

Research article

PPAR γ Expression in Eutopic and Ectopic Endometrium of Reproductive Age Women with Endometriosis

Ekspresi Reseptor PPAR γ Endometrium Eutopik dan Ektopik pada Penderita Endometriosis Usia Reproduksi

Adya F Dilmy, Muharam Natadisastra, Kanadi Sumapradja

Division of Reproductive Immunoendocrinology and Infertility

Department of Obstetrics and Gynecology

Faculty of Medicine University of Indonesia/

Dr. Cipto Mangunkusumo Hospital

Jakarta

Abstract

Objective: To evaluate the expression of PPAR γ receptor and to compare its expression in eutopic and ectopic endometrium in women with endometriosis.

Method: This is a cross sectional study. Ten female subjects with endometriosis that underwent laparoscopy or laparotomy who fulfilled the inclusion criteria were recruited by consecutive sampling. Two samples were taken, eutopic endometrium and ectopic endometrium from endometriosis cyst wall during surgery of each subject. PPAR γ expression was examined by two-step RT-qPCR. Our data was statistically examined using the paired t-test and Pearson's correlation test.

Result: PPAR γ was found to be expressed in eutopic and ectopic endometrium of women with endometriosis using the RT-qPCR method. The expression of PPAR γ was not statistically different in eutopic and ectopic endometrium (1.16 relative fold vs 1.25 relative fold; $p=0.26$). By Pearson's correlation there was a weak positive correlation between PPAR γ expression of eutopic and ectopic endometrium ($r=0.16$).

Conclusion: PPAR γ was detected by two-step RT-qPCR in eutopic and ectopic endometrium of women with endometriosis. Semiquantification of PPAR γ expression showed that there was no significant difference between PPAR γ expression in eutopic and ectopic endometrium of women with endometriosis. There was a weak positive correlation between PPAR γ expression in eutopic and ectopic endometrium of women with endometriosis.

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Keywords: endometriosis, PPAR γ , two-step RT-qPCR

Correspondence: Adya F Dilmy. Department of Obstetrics and Gynecology, Faculty of Medicine University of Indonesia. Jakarta; email: dido_dilmy@gmail.com

Abstrak

Tujuan: Menilai keberadaan reseptor PPAR γ serta membandingkan ekspresi reseptor PPAR γ pada endometrium eutopik dan ektopik pada penderita endometriosis.

Metode: Penelitian ini merupakan penelitian potong lintang (cross sectional). Sepuluh subjek penderita endometriosis yang menjalani laparoskopi atau laparotomi, yang masuk dalam kriteria penerimaan direkrut menggunakan consecutive sampling. Diambil dua sampel, yakni endometrium eutopik dan endometrium ektopik yang berasal dari dinding kista endometriosis saat dilakukan pembedahan, kemudian dilihat ekspresi reseptor PPAR γ dengan two-step RT-qPCR. Ekspresi masing-masing sampel diuji statistik dengan uji t berpasangan dan tes korelasi Pearson.

Hasil: Didapatkan ekspresi reseptor PPAR γ pada endometrium eutopik dan ektopik penderita endometriosis dengan metode RT-qPCR. Ekspresi reseptor PPAR γ endometrium eutopik dan ektopik didapatkan secara statistik tidak berbeda bermakna (1.16 lipatan relatif vs 1.25 lipatan relatif; $p=0.26$). Pada uji korelasi Pearson didapatkan korelasi positif lemah antara ekspresi PPAR γ pada endometrium eutopik dan ektopik ($r=0.16$).

Kesimpulan: Tampilan reseptor PPAR γ pada endometrium eutopik dan ektopik penderita endometriosis didapatkan dengan metode two-step RT-qPCR. Dengan semikuantifikasi ekspresi reseptor PPAR γ tidak didapatkan perbedaan antara ekspresi reseptor PPAR γ pada endometrium eutopik dan ektopik pada penderita endometriosis. Terdapat korelasi positif lemah antara ekspresi reseptor PPAR γ pada endometrium eutopik dan ektopik pada penderita endometriosis.

