been described more than 20 years ago where the risk of endometriosis is more than six fold in women with nuclear families history of severe endometriosis. [2,7]

Endometriosis implants are said to express aromatase, cytochrome p450, an enzyme catalyzing the conversion of androgens into estrogen, causing local production of estrogen and increasing estrogen concentrations that can trigger growth of endometriosis lesions. Recent evidence suggests that polymorphisms in genes that encode enzymes for drug metabolism or certain hormones may affect phenotypic metabolic rate variations. The CYP19 gene, located on the chromosome 15q21, has been reported to be associated with several polymorphisms with varying results, including CYP19 Arg264Cys (C790T), CYP19 C1558T, CYP19 TTTA VNTR, CYP19 Val 80, CYP19 3bp I / D, and CYP19 Trp39Arg (T115C). CYP19 Arg264Cys (C790T) replaces C to T on exon 7, resulting in a change in the amino acid Arg264Cys. [8]

2. Methods

This is an observational case control study using frozen DNA sample from women with endometriosis and/ or adenomyosis who had undergone laparotomy/ laparoscopy at Obstetrics and Gynecology Department Dr. Mohammad Hoesin Hospital Palembang from Januari to November 2013.

Inclusion criteria were those in age 13-45 years and still have period. Endometriosis women who were pregnant, having ovarian carcinoma and myoma were excluded from the study. Controls were patients who undergone laparotomy or laparoscopy for other reasons and proven not having endometriosis and/ or adenomyosis.

Blood samples were collected in EDTA tube and refrigerated in maximum temperature of 4^0C until DNA extraction performed. Extraction was conducted using Chelex-100 method with phosphate buffer saline (PBS) pH 7.4; Safonin 0.5% in PBS; and *Chelex 20%* in ddH_2O pH 10.5.

DNA genome fragments were multiplied in vitro using oligonucleotide amplification primer pair to limit ampification area. We used *forward primer* 5'--AGTAA-CACAGAACAGTTGCA-3' dan *reverse primer* 5'-TCCAGACTCGCATGAAT-TCTCCGTA-3'. PCR was performed using labcycler (Sensquest).[9]

Polymorphism G/A in aromatase enzyme CYP19 Val80 (rs700518:A>G) was detected from RFLP (restriction fragment length polymorphism) using retriction enzyme *Rsa1.* Genotypes were identified by a single band 0f 188bp for AA, double band of 164bp and 188bp for GA genotype, and only 0ne band 0f 164bp for GG genotype.

PCR products with180 bp length were digested by 5.0 unit *Rsa1* with buffer reaction and incubated in 37^0C temperature for 180 minutes. Then 5µl compund was poured into 2% polyacrylamide gel electrophoresis (PAGE) containing ethidium-bromide, then continued with electophoresis and visualized under ultra violet using Gel-Dov (BIO-RAD laboratories USA). Results were then analyzed using Quantity One software.

3. Results

Within periods of January to November 2013, there were 101 subjects, 48 in case group and 53 in control group in previous study.[10] during frozen process, 1 DNA from case group and 6 DNA from control group were excluded because of corrupted.

Mean age in case group of this study was 35.8 years. It corresponded with data showed that average age for endometriosis patients were 35-45 years. Patients in case group have significantly lower BMI than in control. More than half patients with endometriosis experienced infertility (52.1%), while only 6.4% of control group having infertility, $p<0.001$.

It can be said from the table 2. that distribution of mutant homozygote (AA) and heterozygote (GA) genotype were different in case group than in control (20 and 18 vs 8 a. There was significant difference between incidence of polymorphism between case and

control group, p<0.01.

Using bivariate analysis with continuity correction, there was increase risk of endometriosis in case group with alele A mutant (AA homozygote as well as GA heterozygote) with odd ratio 2.393 (CI95% 0.935-6.120); p = 0.107 (p>0.05) (table 3). We assume the result was not statistically significant because of low sample size. When we compared the risk of only homozygote mutant (AA genotype) to wild type (GG genotype), the OR value becomes 4.722, statistically significant (CI95% 1.494-14.930); p = 0.015 (p<0.05).

