

Case Report

**Progesterone Receptor Gene Polymorphism Promoter Region +331G/A
Increases Risk of Endometriosis*****Polimorfisme Gen Reseptor Progesteron Regio Promoter +331G/A
Meningkatkan Risiko Terjadinya Endometriosis***Syifa Alkaf¹, Aerul Chakra¹, Usman Said¹, Irsan Saleh²¹Department of Obstetrics and Gynecology²Health and Medicine Research Unit

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Abstract**Objective:** To identify relationship between progesterone receptor gene polymorphism promoter region +331G/A with the risk of endometriosis.**Method:** An observational case-control study. Population are women with endometriosis and/or adenomyosis who have been performed laparotomy/laparoscopy at Obstetrics and Gynecology Department Dr. Mohammad Hoesin General Hospital Palembang, January-November 2013. Subjects fulfilled inclusion criteria, given informed consent and performed blood sampling continued by PCR-RFLP. 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.**Result:** PCR-RFLP results for +331G/A genotype were 26 (54.1%) in case group and 14 (26.4%) in control. +331A/A genotype was not found in both groups. There was significant increase risk of endometriosis in women carrying genotype +331G/A to those with genotype +331G/G with OR 3.29 ($p < 0.05$).**Conclusion:** Polymorphism on progesterone receptor gene +331G/A increases risk of endometriosis in Malay population in Indonesia. Compared to previous studies, there is interesting finding that polymorphic alleles were found more commonly in our study.

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Keywords: endometriosis, polymorphism, progesterone receptor, +331G/A**Abstrak****Tujuan:** Mengetahui hubungan polimorfisme gen reseptor progesteron regio promoter +331G/A dengan risiko terjadinya endometriosis.**Metode:** Sebuah studi kasus kontrol observasional dengan populasi perempuan penderita endometriosis dan/atau adenomyosis yang dioperasi laparotomi/laparoskopi di Bagian Obstetrik dan Ginekologi RSUP Dr. Mohammad Hoesin Palembang dari Januari sampai November 2013. Subjek yang memenuhi kriteria inklusi, diberikan informed consent selanjutnya dilakukan pengambilan darah dilanjutkan pemeriksaan PCR-RFLP. Hasil pemeriksaan dibagi menjadi genotip A/A (mutan heterozigot), G/A (mutan homozigot), dan G/G (wild type). Analisis data menggunakan program SPSS versi 21.0**Hasil:** 1 PCR-RFLP didapat genotip +331G/A sebanyak 26 (54,1%) pada kasus dan 14 (26,4%) pada kontrol. Pada kedua kelompok genotip +331A/A tidak ditemukan. Terdapat peningkatan risiko endometriosis pada perempuan dengan genotip +331G/A dibanding +331G/G dengan rasio odd 3,29 ($p < 0,05$).**Kesimpulan:** Polimorfisme pada gen reseptor progesteron +331G/A meningkatkan risiko endometriosis pada populasi ras Melayu di Indonesia. Dibandingkan penelitian terdahulu, menarik mengetahui alel polimorfik ditemukan dalam jumlah yang banyak pada studi ini.

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Kata kunci: endometriosis, polimorfisme, reseptor progesteron, +331G/A**Correspondence:** Syifa Alkaf. Obstetrics and Gynecology Department. Faculty of Medicine University of Sriwijaya Palembang. Telephone : +62711-360865, Email: dear.syifa@gmail.com**INTRODUCTION**

Endometriosis is a benign gynecologic condition characterized by presence of endometriotic tissue outside the uterine cavity. Chronic pelvic pain and infertility are two of its most common symptoms. Endometriosis is commonly found and affects about 5-10 % reproductive age women and more than 30% infertile women.¹⁻⁴

Genetics study in endometriosis is attracting researchers in recent years. One of interest is poly-

morphism in progesterone receptor gene. Progesterone is a potent antagonist of estrogen-mediated proliferation in endometrium and plays an important role in pathogenesis of endometriosis. Progesterone regulates this crucial function by its two isoform, A and B, which are expressed by one single gene located in chromosome 11q22-q23. PR-A is commonly found in endometrial stroma while PR-B in glandular which function by inducing cell apoptosis and secretoric change, respectively. PR-B acts as strong transcription activator to progres-

terone target genes, while PR-A is a dominant transrepressor of PR-B and ER- α activity. The antiproliferative effect of progesterone depends on the balance of these two isoforms.^{3,5-8}

McDonnell, et al.⁹ found the mechanism of which PR-A inhibits PR-B transcription activity by competitive inhibition to the general transcription factor of the mineralocorticoid receptor. While in nontranscriptionally active cells, PR-A functions as a glucocorticoid inhibitor, PR-B, and androgen receptor. Thus, repression of transcription activity of the estrogen receptor depends on PR-A expression.¹⁰⁻¹²

Functional polymorphism at the promoter region +331G/A of the progesterone receptor gene (rs10895068), by adding a TATA box to the transcription start site, promotes production of PR-B relative to PR-A. Change in PR-A/PR-B ratio is believed to interfere with stromal differentiation and lead to progesterone resistance. Thus, our hypothesis is that DNA polymorphism in the progesterone receptor gene which interferes with the PR-A/PR-B ratio increases the risk of endometriosis.¹³

METHODS

This was a case-control study that took place in the Obstetrics and Gynecology Department and Microbiology Laboratory of Dr. Mohammad Hoesin Hospital in Palembang from January to November 2013.