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Kata kunci: endometriosis, PPAR γ , two-step RT-qPCR

INTRODUCTION

Endometriosis is an endometrium-like lesion outside of the uterine cavity that is sensitive to the hormonal changes in the menstrual cycle.^{1,2} The prevalence of endometriosis in women of reproductive age is around 10%.^{3,4} This condition is more frequently encountered in women with pelvic pain and infertility, in which 35-50% have en-

dometriosis.² Data from RS Dr. Cipto Mangunkusumo in 2000-2005 showed 111 new cases of endometriosis diagnoses by laparoscopy, 75% of which are found in women aged 25-39 years.^{5,6}

Currently, most cases of endometriosis are treated with hormones, that may influence the menstrual cycle and fertility of the patients.^{1,7} Non-hormonal treatments such as NSAID (non-steroidal

anti-inflammatory drugs) do not interfere with fertility, but long-time treatment with such agents may pose significant side effects.^{1,2,7} A non-hormonal treatment that can be used for a long period without significant side effects and with no impact on fertility is urgently needed for endometriosis patients who wish to conceive. Understanding the pathogenesis of endometriosis may open doors to other treatment modalities, either by methods that are already available or under development.^{1,3,7-9}

One agent that may be used as a modulator is the activation of PPAR γ receptor, which is widely recognized in the treatment of diabetes.^{3,10} The potential role of PPAR γ in the pathogenesis of endometriosis and as an alternative non-hormonal treatment encouraged us to study the profile of PPAR γ receptors in patients with endometriosis.

Researchers have widely known that activation of PPAR γ influences the differentiation of adipocytes and insulin resistance.¹¹ PPAR γ may also influence the production of proinflammatory cytokines by systemic immune cells and cells in peritoneal fluids, acting as an external factor to the development of endometrial tissue in the peritoneum.¹²⁻¹⁶ Development in the last decade showed that PPAR γ may effect the profile of certain genes in eutopic and ectopic endometrium.^{17,18} These facts encouraged us to conduct a research, to find differences between the profile of PPAR γ genes in eutopic and ectopic endometrium and to elaborate the role of PPAR γ in the development of local endometriosis tissue.

Significant gene profile differences have been identified in eutopic and ectopic endometriosis tissue.¹⁹⁻²¹ Several studies have revealed higher aromatase profiles in the nucleus of ectopic glandular endometrium compared to eutopic endometrium.²²⁻²⁴ Factors enhancing neovascularisation, VEGF, and adhesion factors, CAM, are also more abundant in ectopic endometrium.^{25,26} The profile of apoptosis-regulator genes, Bcl-2 and Bax, are also different in eutopic and ectopic endometrium.²⁷

Administration of PPAR γ ligands may inhibit adhesion of endometrial cells to the peritoneum by reducing the expression of ICAM-12.^{28,29} The influence of PPAR γ on implants and neovascularization is proven by reduced VEGF activity, thus inhibiting neovascularization.¹⁹ Activation of PPAR γ also reduces aromatase, thus reducing the production of estrogen in endometriosis lesions and reducing the

proliferation of endometriosis lesions in vitro.³⁰ One study also reported reduction of anti-apoptotic genes in endometriosis lesions which had PPAR γ ligands administered.³¹

Administration of PPAR γ ligand agonists in animal with endometriosis also reduced the size and mass of ectopic endometrium.³² In baboons, it reduced the size and area of pelvic endometriosis.³³ In humans, administration of PPAR γ agonists reduced pain symptoms.³⁵ It has been reported that increased PPAR γ expression (determined through immunohistochemistry) in peritoneal lesions reduced symptoms in endometriosis patients, especially pelvic pain, dysmenorrhea, and dyspareunia.^{34,35}

The role of PPAR γ is determined by both the availability of ligands and expression of its receptor.³²⁻³⁵ Expression of PPAR γ receptor in endometriosis tissue may be useful to study its role in the pathogenesis of endometriosis and as a predictor in determining the response to therapy with PPAR γ ligands.^{34,35} We conducted this study to evaluate the expression of the PPAR γ and to compare its expression in eutopic and ectopic endometrium in reproductive age women with endometriosis.

