

## Research Article

## Methylation Profile of HOXA 11 Gene in Eutopic Endometrium on Endometriosis Patient with Infertility

### *Profil Metilasi Gen Hoxa 11 pada Endometrium Eutopik Pasien Endometriosis dengan Infertilitas*

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#### Abstract

**Objective:** To investigate the HOXA11 gene profile on endometriosis patients with infertility in Indonesia.

**Methods:** This cross sectional study was conducted in Dr. Cipto Mangunkusumo Hospital from July 2015- June 2016. The subjects were endometriosis patients with infertility who have been confirmed histopathological. The control group was taken from non-endometriosis and fertile patients. Eutopic endometrium samples were taken and examined for the methylation of HOXA 11 gene.

**Results:** Both groups consist of six patients. The difference of methylation of HOXA 11 gene between those two groups is statistically significant ( $p=0.03$ ). There was hyper methylation in endometriosis group.

**Conclusion:** There is a hyper methylation of HOXA 11 gene in eutopic endometrium of endometriosis patients with infertility. Thus, possibly can explain the poor endometrial receptivity in endometriosis patient and give a broad research area in epigenetic therapy of endometriosis.

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**Keywords:** endometriosis, epigenetic, HOXA 11, infertility, methylation

#### Abstrak

**Tujuan:** Teori epigenetik yang berkembang adalah terjadi hipermetilasi pada gen HOXA 11 sehingga terjadi penurunan ekspresi gen tersebut.

**Metode:** Penelitian potong lintang ini dilakukan di RS Dr. Cipto Mangunkusumo pada Juli 2015 - Juni 2016. Subjek penelitian adalah pasien endometriosis yang terbukti secara histopatologi dengan infertilitas dan kelompok kontrol merupakan pasien non-endometriosis yang fertil. Status metilasi gen HOXA 11 dari sampel endometrium eutopik pada kedua kelompok ini diperiksa dan dibandingkan.

**Hasil:** Enam pasien endometriosis dan enam pasien kontrol diambil sebagai subjek. Perbedaan tingkat metilasi gen HOXA 11 pada kedua kelompok ini berbeda secara signifikan dengan nilai  $p = 0,03$  dengan perbedaan rerata peningkatan kadar metilasi pada kelompok pasien endometriosis sebesar 33%.

**Kesimpulan:** Gen HOXA 11 yang berperan dalam reseptivitas endometrium mengalami hipermetilasi pada pasien dengan endometriosis dan infertilitas.

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**Kata kunci:** endometriosis, epigenetik, hipermetilasi, HOXA11, infertilitas

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#### INTRODUCTION

Endometriosis is a chronic recurrent disease which impacts to pain and infertility. A tissue like endometrium consisting of stroma and glands arising outside endometrium become the pathophysiology of this disease.<sup>1</sup> Endometriosis and infertility has a clinical association.<sup>2</sup> Previous theories have explained the association between endometriosis and infertility.<sup>3</sup> Latest study showed that there were 25-50% of infertile women suffering from endometriosis and about 30-50% endometriosis women having infertility.<sup>4</sup>

Numerous studies attempted to search the causal-effect relationship between endometriosis and infertility; however, the cause was still controversial. A current concept to estimate this relation is that endometriosis is a part of epigenetic disorder. Several studies conducted to determine the role of epigenetic factor on endometriosis patients with infertility because it caused poor effect to the endometrium receptivity.<sup>1,5,6</sup> Epigenetic means a branch of science focusing on the change of genetic expression on phenotype without any alteration on DNA sequence. This change contributes to variation of pathological symptoms.

