RESEARCH ARTICLE

mRNA expression and DNA methylation level of the MMP-2 gene in peritoneal endometriosis

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Abstract

Objective: Endometriosis is a multifactorial disease that affects 10% of women of childbearing age. Its development is known to be related to the MMP-2 gene. Specifically, the expression of MMP-2 is increase in endometriosis. The aim of this study was to evaluate the correlation between mRNA expression and DNA methylation levels of the MMP-2 gene in peritoneal endometriosis.

Methods: This study used a cross-sectional design. The samples included peritoneal endometriosis tissue from women with endometriosis and normal endometrial tissue from women without endometriosis. Twenty samples of each type were taken, and the women were 20-45 years of age. Peritoneal endometriosis tissue was acquired using the laparoscopic technique, while normal endometrial tissue was taken with the microcuretase technique. The mRNA expression of the MMP-2 gene was analysed with qRT-PCR, and the level of DNA methylation of the MMP-2 gene was analysed with a methylation-specific PCR method.

Results: The mRNA expression of MMP-2 gene in peritoneal endometriosis tissue was increased, and there were significant differences between peritoneal endometriosis tissue and normal endometrial tissue. The MMP-2 gene was hypermethylated, but there was no significant difference ($p = 0.596$) between peritoneal endometriosis tissue and endometrial tissue in terms of methylation. This study did not show a significant correlation between mRNA expression and DNA methylation levels of the MMP-2 gene ($p = 0.769$, $r = 0.070$).

Conclusion: The increase in MMP-2 gene expression in peritoneal endometriosis tissue is likely not only due to hypermethylation; there are other factors that might play a role.

Keywords: endometriosis, MMP-2, DNA methylation, cytoskeleton. (JPMA 71: S-112 [Suppl. 2]; 2021)

Introduction

Endometriosis is a common and complex disease in women that is usually characterised by the presence of endometrial-like tissue, such as stroma and glands located outside the uterus.[1] Globally, the prevalence of endometriosis in women of reproductive or premenopausal age is around 10%, or around 176 million.[2,3] Ping et al., using a microarray method, evaluated female endometrial tissue from endometriosis patients and found 2,255 genes whose expression increased and 408 genes with decreased expression in both focal adhesion and regulation of the actin cytoskeleton.[4] The Matrix Metalloproteinase 2 (MMP-2) gene is involved in cytoskeleton regulation.[4,5] The activation of MMP-2 encourages invasion of the tissue, causing degradation of the extracellular matrix (ECM),[6] which could affect the cytoskeleton.[5] However, the MMP-2 in peritoneal endometriosis tissue has not yet known it's mRNA expression and DNA Methylation level. This study aimed to compare mRNA expression, MMP-2 methylation level, and the correlation between mRNA expression and DNA methylation in peritoneal endometriosis compared to normal endometrial tissue.

Patients and methods

This was a comparative cross-sectional study consisting of two groups: women with endometriosis and women without endometriosis. Determination of the minimum number of samples was based on a sample size formula or an estimated proportion (Lemeshow's formula).[7-9] The prevalence of endometriosis in Indonesia based on data from several hospitals ranges from 13.6 to 69.55%. Thus, the sample size could be determined using the formula $N = (Z\alpha)\ 2p\ (1 - p) / d2$ (N = total sample size, $Z\alpha$ = error rate (1.96), p = proportion of endometriosis (30%), d = precision (20%)), resulting in a total of 20 samples per group, from women aged 20-45 years. The study subjects were recruited from obstetric gynaecology clinics at CiptoMangunkusumo Hospital and Fatmawati Hospital, Jakarta. The study subjects were asked about their willingness to participate in this study before the tissues

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were taken. For women with endometriosis, peritoneal endometriosis tissue was taken using a laparoscopic technique; for women without endometriosis, normal endometrial tissue was acquired using the microcuretase technique.

