

POLYMORPHISMS OF THE PROGESTERONE RECEPTOR GENE

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Polymorphisms of the Progesterone Receptor Gene in Endometriosis Patients of South Sumatra, Indonesia

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Abstrak

Polimorfisme pada gen reseptor progesteron dapat mengubah ekspresi dua isoform reseptor progesteron, yang terlibat dalam regulasi efek antiproliferasi progesteron di jaringan endometriotik. Studi ini bertujuan mengidentifikasi polimorfisme +331G/A gen reseptor progesteron pada pasien endometriosis di Palembang, Sumatera Selatan. Identifikasi *single-nucleotide polymorphism* (SNP) +331G/A dilakukan pada 42 pasien endometriosis dengan menggunakan *polymerase chain reaction* (PCR) dan *restriction fragment length polymorphism* (RFLP). Pada penelitian ini, didapatkan 26 subjek (61,9%) memiliki genotip heterozigot mutan terhadap SNP +331G/A. Tidak ditemukan subjek dengan genotip homozigot mutan terhadap SNP +331G/A. Frekuensi alel polimorfik SNP +331G/A didapatkan sebesar 30,9%. Dapat disimpulkan bahwa polimorfisme +331G/A gen reseptor progesteron teridentifikasi pada pasien endometriosis di Palembang, Sumatera Selatan. Penelitian selanjutnya diperlukan guna menentukan peran polimorfisme ini dalam patogenesis endometriosis, khususnya pada pasien di Indonesia.

Kata kunci: endometriosis, gen reseptor progesterone, polimorfisme, +331G/A

Abstract

Polymorphisms of the progesterone receptor gene alter the expressions of two receptor isoforms involved in the regulation of progesterone's antiproliferative effect in endometriotic tissue. This study aims to identify the +331G/A polymorphism of the progesterone receptor gene in endometriosis patients in Palembang, South Sumatra. Identification of +331G/A single-nucleotide polymorphism (SNP) was conducted on 42 endometriosis patients through *polymerase chain reaction* (PCR) and *restriction fragment length polymorphism* (RFLP). In this study, twenty-six (61.9%) subjects had heterozygous mutant genotype for the +331G/A SNP. No subject with homozygous mutant genotype for the +331G/A polymorphism was identified. The frequencies of polymorphic alleles for the +331G/A polymorphism was 30.9%. In conclusion, the +331G/A progesterone receptor gene polymorphism was present in endometriosis patients in Palembang, South Sumatra. This finding may warrant further studies to determine whether this polymorphism play a role in the development of endometriosis in the Indonesian population.

Keyword: endometriosis, progesterone receptor gene, polymorphism, +331G/A

1. Introduction

Endometriosis is a gynecologic disorder commonly found in women of reproductive age, marked by the presence of ectopic endometrial tissue outside the uterine cavity.¹ The mechanistic theory (i.e. reflux of viable endometrial tissue through the uterine tubes during menstruation), implantation theory, and coelomic metaplasia are regarded as the classic theories explaining possible etiologies of endometriosis. Yet, these theories have not completely elucidated the molecular mechanisms involved in the pathogenesis of endometriosis. Multiple factors have currently been known to be involved in the pathophysiology of endometriosis, including genetic, immunologic and environmental conditions.^{1,2}

As ectopic endometrial tissue responds to hormones and drugs in a similar manner to the eutopic endometrium, estrogen and progesterone have important roles in the development of endometriosis.^{2,3} Estrogen induces endometrial tissue proliferation, which is physiologically inhibited by progesterone.⁴ Progesterone facilitates decidualization, including differentiation of stromal and epithelial cells of the endometrium.⁵ The action of progesterone is mediated through its combined interaction with two functionally distinct receptor isoforms, PR-A and PR-B.⁶ The PR-A and PR-B isoforms are transcription factors belonging to the ligand-dependent nuclear receptor family. These two isoforms are structurally identical, but PR-B has additional 164 amino acids in its amino terminal, which encodes transactivation functions specific to PR-B. The additional region is required to distinguish target genes that can be

activated by PR-B, but not by PR-A. Both PR-A and PR-B are expressed from two distinct promoters in the progesterone receptor (PGR) gene.^{3,7} Alterations in PR-A to PR-B ratio might also alter responsiveness to progesterone.^{7,8}