Epigenetic regulation consists of DNA methylation or histone modification.<sup>7</sup> Every modification of epigenetic is reversible and dynamic. There are several factors influencing DNA methylation such as environment, stress, and lifestyle. Dietary habit can also affect to epigenetic modification e.g. folate deficiency on neural tube defect because folate has a role in DNA methylation reaction.<sup>8</sup>

Several studies stated that there was hypermethylation on gene promoter, for example HOXA10 and HOXA11 gene, causing low expression of the gene. HOX gene is a progesterone target having dysregulation on endometriosis; never the less, this gene is essential in endometrium response to progesterone during decidualization and implantation. The progesterone resistance explained poor support of implantation and failure of treatment on endometriosis.<sup>9</sup>

The study of epigenetic on endometriosis has important role to diagnosis, management, and prognosis in future. Unfortunately, there is still no available data in Indonesia about the profile of methylation on HOXA11 gene. Therefore, this study aims to find out the HOXA11 gene profile on endometriosis patients with infertility in Indonesia.

## METHODS

This was a cross sectional study to determine the difference on profile of methylation on HOXA11 gene on endometriosis patients with infertility and control group. This study was carried out at Dr. Cipto Mangunkusumo hospital from July 2015 to 2016 involving all endometriosis patients with infertility undergoing treatment.

The inclusion for cases group was 20-35-year-old women diagnosed endometriosis and confirmed by surgery and histopathology, having married and diagnosed infertility, having regular

menstruation cycle between 21 and 35 days, and agreed to participate in this study. Meanwhile, for control cases, we recruited 20-35-year-old married women, having history of pregnancy and delivery, not diagnosed as infertility, having regular menstruation cycle between 21 and 35 days, not having dysmenorrhea, pelvic pain, dysuria or pain on defecation, pain during sexual intercourse, having normal gynecology result on ultrasound, Ca-125 result less than 19 U/ml, and agreed to participate in this study.

We excluded all women having endometrial cancer, ovary cancer, gastric cancer, leukemic, having endometritis, history of ectopic pregnancy, ongoing pregnancy, contraceptive user since last 6 months, having tubal occlusion or abnormal sperm analysis result on her couple. The data would be dropped out whether resigning from this study, the broken tissue to be difficult in analysing, and histopathological result on sample not endometriosis.

The subjects were taken by consecutive sampling with 6 subjects each for the minimal number of samples. Data were analysed through SPSS Statistics version 22 on IBM software. The analysis on methylation on HOXA11 gene in eutopic endometrium between endometriosis and control group was performed through independent t-test. This study has been approved by Ethical Committee on Dr. Cipto Mangunkusumo Hospital/ Faculty of Medicine Universitas Indonesia under number 757/UN2.F1/ETIK/2015.

## RESULTS

There were 12 subjects consisting 6 subjects each on endometriosis and control group as inclusion and exclusion criteria. The characteristic data on each subject was shown at Table 1.

**Table 1.** The Subjects Characteristic Participating in this Study

Subjects	Age (years old)	Diagnosis	Fertility Status	Surgery	AFS Scoring
E1	35	Bilateral endometriosis cyst	P1, Secondary infertility for 8 years	Cystectomy laparoscopy, chromotubation, and adhesiolysis	IV
E2	27	Left ovary endometriosis cyst	Primary infertility for 3 years	Cystectomy laparoscopy and chromotubation	III
E3	35	Left ovary endometriosis cyst	Primary infertility for 3 years	Cystectomy laparoscopy and chromotubation	III

Subjects	Age (years old)	Diagnosis	Fertility Status	Surgery	AFS Scoring
E4	32	Bilateral endometriosis cyst	Primary infertility for 10 years	Bilateral cystectomy laparoscopy, chromotubation, and adhesiolysis	IV
E5	31	Left ovary endometriosis cyst	Primary infertility for 4 years	Left cystectomy laparoscopy and chromotubation	III
E6	33	Bilateral endometriosis cyst	Primary infertility for 4 years	Bilateral cystectomy laparoscopy, chromotubation, and adhesiolysis	IV

For methylation profile, the author performed DNA amplification process on promotor region of HOXA11 gene through Methylation-specific polymerase chain reaction (MSP) technique to detect the sequence having methylation. The specific primer was used on promotor region of HOXA11 gene by using software Methprimer. We also applied positive control from Epitech Methylated Human. The MSP result was going on electrophoresis in 2.8% agarose gel with 90 volts for 42 minutes.