METHODS

This a biomedical science study with cross sectional design, and with numerical comparison between two-paired group analysis. We collected data from February to April 2014. Subjects were recruited from general gynecology patients and patients from the fertility clinic who underwent surgery in the Department of Obstetrics and Gynecology, RS Cipto Mangunkusumo, Jakarta. Samples were analyzed in the Integrated Laboratory, Faculty of Medicine University of Indonesia.

Samples were obtained using endometrial biopsy when subjects were in the operating room. Samples were transported, then total DNA extraction was performed. After that, samples were stored in -80°C until analysis was performed. After all samples were collected, frozen endometrial tissues were thawed, and RT-PCR was conducted to assess the expression of PPAR γ receptors in the endometrium.

RT-PCR examination of PPAR γ receptors was initiated by thawing of samples stored in RNA ladder in room temperature, followed by RNA isolation.

The result of RNA isolation was total RNA of samples in elution buffer solution.

Development of cDNA from total RNA was started by assessment of concentration and purity of RNA by using spectrum analyzer in 260 nm and 280 nm wavelength. To assess the expression of PPAR γ with qPCR, we designed primers in silico (with computers) with IDT (Integrated DNA technologies) software, and we obtained the sequence of target and reference genes.

All qPCR were performed with Rotor Gene Q[®] machine. Quantification for each sample was calcu-

lated by using the CT (Cycle threshold) from target genes (PPAR γ) and the CT from reference genes (GAPDH). Semiquantification of gene expression was carried out with Livak method.³⁷

RESULTS

We identified 49 patients who underwent surgery for endometriosis cysts from February to April 2014. Eighteen patients were excluded: 12 did not sign the consent form, 2 patients had tubo-ovarian abscess, 2 patients had obesity, and 2 patients were smokers. From the remaining 31 patients, we did not obtain adequate endometrial samples (nil or less than 0.5 ml). Seventeen patients were adequate for analysis, however due to limited funds for reagents and material, only 10 patients (20 samples) were analyzed. The remaining 7 pair of samples were stored in -80°C for further studies.

This study included 10 subjects. All subjects underwent surgery for endometriosis cysts. The mean age of patients was 31.8 ± 4.15 years, with BMI 22.21 ± 1.38 kg/m², waist-hip ratio 0.81 ± 0.52 cm, and mean leukocyte count $8,033 \pm 1,897$. All subjects were stage III-IV endometriosis patients with endometriosis cysts; the mean size of the cysts was $6.8 \pm 2,1$ cm and all subjects experienced menstrual pain with a mean visual analogue scale (VAS) 6.3 ± 1.15 . It could be concluded that the subjects belonged to a homogenous group, which is women of reproductive age with menstrual pain and endometriosis cysts, with normal BMI and without other comorbidities.

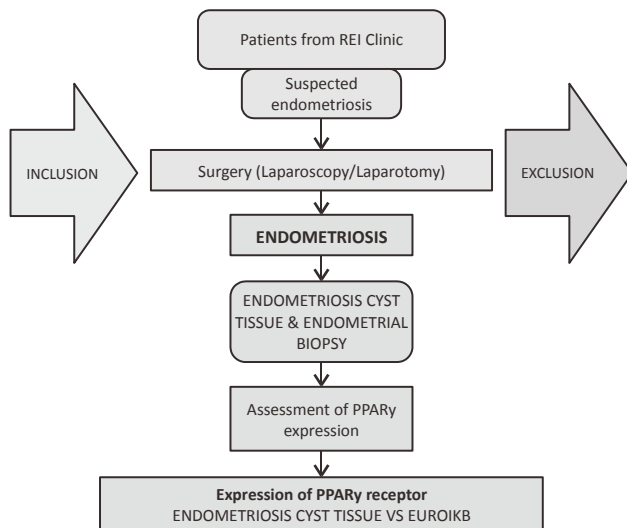


Figure 1. Research workflow.

