Research Article

The Role of Matrix Metalloproteinase-9 (MMP-9) in Endometriosis

Peran Matriks Metalloproteinase-9 (MMP-9) pada Endometriosis

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Abstract

Objective: To investigate the role of MMP-9 expression in endometriosis.

Methods: The study was conducted from October 2015 to March 2016, an observational study with cross-sectional design. Samples are all endometriosis patients who underwent laparoscopic surgery in Dr. Wahidin Sudirohusodo Hospital and several other hospitals in Makassar. Samples were stored and fixed in the Grand Medika Histopathology Laboratory Makassar for examination the expression of MMP-9 using immunohistochemical methods. Conducted an analysis of 50 samples, of which 11 samples of stage II, 21 stage III samples, and 18 samples of stage IV. The data obtained and analyzed statistically using Mann Whitney and Chi Square test with a significance level of $p < 0.05$.

Results: The results reported rankings mean the expression of MMP-9 in stage I-II = 16.68, stage III-IV 27.99 ($p = 0.013$). There were differences in the expression of MMP-9 based on the stage. Stage I-II endometriosis had a more positive 2 expression of MMP-9 (45.5%), stage III-IV endometriosis have more positive 3 expression of MMP-9 (59.0%). The results of chi square test ($p = 0.043$).

Conclusion: Higher expression of MMP-9 is significantly associated with higher degree of endometriosis.

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Keywords: matrix metalloproteinase-9, stages of endometriosis

Abstrak

***Tujuan**: Mengetahui peran matriks metalloproteinase-9 (MMP-9) dengan derajat berat ringannya endometriosis.*

***Metode**: Penelitian dilakukan dari bulan Oktober 2015 sampai Maret 2016, merupakan penelitian observasional dengan desain potong lintang (cross sectional study). Sampel adalah semua penderita endometriosis yang menjalani operasi laparoskopi di RS Dr. Wahidin Sudirohusodo dan beberapa rumah sakit lain di Makassar. Sampel disimpan dan difiksasi pada Laboratorium Histopatologi Grand Medika Makassar untuk pemeriksaan ekspresi MMP-9 jaringan dengan menggunakan metode imunohistokimia. Dilakukan analisis terhadap 50 sampel, di mana 11 sampel stadium II, 21 sampel stadium III, dan 18 sampel stadium IV. Data yang diperoleh kemudian dianalisa statistik menggunakan uji Mann Whitney dan uji Chi Square dengan tingkat kemaknaan $p<0,05$.*

***Hasil**: Penelitian menunjukkan Rerata rangking ekspresi MMP-9 pada stadium I-II = 16,68, stadium III-IV 27,99 ($p = 0,013$). Stadium endometriosis I-II lebih banyak mempunyai ekspresi MMP-9 positif 2 (45,5%), stadium endometriosis III-IV lebih banyak mempunyai ekspresi MMP-9 positif 3 (59,0%).*

***Kesimpulan**: Ekspresi MMP-9 berkaitan dengan derajat keparahan endometriosis.*

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***Kata kunci**: matriks metalloproteinase-9, stadium endometriosis*

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INTRODUCTION

Endometriosis is a chronic gynecological disorder that is dependent on estrogen levels (estrogen-dependent). It is usually associated with pelvic pain and infertility. It is characterized by the presence of endometrial tissue on the outside of the uterus, most commonly in the pelvic, peritoneum or ovaries, but can also occur in recto-vaginal septum and rare in pleural, pericardial or brain. Its prevalence is estimated to be 6-10% in general woman population and 35-50% of patients experience pain and or infertility. The actual prevalence and incidence of endometriosis in the general population is not clear. Preciado et al investigated the infertile woman with endometriosis at age 30.3 ± 3.9 years, while the incidence of endometriosis in 197 infertile women was 68 people (34.5%).[1]

At the clinical level, endometriosis has a significant impact on a variety of subjective and objective changes, especially in terms of pain (dysmenorrhea, dyspareunia, diskezia), infertility and menstruation impaired. Social impact of this disease is the other side need to be considered by practitioners. Since the etiology and pathogenesis of endometriosis is still an enigma, the treatment is still a controversy.

Endometriosis is accompanied by increased secretion of pro-inflammatory cytokines, impaired cell-mediated immunity, neo-angiogenesis and endometrial reflux anomalies. To date, many of analysis of cytokines have been done thought to be involved in endometriosis. Many factors contribute to triggering, one of them is a metalloproteinase matrix (MMP), a family of zinc-dependent endopeptidase that regulate the integrity and composition of the extracellular matrix. MMP plays an important role in cell proliferation, migration, differentiation, angiogenesis, apoptosis, and immune system. Dysregulation of MMP has implications in various diseases including tumor growth. The initial phase of endometriosis is the degradation and remodeling of the extracellular matrix and increased expression of MMP.[2]

Among the various MMP, found over-expression of metalloproteinase-9 matrix (MMP-9) in different types of tumor. In mouse model, found the role of MMP-9 in terms of the process of invasion, aggression, and tumor metastases.[3] High expression of MMP-9 is an indicator of the progression of tumor growth, reduced survival rate, and increased metastases.