The mRNA expression of the MMP-2 gene was measured using the RT-PCR technique. First, all collected samples were in RNA isolation using the Quick-RNA Miniprep Plus Kit (Zymo), and then the RNA was transcribed into cDNA using an Ace qPCR ReverTra RT Master Mix with gDNA Remover kit (Toyobo). Finally, the expression of mRNA was determined using the RT-PCR technique with a Thunderbird SYBR qPCR Mix kit (Toyobo).

The level of methylation of the MMP-2 gene was determined using the methylated specific polymerase chain reaction method (MSP PCR). First, all collected samples were isolated using a gSYNC DNA Extraction kit (Geneaid). Then, the isolated DNA underwent bisulfite conversion using an EpitectBisulfite kit (Qiagen). Finally, for the analysis of the methylation level of the MMP-2 promoter, MSP PCR was performed using an Epitect MSP kit (Qiagen). Amplification products from the MSP PCR step were confirmed by electrophoresis with 1.5% agarose gel, and the final results were visualised using an ultraviolet transluminator and photographed with a camera. The ImageJ application was used to measure band intensity. The ratio between the band intensity of samples and the positive control was determined as the DNA methylation level. Statistical Programme for Social Science (SPSS) version 23 software was used for data analysis. A t-test was used to analyse differences in mRNA expression and DNA methylation levels of MMP-2 genes between peritoneal endometriosis tissue and normal endometrial tissue for normally distributed data. However, if the data distribution was abnormal, a Mann-Whitney test was used for analysis. A Pearson test was used to analyse the correlation between mRNA expression and DNA methylation of the MMP-2 gene if the data distribution was normal; otherwise, a Spearman test was performed.

Results

Analysis of the mRNA expression of the MMP-2 gene was performed with real-time PCR using an amplification curve depicting the cycle threshold (CT) value. MMP-2 gene expression increased. The results of the statistical analysis, based on an unpaired t-test, showed that there was a significant difference between peritoneal endometriosis tissue and normal endometrial tissue ($p = 0.001$).

DNA methylation of the MMP-2 gene was analysed using the MSP method. The amplicon size of the MMP-2 gene was 193 bp, and the annealing temperature after optimisation was 49°C for methylation primers and 48°C for unmethylated primers. The MMP-2 promoter methylation levels of the peritoneal endometriosis and normal endometrium tissues were 55% and 50%, respectively. The band intensities of the two types of samples (peritoneal and eutopic endometrium) and of control normal endometrium are depicted in Figure-1. We compared peritoneal endometriosis tissue with normal endometrial tissue. The t-test revealed no significant differences ($p = 0.596$). Pearson's test of the correlation between mRNA expression and DNA methylation showed no correlation between mRNA expression with DNA methylation in peritoneal endometriosis tissue ($p = 0.769$, $r = 0.070$) (Figure-2).
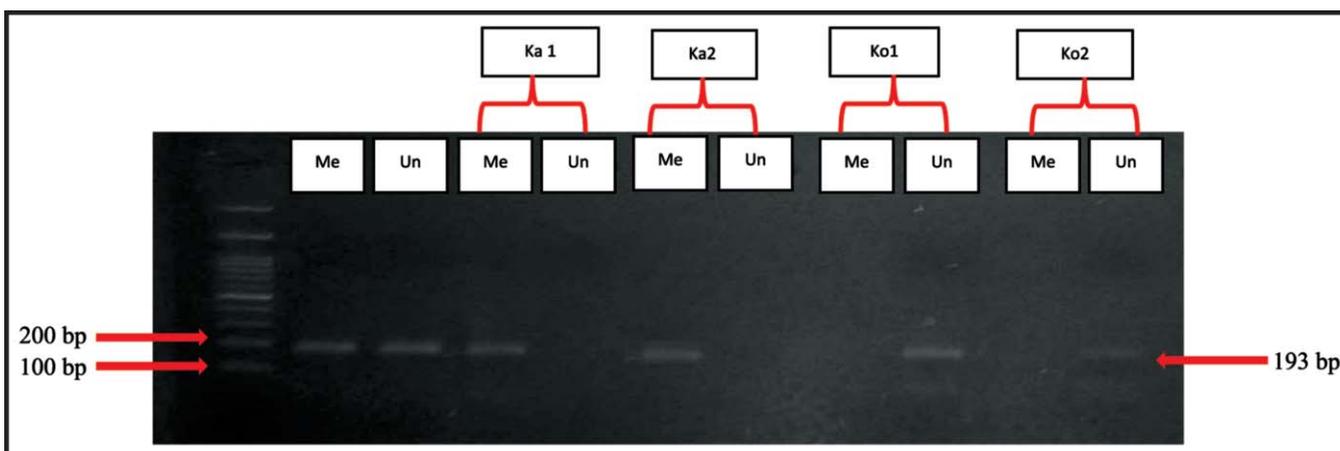
Case samples (Ka) = peritoneal endometriosis tissue; Control (Ko) = normal endometrium tissue; (+) = positive control; (-) = negative control; Me = Methylation; Un = Unmethylation