The PGR gene is located in chromosome 11, and had been known to be highly polymorphic. Several polymorphisms had been studied as a risk factor of endometriosis.⁹ The +331G/A polymorphism (rs10895068) is a single-nucleotide polymorphism (SNP) located between the transcriptional start site of PR-B (nucleotide +1) and PR-A (nucleotide +751). This polymorphism causes an increase in PR-B synthesis through the formation of an additional transcription factor binding site for TBP (TATA binding protein), initiating RNAPol II binding to TATA-box and transcriptional increase of PR-B, decreasing PR-A/PR-B ratio.^{4,8,10,11} Changes in the ratio may have an impact on the ligands and disturb hormone binding functions.¹²

Genetic variability may affect the association of the +331G/A polymorphism with endometriosis. This far, there had been limited data on the genotype and allotype distribution of these polymorphisms in endometriosis patients in Asia, especially in Indonesia. Thus, identification of the +331G/A polymorphism is required to contribute more data on the distribution of this polymorphism in Indonesian endometriosis patients.

2. Methods

Ethical clearance for this study had been approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Sriwijaya prior to the commencement of this study (Certificate No. 171/kepkrsmhfkunsri/2013).

Endometriosis patients seeking treatment at Dr. Mohammad Hoesin General Hospital's Obstetrics and Gynecology Department between August and November 2013 (n = 42, age range 23-57 years, median age: 35 years) were recruited into the study. Endometriosis patients with uterine leiomyoma and carcinoma as comorbidities were excluded. After informed consent was obtained, 2 mL of venous blood was drawn from each patient and collected in EDTA-coated tubes for DNA isolation. In addition, demographic data (marital status, ethnicity) and clinical data (severity of endometriosis based on ASRM diagnostic criteria, presence of dysmenorrhea, dyspareunia, and other forms of pelvic pain) of all recruited subjects were noted. DNA isolation from blood samples was performed using Chelex-100 resin (BioRad, USA) following instructions from the manufacturer. Obtained DNA was then stored at -20°C for later analysis. Amplification of the +331G/A SNP was performed by polymerase chain reaction (PCR), using 25 µL mixtures containing 10 µl dNTP and *GoTaq* Green polymerase (Promega, USA), 9 µl ddH₂O, a pair of primers, and 5 µl template DNA. The *LabCycler* machine (Sensoquest,

Göttingen, Germany) was used in the amplification. The primer pair sequences were 5'-CACTCATGGGATCTGAGAATC-3' (forward) and 5'-CACAAGTCCGGCACTTGAGT-3' (reverse), and amplification was performed under conditions described by De Vivo et al.¹⁰ after optimization.

Detection of the +331G/A SNP was performed by using restriction fragment length polymorphism (RFLP). The PCR product was digested with *NlaIV* endonuclease restriction enzyme at 37°C. The presence of +331G/A SNP would cause the loss of the 5'-GGNNCC-3' restriction site recognized by *NlaIV*. Digestion product was then loaded into 4% agarose gel stained with 0.1% ethidium bromide for electrophoresis and visualized under UV transillumination.

3. Results

Separation with 4% agarose gel showed bands of digestion products from subjects with the G allele (two bands, 170 and 160 bp), and a 330 bp band from subjects with A allele due to the loss of *NlaIV* restriction site in the amplified sequence (Figure 1). In this study, 26 subjects (61.9%) had heterozygous (GA) genotype, and 16 others (38.1%) had the wild type (GG) genotype, with the heterozygous genotype more commonly identified in subjects with grade IV endometriosis. No subject with homozygous mutant (AA) genotype was identified. In addition, 29 (30.9%) A alleles were identified.