The previous studies evaluating the role of MMP in endometriosis had been done with different techniques and materials. Increased expression of MMP-9 was higher in ectopic endometrium (endometriosis) than eutopic endometrium and higher in severe endometriosis.[4,5] However, the study of the role of MMP-9 as endometriosis marker still less common in Indonesia, especially in South Sulawesi.

This study aimed to evaluate the correlation between the expression of Metalloproteinase Matrix-9 (MMP-9) and the severity of endometriosis.

METHODS

This was a cross-sectional study. This study was conducted at several teaching hospitals in Makassar, South Sulawesi, including Dr. Wahidin Sudirohusodo Hospital and other private hospitals used as the teaching hospital networking. The tissue samples of endometriosis cyst that had been fixed were kept at Histopathology Laboratory Grand Medika Makassar for examination the expression of MMP-9. This study was conducted from October 2015 to March 2016. Samples were all patients with endometriosis who underwent laparoscopic surgery. Data were analyzed using Mann Whitney and Chi Square test. P values less than 0.05 were considered as statistically significant.

RESULTS

Table 1 shows the characteristics of the patients. Majority of the patients were in stage III-IV of endometriosis (78%), while stage I-II of 22%. The percentage of women who experienced dysmenorrhea were 54%, a history of infertility (56%), the age of older 35 years old (56%), married (92%), normal weight (56%) and did not use contraception (82%).

Table 1. The Characteristics of the Patients.

Characteristics	Amount (n)	Percentage (%)
Stage of Endometriosis		
I - II	11	22
III - IV	39	78
Dysmenorrhea		
Yes	27	54
No	23	46
Infertile		
Yes	28	56
No	22	44
Age Group		
≤ 35	22	44
> 35	28	56
Marital Status		
Yes	46	92
No	4	8
BMI		
Normoweight	28	56
Overweight	22	44
Contraception		
Yes	9	18
No	41	82

Table 2 shows the stages of endometriosis based on the characteristics of the patients. Most of the patients stage I-II endometriosis may experience more dysmenorrhea (63.6%), infertility (72.7%), the age of less than or equal to 35 years old

(54.5%), married (90.9%), normal weight (72.7%) and did not use contraception (100%). Patients stage III-IV endometriosis may experience more dysmenorrhea (51.3%), infertility (51.3%), the age of older 35 years old (59.0%), married (92.3%), normal weight (51.3%) and did not use contraception (76.9%).

Table 2. Stages of Endometriosis Based on the Characteristics of the Patient.

Characteristics	I-II		III-IV			
	n	%	n	%	Amount	%
Dysmenorrhea						
Yes	7	63.6	20	51.3	27	54
No	4	36.4	19	48.7	23	46
Infertile						
Yes	8	72.7	20	51.3	28	56
No	3	27.3	19	48.7	22	44
Age Group						
≤ 35	6	54.5	16	41.0	22	44
> 35	5	45.5	23	59.0	28	56
Marital Status						
Yes	10	90.9	36	92.3	46	92
No	1	9.1	3	7.7	4	8
BMI						
Normoweight	8	72.7	20	51.3	28	56
Overweight	3	27.3	19	48.7	22	44
Contraception						
Yes	-	-	9	23.1	9	18
No	11	100.0	30	76.9	41	82

Table 3 shows the differences between the average ranking of expression of MMP-9 based on the stage of endometriosis. The average ranking of the expression of MMP-9 in stage I-II was 11.36, stage III-IV of 29.49. Statistical test results obtained by Mann Whitney, p value = 0.000. It is suggested that there are differences in the expression of MMP-9 based on the stage, where the higher stages of endometriosis, then the higher the expression of MMP-9.

Table 3. The Differences between the Average Ranking of Expression of MMP-9 Based on the Stage of Endometriosis

Stage of Endometriosis	The Average Ranking Of MMP-9	Significance (p)
Stage I-II	16.68	0.013
Stage III-IV	27.99	

Table 4 shows the association between the stage of endometriosis and the expression of MMP-9. The Patients who had stage I-II endometriosis have more expression of MMP-9 positive 2 (45.5%), stage III-IV endometriosis have more expression of MMP-9 positive 3 (59.0%). The result of Chi Square test was obtained value p = 0.043. This suggested that there is an association between the stage of endometriosis and the expression of MMP-9.

Table 4. The Association between the Stage of Endometriosis and the Expression of MMP-9.