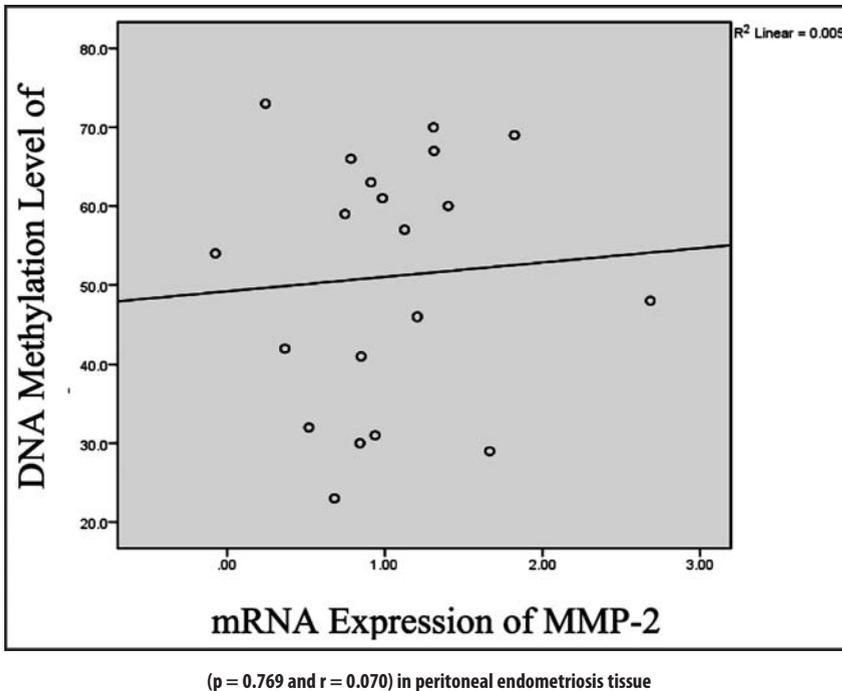**Figure-1:** Product electrophoresis visualisation results from MSP MMP-2.

(p = 0.769 and r = 0.070) in peritoneal endometriosis tissue

Figure-2: Correlation of mRNA expression with DNA methylation of MMP-2 gene.