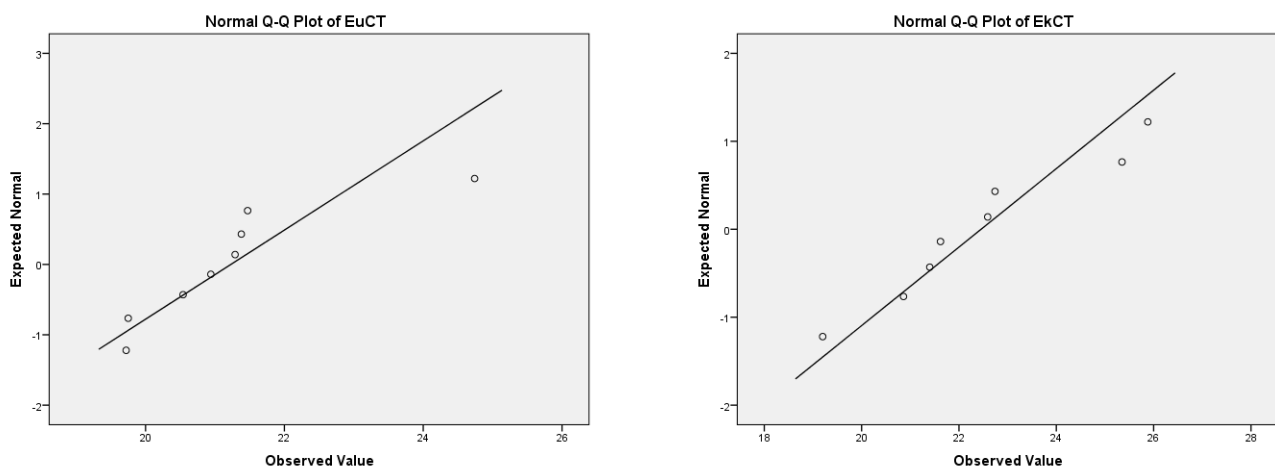


Figure 2. Normality plots of CT (Cycle Threshold) qPCR PPAR γ in Eutopic (left) and Ectopic Endometrium (right)

Quantification of receptor expression was performed with Livak method by using the following formula: $2^{-\Delta\Delta CT}$ [$\Delta\Delta CT = \Delta CT - (\text{median } CT_{PPARy} - \text{median } CT_{GAPDH})$].³⁷ The result of this formula is a relative time-fold towards the reference gene (GAPDH), a housekeeping gene whose expression is constant in all tissues.

The mean expression of PPARy in eutopic and ectopic endometrial tissue was 1.16u and 1.25u, respectively. Normality tests towards the CT (Cycle threshold) data using Kolmogorov-Smirnov and Shapiro-Wilks revealed normal distribution in both groups ($p > 0.05$).

As the data was normally distributed and obtained from the same patient (eutopic and ectopic tissue), we conducted a paired t-test to find the difference between both groups. We found that the expression of PPARy in ectopic endometrium was higher than in eutopic endometrium, but the difference was not significantly different (1.25 vs. 1.16, $p = 0.26$) (Table 1).

Table 1. Comparison of PPARy Receptor Expression

	Mean PPARy receptor expression (Unit= relative-folds to GAPDH, a reference gene)	p	Difference of mean
Eutopic tissue	1.16	0.26	0.09
Ectopic tissue	1.25		

On Pearson correlation test we found a weak correlation between the expression of PPARy receptor in ectopic endometrium and eutopic endometrium ($r = 0.16$), as shown Table 2.