The population consisted of women with endometriosis and/or adenomyosis who had undergone laparotomy or laparoscopy in the Obstetrics and Gynecology Department of Dr. Mohammad Hoesin General Hospital in Palembang.

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

Blood samples were collected in EDTA tubes and refrigerated at a maximum temperature of 4°C until DNA extraction was performed. Extraction was conducted using the Chelex-100 method with phosphate buffered saline (PBS) pH 7.4; Safonin 0.5% in PBS; and Chelex 20% in ddH₂O pH 10.5.

DNA genome fragments were multiplied *in vitro* using an oligonucleotide amplification primer pair to limit the amplification area. We used 18 pmol primer

Forward 5'-CACTCATGGGATCTGAGAATC-3' and 18 pmol reverse primer 5'-CACAAGTCCGGCACTTGAGT-3'. PCR was performed using a lab cycler (Sensquest).

Polymorphism G/A in the progesterone receptor promoter region +331 was detected from RFLP (restriction fragment length polymorphism) using the restriction enzyme NlaIV.

PCR products with a 330 bp length were digested by 1.0 unit NlaIV (isoschizomer BspLI, fermentas) with buffer reaction and incubated at 37°C temperature for 180 minutes. Then 5 μ l of the compound was poured into a 2% polyacrylamide gel electrophoresis (PAGE) containing ethidium-bromide, then continued with electrophoresis and visualized under ultra violet using Gel-Dov (BIO-RAD laboratories USA). Results were then analyzed using Quantity One software.

RESULTS

Within the periods of January to November 2013, there were 101 subjects, 48 in the case group and 53 in the control group.

Mean age in the case group of this study was 35.8 years. It corresponded with data showing that the average age for endometriosis patients was 35-45 years. Patients in the case group had significantly lower BMI than in the control. More than half of the patients with endometriosis experienced infertility (52.1%), while only 7.5% of the control group had infertility, $p < 0.001$.

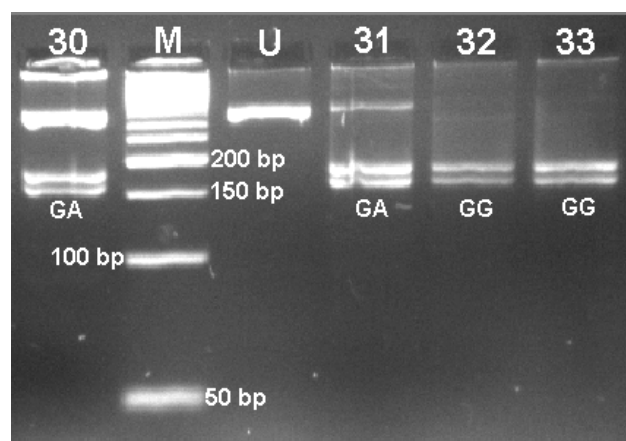


Figure 1. Visualization Result after pcr-RFLP

Table 1. Subject Sociodemographic Characteristic

Characteristic	Case		Control		p
	Δ	%	Δ	%	
Mean age (year)	35,8		28,11		<0.001
Parity					
Nulliparous	33	68.8	24	9.4	<0.001
Primigravid	7	14.6	24	45.3	
Multigravid	8	16.7	24	45.3	
Mean BMI	22.96		25.47		0.003
Ethnic group					
South Sumatra	37	77.1	39	73.6	0.358
Jawa	8	16.7	10	18.9	
Minang	1	2.1	2	3.8	
Cina	2	4.2	0	0	
Others	0	0	2	3.8	
Marital status					
Married	44	91.7	50	94.3	0.597
Unmarried	4	8.3	3	5.7	
Abortus history					
(+)	3	6.3	9	17.0	0.128
(-)	45	93.8	44	83.0	
Hormonal contraception					
(-)	36	75.0	41	77.4	0.255
(+)	12	25.0	12	22.6	
Infertility					
(+)	25	52.1	4	7.5	<0.001
(-)	23	47.9	49	92.5	
Smoking					
(+)	1	2.1	2	3.8	1.00
(-)	47	97.9	51	96.2	
Family history					
(+)	3	6.3	5	9.4	0.718
(-)	45	93.8	48	90.6	

Table 2. Distribution and Analysis of Progesterone Receptor Genotype

Genotype	Case	Control	Total
Genotype +331G/A	26 (54.1%)	14(26.4%)	40
Genotype +331G/G	22 (45.8%)	39 (73.5%)	61
Total	48	53	OR: 3.292 (95% CI 1.43-7.58), p<0,01

It can be said from the Table 2. that distribution of mutant heterozygote genotype was found more commonly in case group than control, $p < 0,01$. There was increase risk of endometriosis in women carrying progesterone receptor gene promoter region +331G/A, with odd ratio 3.29 (95% CI.43-7.58). Homozigote mutant genotype (+331A/A) was not found in either group. Maybe because limitation of number of subjects studied.