Table 1. Subject Sosiodemographic Characteristic

Characteristic	Case		Control		p
	Δ	%	Δ	%	
Mean age (year)	35.4		27.8		<0.001
Parity					
Nulliparous	33	70.2	5	10.6	
Primigravid	7	14.8	22	46.8	<0.001
Multigravid	8	17.0	20	42.5	
Mean BMI	22.9		25.47		0.003
Etnic group					
South Sumatra	36	76.6	35	74.4	
Jawa	8	17.0	8	17.0	
Minang	1	2.1	2	4.3	0.358
Cina	2	4.2	0	0	
Others	0	0	2	4.3	
Marital status					
Married	43	91.5	45	95.7	0.597
Unmarried	4	8.5	2	4.3	
Abortus history					
(+)	3	6.4	6	12.7	0.128
(-)	44	93.6	41	87.2	
Hormonal contraception					
(-)	35	74.5	38	80.9	0.255
(+)	12	25.5	9	19.1	
Infertility					
(+)	24	52.1	3	6.4	<0.001
(-)	23	47.9	44	93.6	
Smoking					
(+)	1	2,1	2	4.3	1.00
(-)	46	97.9	45	95.7	
Family history					
(+)	3	6.4	4	8.5	0.718
(-)	44	93.6	43	91.5	

Table 2. Distribution of CYP 19 Val80 genotype polymorphism

Genotype	Case	Control	Total
A/A	20	8	28
G/A	18	22	40
G/G	9	17	26
Total	47	47	94

P= 0.018 (p<0.05)

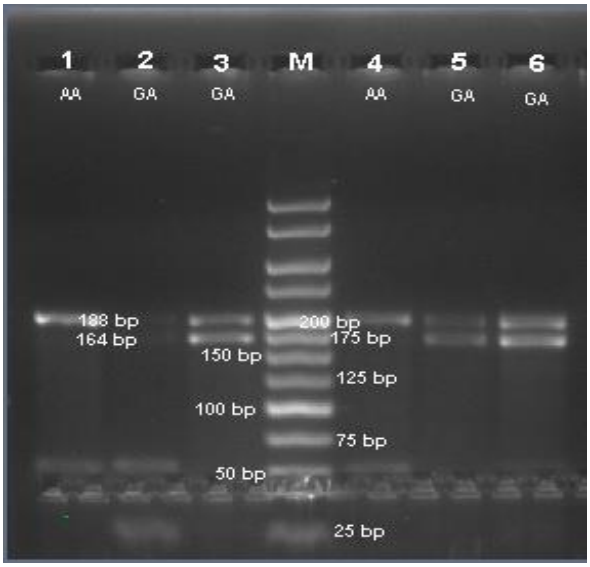

Figure 1. Visualization result after PCR-RFLP

Table 3. Endometriosis risk between polymorphism in case and control group

Genotype polymorphism	Case	Control	Total
A/A + GA	38	30	28
G/G	9	17	26
Total	47	47	94

OR= 2.393 (CI95% 0.935-6.120); p=0.107 (p>0.05)

4. Discussion

The susceptibility to endometriosis is influenced by genetic as well as ecogenic interactions. Various single nucleotide polymorphisms (SNPs) have been associated with. Somes studies focused on polymorphism on estrogen related genes including aromatase gene, including cathecol-O-methyltransferase (COMT), 17-β-hydroxysteroid dehidrogenase, Cytochrom p450 (CYP) 17 A2, CYP 19-2, CYP1B1, and estrogen receptor (ER) alpha.[11]

CYP17 and CYP19 were studied because they were involved in estrogen metabolism . HSD17 catalyzes the final step of estradiol (E2) biosynthesis, the conversion of estron E2. While aromatase (CYP19) catalyzes conversion of testosterone into estradiol. Genetic polymorphism involved in estrogen synthesis and metabolism may play an important role in the variation of endometriosis among individuals by altering local estrogen production or cicrulating estrogen levels.[12] Here we evaluate whether polymorphism in CYP19 Val 80 (is associated with the risk of endometriosis.

In our case control study, we found increased risk of endometriosis of mutant and heterozygote genotype with endometriosis. and the risk for mutant homozygote only was higher with significant value (OR 4.722, p<0.05). only few studies have investigated association of CYP19 Val80 gene with endometriosis risk. Vietri MT, et al[6] found in their study that the polymorphism of CYP 19 were significantly represented in Val80 and C1558T in patients affected with endometriosis.

