

# Analisis genetik dan epigenetik gen pengkode faktor nyeri Nerve Growth Factor (NGF), Transient Receptor Potential Cation Channel Subfamily Ankyrin Member 1 (TRPA1), dan Purinergic Receptor P2x Ligand-Gated Ion Channel 3 (P2RX3) pada endometriosis = Genetic and epigenetic analyses of Nerve Growth Factor (NGF), Transient Receptor Potential Cation Channel Subfamily Ankyrin Member 1 (TRPA1), and Purinergic Receptor P2x Ligand-Gated Ion Channel 3 (P2RX3) as a pain protein coding in endometriosis

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## Abstrak

Endometriosis sering dikaitkan dengan nyeri menstruasi dan nyeri pelvis. Penyakit ini terjadi sekitar 10-15% pada perempuan usia reproduksi. NGF, TRPA1 dan reseptor P2RX3 aktivitas gen terlibat dalam respon nyeri. Penelitian ini bertujuan untuk menganalisis tingkat ekspresi mRNA gen NGF, reseptor TRPA1 dan reseptor P2RX3 yang diduga disebabkan oleh perubahan tingkat metilasi DNA promotor gen tersebut, serta hubungannya pada intensitas nyeri subjek endometriosis. Sampel jaringan endometrium dan susukan endometriosis peritoneum diperoleh dari 20 subjek endometriosis, sementara jaringan endometrium kontrol diperoleh dari 20 subjek nir endometriosis. Metode yang digunakan untuk analisis metilasi DNA yaitu metode MSP dan perangkat lunak Image-J digunakan untuk menganalisis intensitas pita metilasi dari gen NGF, reseptor TRPA1 dan reseptor P2RX3R, selanjutnya digunakan metode qRT-PCR untuk analisis tingkat mRNA gen-gen tersebut. Penilaian intensitas nyeri dilakukan dengan menggunakan kuesioner standar skala penilaian numerik (NRS) melalui wawancara dengan pasien. Pada penelitian ini didapatkan hasil terdapat hubungan yang bermakna antara intensitas nyeri dan kejadian endometriosis ( $p > 0,001$ ). Hasil tersebut dibuktikan dengan terdapat perbedaan yang bermakna tingkat ekspresi relatif mRNA gen NGF, reseptor P2RX3 antara jaringan endometrium endometriosis dibandingkan dengan subjek nir endometriosis masing-masing nilai  $p = < 0,05$ . Terdapat juga perbedaan yang bermakna tingkat metilasi DNA promotor gen NGF dan reseptor P2RX3 antara jaringan endometrium endometriosis dengan endometrium nir endometriosis ( $p < 0,05$ ). Selanjutnya, terdapat hubungan yang bermakna antara ekspresi mRNA NGF dan reseptor Reseptor P2RX3 dengan intensitas nyeri pada endometriosis ( $p < 0,05$ ), namun tidak terdapat hubungan antara tingkat metilasi DNA dengan ekspresi relatif mRNA gen NGF, reseptor reseptor TRPA1 pada endometriosis begitupun antara metilasi DNA dengan intensitas nyeri pada ketiga gen tersebut ( $p > 0,05$ ). Terjadi perubahan ekspresi relatif mRNA gen NGF, reseptor TRPA1 dan reseptor P2RX3 pada subjek endometriosis yang berhubungan dengan peningkatan intensitas nyeri pada endometriosis, namun mekanisme epigenetik metilasi DNA pada penelitian tersebut tidak berhubungan pada intensitas nyeri endometriosis.

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Endometriosis is often associated with both cyclic menstrual pain and pelvic pain. It affects 10% of reproductive age women. NGF, TRPA1 and P2RX3 receptors gene activity are found to be involved in pain response. This study aims to analyze the methylation level of NGF gene, TRPA1 and P2RX3 receptors that might alter the mRNA expression in peritoneal endometriosis and endometrial tissue, as well as its

correlation to the pain level in endometriosis patients. 20 endometrial tissues and 20 peritoneal endometriosis tissues were obtained from patients, while 20 endometrium tissues as control were obtained from healthy women. First, each participant was given informed consent before the research begin. We used methylation specific PCR (MSP) and Image-J software to analyze the methylation level of NGF gene, TRPA1 and P2RX3 receptors; electrophoresis to analyze the band intensity; qRT-PCR to evaluate the mRNA level in each gene. Finally, we evaluated the pain level using the standardized questionnaire of numeric rating scale (NRS) by doing interviews with patients. In this study, it was found that there is a significant relationship between pain intensity and the incidence of endometriosis ( $p > 0.001$ ). This results is proven by a significant difference in the mRNA expression level of NGF gene and P2RX3 receptor between endometrial endometriosis and endometrial non-endometriosis tissues, with each p value =  $< 0.05$ . There is also a significant difference in the DNA methylation level of NGF gene and P2RX3 receptor between endometrial endometriosis and endometrial non-endometriosis tissues ( $p < 0.05$ ). There is a significant relationship between the mRNA expression level of NGF gene, P2RX3 receptor and pain intensity in endometrial endometriosis tissues ( $p < 0.05$ ). However, the results showed that there is no correlation between the DNA methylation level and the mRNA expression of NGF gene, TRPA1 and P2RX3 receptors. There is also no correlation between the DNA methylation level of NGF gene, TRPA1 and P2RX3 receptors and pain intensity in endometriosis tissue. There is an alteration of mRNA expression of NGF gene, TRPA1 and P2RX3 receptors which correlates to pain intensity in endometriosis patients. However, there is no correlation between DNA methylation level and pain intensity in endometriosis patients.