The electrophoresis result was changed into methylation area using software "Image J". Every methylation level was the comparison between methylation region and positive control area (20944.78); then, we counted the mean of intensity on endometriosis group (64.02%) and control group (31.99%). The result pointed out that there was an increase of methylation in endometriosis group as 32.03% (Table 2).

Normality test using Shapiro Wilk showed both

groups had significant value of 0.664 and 0.443. Therefore, these group had normal distribution. Independent t-test was run to determine the difference on methylation level between endometriosis and control group. Statistical analysis revealed there was significant difference between these group ( $p=0.036$ ).

## DISCUSSION

The strength of study was the first research in Indonesia approaching methylation profile on HOXA11 gene on endometriosis patients with infertility. There was significant difference on methylation percentage of HOXA11 gene which was found higher on endometriosis group. Epigenetic theory on DNA methylation stated that silencing gene could be found in increasing of methylation.<sup>10,11</sup> Nonetheless, this study did not find out the mRNA expression on HOXA gene due to budget limitation.

**Table 2.** Analysis Result on Semi Quantitative Data on Methylation Level of HOXA11 Gene

Sample Code	Status	MSP Result		Image J Semi Quantitative		Methylation Percentage (%)
		Methyl	Un-methyl	Methyl	Un-methyl	
E1	case	v	v	15305.5	13.739.87	73.08
E2	case	v	v	14257.7	12049.12	68.07
E3	case	v	v	12754.49	11752.82	60.90
E4	case	v	v	13591.21	14184.31	64.89
E7	case	v	x	10677.68	0	50.98
E8	case	v	v	13868.53	12.846.92	66.21
K1	control	x	v	0	5260.27	0.00
K2	control	v	v	11187.6	14464.78	53.41
K3	control	v	v	13982.06	19.451.57	66.76
K4	control	v	v	8886.09	13510.06	42.43
K5	control	v	v	6148.57	26450.88	29.36
K8	control	x	v	0	6843.34	0.00

X: band (-)

V: band (+)

Positive control: 20944.78

Recently, epigenetic study in endometriosis aims to understand infertility on endometriosis targeting to promising therapy invention. HOXA11 gene has their own role in implantation disruption on infertility cases caused by endometriosis.<sup>12</sup> Some studies revealed that gene silencing of HOXA11 was led due to hyper methylation.<sup>10,12</sup>

Progesterone resistance on endometrium is a common condition found in endometriosis cases. Apart from that, endometriosis is known as estrogen-dependent disease to grow and reserve the tissue. Ovary and several other tissues such as adrenal and adipose produced estrogen. Previous study showed that there were inflammation reaction increasing aromatase activity in endometriosis; thus, it produced more estrogen on local tissue. Meanwhile, expression of HOXA11 gene was set by endogenous estrogen and progesterone.<sup>13,14</sup>

The HOXA11 gene is not a single epigenetic aberration responsible for infertility incidence on endometriosis patients. Several studies had shown the DNA methylation involvement on some genes to develop endometriosis, including HOXA10, E-cadherin, ER- $\alpha$ , SF-1, and PGR.<sup>10</sup> Natadisastra, et al. stated that methylation of HOXA10 gene in Indonesia was similar with other studies abroad. There was an increase of methylation on endometriosis patients compared to control group ( $p=0.03$ ).<sup>15,16</sup>

Epigenetic study certainly offers hope to endometriosis patients with infertility. In addition to that, researchers have broad chances to investigate epigenetic both for therapy and prognosis. It hopes that further studies about gene target therapy on enzyme affecting epigenetic change. Therefore, it can manipulate the expression of HOXA11 gene to repair the methylation aberration as promising therapy to repair the endometrium receptivity on endometriosis patients with infertility.

### CONCLUSION

There was an epigenetic role including DNA methylation on HOXA11 gene in endometriosis patients with infertility. The methylation degree of HOXA11 gene on endometriosis group with infertility shows higher significantly than control group.

### CONFLICT OF INTEREST

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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