Discussion

This results of this study are in line with those of previous studies discussed below. Women with endometriosis showed an increase in MMP-2 expression.[10] Using a mouse model, Lu et al. also found increased MMP-2 expression in peritoneal endometriosis tissue.[11] Ria et al. found similarly increased expression of MMP-2 in endometriosis tissues.[12] Huang et al. reported similar results, finding that MMP-2 expression in peritoneal fluid was higher in women with endometriosis than in women without endometriosis.[13] Tang et al. showed increased MMP-2 expression in ovarian endometriosis.[14] Likewise, a study conducted by Sotnikova et al., who used a murine mouse model, reported that MMP-2 expression increased in ectopic endometriosis tissue.[15]

This findings regarding DNA methylation in the MMP-2 gene are not the same as those of Tang et al., who found that MMP-2 gene hypomethylation occurred in ectopic endometriosis tissue.[14] Likewise, the study conducted by Lin et al. showed that MMP gene hypomethylation occurred in ischaemic stroke.[16] This could be because the type of tissue was different in this study than that of Tang et al. Thus, the sensitivity of gene methylation might depend on the location of the endometriotic lesion.

In our study, there was a positive correlation between mRNA expression and DNA methylation levels of MMP-2 in peritoneal endometriosis tissue, but it was not statistically significant. This differs from the results of Tang et al., which showed a statistically significant correlation between mRNA expression and MMP-2 DNA methylationin ovarian endometriosis tissue.[17] The lack of correlation between mRNA expression and DNA methylation in this study is unusual, as it is known that increased expression could be caused by hypomethylation. Currently, the biological basis and mechanism of increased expression due to hypermethylation of the promoter remains largely unknown. A possible explanation is that methylation that occurs in the promoter area close to the initial site of transcription leads to a three-dimensional change in the chromatin conformation in this region, which may result in increased transcription; or, methylation could prevent repressor binding, which normally prevents gene expression in normal cells.[18]

The MMP-2 DNA methylation mechanism is not one of the main drivers of changes in gene expression in peritoneal endometriosis. Such changes could be due to other epigenetic mechanisms, such as histones and microRNA (miRNA) modifications, as well as mutations and polymorphisms. The histone modifications and changes in the expression of histone deacetylases that occurred have been found in endometriosis.[4,19] MMP-2 is involved in the regulation of actin cytoskeleton, which could be activated by a variety of growth factors, including epidermal growth factor receptor (EGFR) through the MAPK pathway.[5,4,20] The activation of MMP-2 encourages tissue invasion, causing degradation in the ECM.[6] It is has been shown that the ECM affects the organisation of the actin cytoskeleton in non-muscle cells, smooth muscle cells, and striated muscle cells. In turn, ECM degradation could affect the cytoskeleton, causing it to change cell shape, motility and function. The interaction between the ECM and actin cytoskeleton is mediated by integrin receptors, which are transmembrane heterodimers capable of transmitting mechanical signals from the ECM to cells and transferring them to intracellular stimulation, which leads to changes in cell phenotype.[5]

Conclusion

There was an increase in the expression and level of methylation (hypermethylation of the MMP - 2 gene) in the peritoneal endometriosis tissue.

References

1. Wang YM, Wu MJ, Lin YH, Chen J. Association of epidermal growth factor receptor (EGFR) gene polymorphisms with endometriosis. Medicine (Baltimore) 2019; 98:e15137. doi: 10.1097/MD.0000000000015137.

2. Eisenberg VH, Weil C, Chodick G, Shalev V. Epidemiology of endometriosis: a large population-based database study from a healthcare provider with 2 million members. BJOG 2018; 125:55-62. doi: 10.1111/1471-0528.14711.

3. Marinho MCP, Magalhaes TF, Fernandes LFC, Augusto KL, Brilhante AVM, Bezerra LRPS. Quality of Life in Women with Endometriosis: An Integrative Review. J Womens Health (Larchmt) 2018; 27:399-408. doi: 10.1089/jwh.2017.6397.

4. Ping S, Ma C, Liu P, Yang L, Yang X, Wu Q, et al. Molecular mechanisms underlying endometriosis pathogenesis revealed by bioinformatics analysis of microarray data. Arch Gynecol Obstet 2016; 293:797-804. doi: 10.1007/s00404-015-3875-y.

5. Bildyug N. Matrix metalloproteinases: an emerging role in regulation of actin microfilament system. Biomol Concepts 2016; 7:321-29. doi: 10.1515/bmc-2016-0022.