Supporting this result, Wang Ledan et al[13] reported that among four CYP19 SNPs polymorphisms studied, rs700518 (Val80) AA genotype in the endometriosis-related infertility group was significantly higher than in the control group (55.4 vs 25.3%), whereas the

frequency of the AG genotype in the infertility group was much lower in the control group (21.5 vs 55.6%) ($p<0.001$). Different result showed by Tsuciya m, et al[12], they found no statistically significant assocuiation was found in CYP19 polymorphism. Though, the polymorphism used in thier study was Arg264Cys polymorphism, out of Val80.

Our study used strict criteria for the definition of cases and controls. Women with endometriosis and/or adenomyosis included in this study were already proved by surgical (laparotomy or laparoscopy findings). Controls were included from those who performed surgical procedure of special reasons but gynecology. Limitation of our study were limited sample size, thus further study with larger sample size and multi center would be beneficial to support this result. For genetic polymorphism varies among ethnic groups–our study conducted in Malay population, a multi center study involving various race and ethnic group would be beneficial.

5. Conclusion

Polymorphism in aromatase gene CYP19 Val80 increases risk of endometriosis. Homozygote mutant shows significant relation with endometriosis risk compared with homozygote wild type. Further research with multicenter to recruit more samples will be considered beneficial to support this study result.

6. References

1. Barbosa CP, De Souza AMB, Bianco B, Christofolini DM. The effect of hormones on endometriosis development. Minerva Ginecol. 2011;63:375-86.
2. Cramer DW, Missmer SA. Cramer & Mismer: Epidemiology of endometriosis. Annals New York Academy of Sciences. Harvard Medical School. Boston.
3. Vigano P, Prazzini F, Somigliana E, Vercellini P. Endometriosis: epidemiology and aetiological factors. Best Prac Research Clin Obstet Gynecol. 2004;18(2):177-200.
4. Mohammed A, Bedaiwy, Falcone T. Laboratory testing for endometriosis. Clin Chemica Acta. 2004;340: 41-56.
5. Materese G, Placido GD, Nikas Y, Alviggi C. Pathogenesis of endometriosis: natural immunity dysfunction or autoimmune disease? Trends in Mol Med. 2003;9(5):223-228.
6. Vietri MT, Cioffi M, Sessa M, Simeone S, Bontempo P, Trabucco E, et al. CYP17 and CYP19 gene polymorphisms in women affected with endometriosis. Fertil Steril.2008;92(5):1532-1535.
7. Kaam KV. Romano A, Schouten JP, Dunselman GAJ, Groothuis PG. Progesterone receptor polymorphism +331G/A is associated with decreased risk of deep infiltrating endometriosis. Human Reprod;22:129-35.
8. Kado N, Kitawaki J, Obayashi H, Ishihara H, Koshiba H, Kusuki I, et al. Association of the CYP17 gene and CYP19 gene polymorphisms with risk of endometriosis in Japanese women. Human Reproduction. 2002;17(4):897-902.
9. Somner J, McLelan S, Cheung J, Mak YT, Frost ML, Knapp KM. Polymorphisms in the P450 c17 (17-Hydroxylase/17,20-Lyase) and P450 c19 (aromatase) genes: association with serum sex steroid concentrations and bone mineral density in postmenopausal women. J of Clin Endocrinol Metabolism. 2004;89(1):344-351.
10. Alkaf S, Chakra A, Said U, Saleh I. Progesteron receptor gene polymorphism promoter region +331 G/A increases risk of endomertriosis. Indonesian J Obstet Gynecol. 2016;4(1):52-56.
11. Huber A, Keck CC, Hefler LA, Schneeberger C, Huber JC, Bentz EK, et al. Ten estrogen-related polymorphisms and endometriosis. Obstet Gynecol. 2005;106(1):1025-1031.
12. Tsuchiya M, Nakao H. Katoh T, Sasaki H, Hiroshima M, Tanaka T. Association between endometriosis and genetic polymorphisms of the estradiol-synthesizing enzyme genes HSD17B1 and CYP19. Human Reproduction. 2005;20(4):974-978.

13. Wang L, Lu X, Danhan W, Qu W, Li W, Xu X, et al. CYP19 gene variant confers susceptibility to endometriosis-associated infertility in Chinese women. Experimental & Molecular Medicine. 2014;46:e103.