MMP-9 Expression	Stage of Endometriosis						Significant (p)
	I-II		III-IV		Total		
	n	%	n	%	n	%	
Positive 1	4	36.4	5	12.8	9	18.0	0.043
Positive 2	5	45.5	11	28.2	16	32.0	
Positive 3	2	18.2	23	59.0	25	50.0	
Total	11	100.0	39	100.0	50	100.0	

DISCUSSION

The study showed an increased expression of MMP-9 in the tissue and parallel with increasing the stage of endometriosis. More than 10% of women have endometriosis. Most of them have no or only little complains of symptoms, thus the prevalence of severe endometriosis (stage III-IV) more than mild endometriosis (stage I-II). Some women complain of severe dysmenorrhea, chronic pelvic pain, and dyspareunia. Symptoms depend on the location, distribution, and depth of endometrium implantation, the presence and severity of adhesions, the presence and size of ovarian endometrioma. A study by Ragab et al involving 654 young adult women with endometriosis found that 48.9% of the subjects complained menstrual pain, and 68.8% reporter severe dysmenorrhoea.[6] A retrospective study by Andres et al involving 394 patients showed that dysmenorrhoea affects 80.9% young adult patients (severe dysmenorrhoea of 33.3%) and chronic pelvic pain of 66.6%.[7]

The mean age of patients with endometriosis varies 31.1 ± 10.4 years (between 17-53 years old), mostly 30-35 years.[8] According to the theory, the true prevalence of endometriosis is unknown, but approximately 5-10% of women in reproductive age (menarche to menopause). The peak age is 25-

30 years old, and is rare in young women or post-menopausal women.

Macer et al revealed that women with mild endometriosis showed a decrease in the pregnancy rate after 3 years compared to women with unexplained infertility (36 vs 55%).[9] Brosens et al conducted a study of IVF, and they concluded that in women with severe endometriosis, decreased ovarian reserve, decreased embryo quality and total of oocytes, and decreased the implantation ratio. The correlation between endometriosis and infertility is supported by the literature.[10]

The study by Yi et al, including 481 women with endometriosis, stage I by 153 women, stage II by 113, stage III by 110, and stage IV by 105, showed that women with severe stage (III-IV) were associated with low BMI, compared to patients with minimal or mild stage (I-II), and BMI was significantly related to the stage of disease.[11]

In this study, there was significant association between the expression of MMP-9 based on the stage of endometriosis, where the higher stages of the disease, the higher the expression of MMP-9. The immunohistochemical study of 20 cases with endometriosis showed that MMP-1, -2, and -9 were strongly detected in the stroma and epithelial cells.[12] This study supported by Chen et al, showed that increased MMP-9 occurred in ectopic endometrium. Collette et al. revealed that increased proteolytic activity of eutopic endometrium occurred in women with endometriosis than normal women, where found an increase in MMP-9 and a decrease in its natural inhibitor.[13]

The pathogenesis of endometriosis regarding attachment-aggression-angiogenesis (AAA) scheme has been generally accepted. In this process, the role of MMP was uncontested. MMP is a family of zinc-dependent endopeptidase that can degrade extracellular collagen and matrix components. The formation of ectopic endometrial tissue mediated by factors that facilitate adhesion to peritoneal cavity include cell growth, increased aromatase activity, angiogenic, neuralgenic/ lymphogenic factors, and reinforced by the activity of MMP. MMP regulated migration, infiltration, proliferation and apoptosis of cells. MMP-2 and MMP-9 are two members of the MMP family that is the most powerful in degrading collagen IV. In order to occurred adhesion and infiltration, structural changing should be occurred in the tissue by the extracellular membrane degradation and MMP play a role in accelerating neovascularization.[13,14]

Swarnakar et al stated that the synthesis of MMP-9 was correlated positively to the stage of the disease, where a correlation between focal inflammatory pelvic endometriosis was found, as consequence found improved functioning of immune cells in the peritoneal cavity.[15] MMP-9 is mainly synthesized and secreted by macrophages and neutrophils.[16] In peritoneal fluid, found a large number of derivate-macrophages substance, resulting in increased levels of MMP-9 in the peritoneal fluid of patients with endometriosis, in line with the severity of the stadium. Once ectopic endometrium was implanted in the peritoneum, a large number of MMP was expressed by autocrine and paracrine cytokine activation, thereby increasing the extracellular matrix degradation that contribute to further growth of the endometrium. This is also consistent with a recent study by Liu et al, involving 100 patients with endometriosis, including ectopic endometrium and eutopic endometrium, and patients with uterine myoma as controls. Blood and acites fluid sampling were performed to evaluate the level of MMP-9 using zimogram and enzyme-linked immunosorbent assay (ELISA). The results showed a significant correlation between MMP-9 and the location of the endometrium, clinical stage, and the proliferation cycle ($p < 0.005$).[4]

The different result reported by Gilabert-Estelles et al, that get the unsignificant expression of MMP-9 in the immunohistochemical examination of endometrial and ovarian tissue of endometriosis patients, the study revealed a new theory that the activity of proteolytic enzymes such as protease and gelatinase (MMP 2 and MMP-9) is likely to be only increased in the early formation of endometriosis implants, but when cysts/ endometrioma formed, proteolytic activity was not found anymore, only the levels of inhibitors such as PAI-1 and TIMP-1 is relatively increased, although not in all cases. These findings may elucidate the operative clinical implications where endometrioma cysts are often found in the absence of adhesions and expansion into the surrounding tissue of the ovary.[17]

CONCLUSIONS

We found that higher MMP-9 expression is associated with higher degree of endometriosis.

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