We then further analyzed relationship between these two genotypes found in case groups with other variables such endometriosis level, infertility, and dysmenorrhae. Correlation value between genotype +331G/A and endometriosis level based on ASRM was weak (0.27) with $p > 0.05$ (Table 3). Genotype +331G/A had also weak correlation with dysmenorrhae level, $r = 0.32$ with $p > 0.05$.¹⁴

Table 3. Distribution and Correlation Test between Genotypes and ASRM Endometriosis Level, and Genotypes with Dysmenorrhoea Level in Case Group

Genotype	Endometriosis level					Dysmenorrhoea level				
	I	II	III	IV	Total	No pain	Mild	Moderate	Severe	Total
+331 G/A	1 4.5%	6 27.3%	6 27.3%	9 40.9%	22	4 15.3%	6 23.0%	8 30.7%	8 30.7%	26
+331 G/G	0 0.0%	4 19.0%	3 14.3%	14 66.7%	21	1 4.5%	1 4.5%	10 45.5%	10 45.5%	22
Total* (%)	1	10	9	23	43 p= 0.33 r=0.27	5	2	23	18	48 p=0.14 r=0.32

*five adenomyosis only patients were not included

DISCUSSION

There was significant age difference between case and control group. This can be explained because most subjects in control group were primiparous where as the others were mostly women who seeking for help after years of infertility. More than half of case group were infertile. This finding was along with the fact that endometriosis is one of commonest cause of infertility.¹⁵

Some previous researchers reported of specific group of women with tall and thin posture, short ovulatory cycle, and heavy menstrual bleeding as to be "endometriosis phenotype". Women with normal BMI tend to have more regular cycle than those with higher BMI. From our findings, BMI average in case group was significantly lower than those in control group.

We also find in this study that presence of +331G/A genotype increased the risk of endometriosis with OR 3.29. While correlation of this genotype with the ASRM level and dysmenorrhea score was weak. Limitation of sample size and consecutive sampling methods were rationale of this result.¹⁶

Gentilini et al.¹⁷ showed that this +331G/A polymorphic gene increased risk for deep infiltrating endometriosis with high invasive potential. It was assumed that PR-B, with a nontranscriptional mechanism, provokes cell development through interaction with estrogen receptor and stimulates Src/P21ras/ERK pathway. In investigating progesterone receptor on uterine myoma, Renner, et al.¹⁴ found that +331G/A genotype was related to endometriosis incidence in myoma patients, which means this genotype was found more common in myoma patients having endometriosis.

De Vivo, et al.¹⁸ stated that progesterone receptor gene polymorphism enhanced risk for endometrial cancer by increasing PR-B receptor expression.

This polymorphism was not related to cell ability to implant on ectopic sites but rather enabling cell invasiveness by altering PR-A and PR-B balance.

Different from those findings, Kaam, et al.¹³ detected that +331A allele reduced risk of deep infiltrating endometriosis and adenomyosis. From our consideration, this result might be bias since allele +331A only found in 2 subjects. It was also re-

ported from this study that ovarian ca patients did not show increase on +331A allele significantly.

Berchuck, et al.¹¹ reported lessen endometriosis risk on +331A variant allele in women participating in the study of ovarian carcinoma. Treloar, et al.¹⁹ found there was no relationship between endometriosis risk with this variant.

Difference on study results were probably from different sample size, population and demography thus evoked variations in polymorphism pattern reported. In this study, conducted in Indonesia, we assumed to represent Malay population in Indonesia. On our best knowledge, this study was the first taken place in Indonesia. Previous researchers in different countries and ethnic group reported various results. As we know, single nucleotide polymorphism is an inherited form and the incidence will be different among races.²⁰⁻²⁴

From this study we can conclude an interesting fact that polymorphism frequency in progesterone receptor gene promoter region +331G/A were found frequently in subjects, 54.1% in case group and 26.4% in control. Remembering that polymorphism is variable among groups and places, this study took place in Sumatera Selatan Indonesia revealed an attracting result.

Limitation of this study is that we did not analyze progesterone receptor gene expression in endometriotic tissues. This gene expression examination is crucial to see how significant genotype polymorphism on tissue level, and to evaluate factors contributing to this.

CONCLUSION

Polymorphism on progesterone receptor gene promoter region +331G/A has significant relation with endometriosis. There is significant increase risk of endometriosis on Malay population in Indonesia having +331G/A gene compared to +331G/G gene. While endometriosis level based on ASRM as well as dysmenorrhea level are on weak correlation with this gene polymorphism.

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