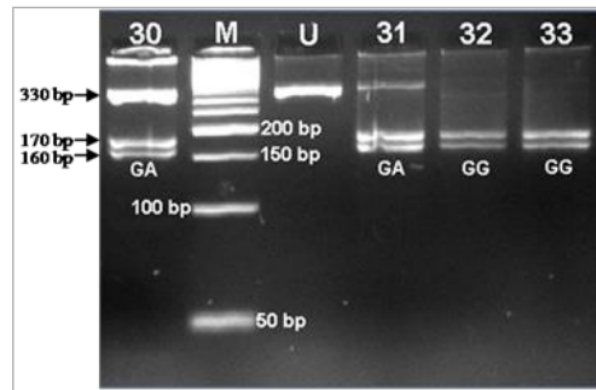


Figure 1. Visualized RFLP product for the +331G/A SNP. M: 50 bp marker ladder, U: uncut (undigested PCR product), 30-33: sample number. Samples 30 and 31: heterozygous, samples 32 and 33: wild type.

4. Discussion

The GA genotype was more commonly identified in comparison to the GG genotype, and the AA genotype was not identified. This finding is similar to another Indonesian study¹⁰ and differs from a previous study on the +331G/A polymorphism conducted in Dutch Caucasians where the GG genotype (96.9%) was more commonly identified than the GA genotype (1.5%), and the AA genotype was identified only in one subject.¹⁴ This finding also differs from an Iranian study, where the GA genotype was only identified in 1.96% of subjects.⁶

Progesterone resistance had been postulated as one of the contributing factors to the development of endometriosis. One proposed mechanism of progesterone resistance is through loss or changes in progesterone receptor expression and isoform ratio.² The +331G/A SNP causes alterations of the PR-A to PR-B ratio through the creation of an additional TATA box, increasing PR-B's transcriptional activity and expression

with no effect on PR-A.^{4,8,10} An increase in PR-B expression is known to antagonize PR-A's repression of endometrial cell proliferation.¹⁵ However, it has also been proposed that this increase in PR-B might promote cell growth via non-transcriptional mechanism by interacting with estrogen receptors.⁴

However, existing studies on the association of +331G/A SNP with endometriosis remained contradictory. Studies conducted in the Netherlands and the United States suggested the A allele's protective properties against endometriosis, possibly due to increase in PR-B transcription favored by the polymorphic allele's presence.^{4,16} A study in Indonesian patients showed an increased endometriosis risk in subjects carrying the polymorphic allele.¹⁰ An Iranian study failed to find a significant association between the +331G/A SNP and endometriosis presence in general, yet the same study elucidated alterations in the PR-A/PR-B expression at the presence of the polymorphic allele A.⁸ It has also been suggested that the polymorphic allele A

might only affect the development of deep infiltrating endometriosis by altering the invasive tendencies of endometrial cells.⁴ It should be considered that existing studies on the +331G/A SNP were chiefly conducted in Caucasian populations, and there had been minimal data available on the distribution of the +331G/A SNP in endometriosis patients from other populations.¹⁵

Alterations in cell response to progesterone might be involved in the mechanism of endometriosis-associated pelvic pain. Estrogen dominance in endometriosis has been found to be involved in the upregulation of several factors involved in nerve growth, including nerve growth factor (NGF), vascular endothelial growth factor (VEGF), and brain-derived neurotrophic factor (BDNF), and is implied to have a role in causing neuroinflammation that manifests as pelvic pain. Alterations in signaling involving the progesterone receptor might lead to reduced capacity for

progesterone to oppose estrogen-induced changes.⁶

It should be noted that this study was limited to a descriptive study and identification of the polymorphisms, warranting further studies to determine its direct effect on the expression of progesterone receptors or alterations in progesterone receptivity in the patients. Considering environmental factors' influence on the development of endometriosis, future studies should be conducted while taking environmental factors into account.

5. Conclusion

The +331G/A SNP polymorphism was identified in endometriosis patients in Palembang, South Sumatra. Further investigations are necessary to determine whether these polymorphisms play an important role in the risk of endometriosis in Indonesian population.

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