Analisis Ekspresi mRNA dan Metilasi DNA Promoter Gen TNF-?, Sitokin CXCL16 dan P53 pada Darah Menstruasi Penderita Endometriosis dengan Nyeri dan Nir-Endometriosis = Analysis of mRNA Expression and Promoter DNA Methylation of TNF-Ĩ±, CXCL16 and P53 Genes in Menstrual Blood of Endometriosis Patients with Pain and Non-Endometriosis

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Abstrak

Latar Belakang: TNF- dan CXCL16 terlibat dalam patofisiologi endometriosis melalui regulasi respon inflamasi dan pengkode nyeri endometriosis. Peningkatan TNF- berperan dalam jalur pensinyalan P53 untuk apoptosis. Darah menstruasi sebagai pelepasan jaringan endometrium dapat digunakan dalam mengidentifikasi biomarker untuk diagnosis penyakit endometriosis tanpa memerlukan biopsi. Metode: Sampel darah menstruasi subjek dikumpulkan dengan menggunakan pembalut kertas saring dan jaringan endometrium dikumpulkan dengan melakukan biopsi, yang kemudian diekstraksi DNA dan RNA-nya. Tingkat metilasi DNA diukur dengan menggunakan metode pyrosequencing. Tingkat ekspresi mRNA diukur dengan menggunakan metode qPCR dan dianalisis dengan metode Livak Hasil: Ekspresi mRNA gen TNF- pada darah menstruasi pasien endometriosis meningkat signifikan 3,73 kali lipat dibandingkan ekspresi pada kontrol (p=0,005). Gen TNF- mengalami hipermetilasi dan berbeda bermakna dalam darah menstruasi pasien endometriosis dibandingkan kontrol (p=0,008). Sedangkan ekspresi mRNA gen CXCL16 pada darah menstruasi pasien endometriosis meningkat 2,42 kali (p=0,030) dibandingkan ekspresi mRNA darah menstruasi pada kontrol. Gen CXCL16 mengalami hipometilasi (p=0,004). Pada P53 terjadi terjadi peningkatan ekspresi gen P53 1,52 kali. Ekspresi mRNA gen TNF- dan CXCL16 pada subjek nyeri berat lebih tinggi dibandingkan subjek nyeri sedang, dan terdapat korelasi positive. Kesimpulan: Penelitian ini menunjukkan bahwa peningkatan ekspresi mRNA TNF- dan CXCL16 dalam darah menstruasi pasien endometriosis dapat menjadi penanda langsung untuk mendiagnosis endometriosis. Namun, untuk memvalidasi lebih lanjut temuan ini dan mengeksplorasi potensi sebagai alat diagnostik, penelitian tambahan yang melibatkan kelompok pasien yang lebih besar diperlukan

.....Background: TNF- and CXCL16 are implicated in the pathophysiology of endometriosis through the regulation of inflammatory response and the coding of endometriosis pain. Elevated TNF- is implicated in the P53 signaling pathway for apoptosis. Menstrual blood, as a discharge of endometrial tissue, presents an opportunity for identifying biomarkers for the diagnosis of endometriosis without resorting to biopsy. Method: Menstrual blood samples were collected using filter paper pads, and endometrial tissues were obtained via biopsy, from which DNA and RNA were extracted. DNA methylation levels were assessed using the pyrosequencing method after bisulfite conversion treatment. Meanwhile, mRNA expression levels were measured using the quantitative polymerase chain reaction (qPCR) method and analyzed using the Livak method. Results: The mRNA expression of the TNF- gene in menstrual blood of endometriosis patients increased significantly by 3.73 times compared to controls (p=0.005). The TNF- gene exhibited hypermethylation, significantly differing in menstrual blood of endometriosis patients compared to controls (p=0.008). The mRNA expression of the CXCL16 gene in menstrual blood of endometriosis patients

increased by 2.42 times (p=0.030) compared to controls, although there was no significant difference in expression between menstrual blood and endometrial tissue in endometriosis patients (p=0.173). The CXCL16 gene displayed hypomethylation (p=0.004). There was an increase in P53 gene expression, which was 1.52 times higher than in control menstrual blood. The mRNA expression of TNF- and CXCL16 genes in subjects experiencing severe pain was higher than in those with moderate pain, and there was a positive correlation. Conclusion: This study suggests that increased mRNA expression of TNF- and CXCL16 in menstrual blood of endometriosis patients may serve as direct markers for diagnosing endometriosis. However, further validation of these findings and exploration of their potential as diagnostic tools requires additional studies involving larger patient cohorts.