Table 2. Correlation between PPARy Expression in Ectopic and Eutopic Endometrium in Endometriosis Patients

	Mean	r
PPARy receptor expression in eutopic endometrium	1.16	0.16
PPARy receptor expression in ectopic endometrium	1.25	

DISCUSSION

MacLaren et al found that PPARy can be found in the reproductive system of mice and bovine.³⁷ Pritts et al reported that PPARy was ultimately expressed in the nucleus of cultured endometrial tis-

sue.¹⁷ Peeters et al assessed the expression of PPARy in cultured endometrial cells using immunohistochemistry and RT-PCR, and its effect on administration of PPARy agonists and expression of VEGF.¹⁸ Until now, no study have assessed the expression of PPARy in ectopic and eutopic endometrial tissue in women with endometriosis and identifying the correlation between both groups.

In this study we obtained the expression of PPARy receptor in eutopic and ectopic endometrium in patients with endometriosis, which indicates that the PPARy ligand may directly influence endometrial tissue or locally, and may be the background for the administration of PPARy agonists in patients with endometriosis. Ohama et al found that administration of PPARy agonists may lower TNFy and IL-8 in endometrial tissue.³⁸ Lebovic et al proved that PPARy agonists significantly reduce the size of endometriosis lesions in mice and primates.^{32,33} One of the possible mechanism for their findings are through the role of PPARy receptors in target tissue (eutopic and ectopic endometrium), as we have found in this study.

This study also revealed that the expression of PPARy receptors in eutopic and ectopic endometrium was not significantly different ($p > 0.05$), which shows a correlation between the profile of eutopic and ectopic endometrial tissue. This is in line with other studies stating that endometriosis is a disease of eutopic endometrium.^{19,20} Afshar et al found that gene expression abnormalities in eutopic endometrium may influence the behaviour of ectopic endometrium in primates.¹⁹ Furthermore, Sha et al discovered that gene expression abnormalities identified by qPCR in eutopic endometrium of endometriosis patients compared to endometrium in patients without endometriosis may lead to the survival of endometrium undergoing retrograde menstruation.²⁰ Wren et al also concluded from 562 genes analyzed with microarray from several studies, that there are multiple correlations between expression of those genes in eutopic and ectopic endometrium.²¹ Beside our study, until now there has been no study assessing the expression of PPARy receptors in eutopic and ectopic endometrium.

We found a positive weak correlation between expression of PPARy receptor in eutopic and ectopic endometrium of endometriosis patients ($r = 0.16$). This shows that only 16% of PPARy receptor expression in eutopic or ectopic endome-

trium can be predicted from the PPAR γ receptor expression in their counterparts in patients with endometriosis. This means that the expression of PPAR γ in eutopic endometrium samples did not entirely represent its expression in ectopic endometrium samples, and vice versa. A further study on PPAR γ receptor expression in endometrial samples from subjects without endometriosis is needed to evaluate the correlation between PPAR γ receptor expression in eutopic and ectopic endometrium of endometriosis patients and patients without endometriosis.

CONCLUSION

Expression of PPAR γ receptor in eutopic and ectopic endometrial tissue was not significantly different. Therefore, we can conclude that eutopic and ectopic endometrial tissue in endometriosis patients have several similarities. This also means that PPAR γ receptor may have a role in the pathophysiology of endometriosis in eutopic endometrium of endometriosis patients.

Our results also demonstrates the potential role of PPAR γ in the pathophysiology and pathogenesis of local endometriosis in endometriosis lesions and eutopic endometrium of endometriosis patients. Knowledge on pathophysiology of endometriosis is essential to understand the pathogenesis, diagnosis, and treatment of endometriosis. Each study trying to solve the puzzle of endometriosis may further the progress towards the understanding of endometriosis.

This study could be continued with samples from patients without endometriosis as the control group, if it is ethically approved. Further studies using samples from various endometriosis samples such as superficial lesions, deeply infiltrating endometriosis, and from endometriosis patients with various stages of endometriosis could also provide significant data on cracking the puzzle of endometriosis.

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