6. Byeon HK, Ku M, Yang J. Beyond EGFR inhibition: multilateral combat strategies to stop the progression of head and neck cancer. Exp Mol Med 2019; 51:1-14. doi: 10.1038/s12276-018-0202-2.

7. Naing NN. Determination of sample size. Malays J Med Sci 2003; 10: 84-6.

8. Lemeshow S, Hosmer Jr DW, Klar J, Lwanga SK. Adequacy of sample size in health studies. Chichester, England: John Wiley & Sons Ltd, 1990; pp 1-4.

9. Lwanga SK, Lemeshow S. Sample size determination in health studies, A practical manual. Geneva, Switzerland: WHO's Press, 1991; pp 1-3.

10. Bruner-Tran KL, Eisenberg E, Yeaman GR, Anderson TA, McBean J, Osteen KG. Steroid and cytokine regulation of matrix metalloproteinase expression in endometriosis and the establishment of experimental endometriosis in nude mice. J Clin Endocrinol Metab 2002; 87: 4782-91. doi: 10.1210/jc.2002-020418.

11. Lu XE, Ning WX, Dong MY, Liu AX, Jin F, Huang HF. Vascular endothelial growth factor and matrix metalloproteinase-2 expedite formation of endometriosis in the early stage ICR mouse model. Fertil Steril 2006; 86 (Suppl 4): 1175-81. doi: 10.1016/j.fertnstert.2005.12.083.

12. Ria R, Loverro G, Vacca A, Ribatti D, Cormio G, Roccaro AM, et al. Angiogenesis extent and expression of matrix metalloproteinase-2 and -9 agree with progression of ovarian endometriomas. Eur J Clin Invest 2002; 32: 199-206. doi: 10.1046/j.1365-2362.2002.00960.x.

13. Huang HF, Hong LH, Tan Y, Sheng JZ. Matrix metalloproteinase 2 is associated with changes in steroid hormones in the sera and peritoneal fluid of patients with endometriosis. Fertil Steril 2004; 81: 1235-9. doi: 10.1016/j.fertnstert.2003.10.027.

14. Tang L, Xiang Y, Zhou Y, Mu J, Zai M, Xing Q, et al. The DNA methylation status of genes encoding Matrix metalloproteinases and tissue inhibitors of Matrix metalloproteinases in endometriosis. Mol Reprod Dev 2018; 85: 17-25. doi: 10.1002/mrd.22931.

15. Ba?kowiec M, Maksym RB, W?odarski PK. The bimodal role of matrix metalloproteinases and their inhibitors in etiology and pathogenesis of endometriosis (Review). Mol Med Rep 2018; 18: 3123-36. doi: 10.3892/mmr.2018.9303.

16. Lin HF, Hsi E, Huang LC, Liao YC, Juo SH, Lin RT. Methylation in the matrix metalloproteinase-2 gene is associated with cerebral ischemic stroke. J Investig Med 2017; 65: 794-99. doi: 10.1136/jim-2016-000277.

17. Hestiantoro A, Sumparadja K, Wiweko B, Pratama G, Harzif AK. Best practice on imperial, 1st ed. Jakarta, Indonesia: Sagung Seto, 2012; pp 25.

18. Weng X, Zhang H, Ye J, Kan M, Liu F, Wang T, et al. Hypermethylated Epidermal growth factor receptor (EGFR) promoter is associated with gastric cancer. Sci Rep 2015; 5: 10154. doi: 10.1038/srep10154.

19. Oliva R. Reproductive Disease Epigenetics. In: Tollefsbol TO, eds. Medical Epigenetics. California, United States: Elsevier Inc, 2016; pp 375-90.

20. Hudson LG, Moss NM, Stack MS. EGF-receptor regulation of matrix metalloproteinases in epithelial ovarian carcinoma. Future Oncol 2009; 5: 323-38. doi: 10.2217/fon.09.10.