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Abd Elatif M. Elbalshy
Menofia University

Ashraf M. Kansouh
Shibeen Elkom Teaching Hospital, enasmaths1974@yahoo.com

Samar E. Ghanem
Menofia University

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Original Article

Significance of matrix metalloproteinase-9 expression in the ectopic endometrial tissue of women with endometriosis

Ashraf M. Kansouh[a], Abd Elatif M. Elbalshy[b], Samar E. Ghanem[c]

[a]Department of Obstetrics and Gynecology, Shibeen Elkom Teaching Hospital, Shibeen El-Kom, [b]Department of Pathology, Faculty of Medicine, Benha University, [c]Lecturer of Clinical Biochemistry, National Liver Institute, Menofia University, Banha, Egypt

Abstract

Background

Degradation of the extracellular matrix is a basic step in the formation of endometriosis. The extracellular matrix degradation operated by matrix metalloproteinases (MMPs) Because matrix metalloproteinases (MMPs) are essential in orchestrating proper physiological functioning of the endometrium. MMP-9 is affected by many factors such as prostaglandin E2, tumor necrosis factor-α, interleukin 1β, interferon-γ, and tissue inhibitors of matrix metalloproteinase.

Objective

The aim of the present study was to evaluate MMP-9 forms in the ectopic and eutopic endometrial tissue of women with endometriosis.

Patients and methods

The study was carried upon 56 patients. Patients were divided into two groups (study and control). The study group included 28 patients, which were infertile, and in whome diagnostic laparoscopy, open laparotomy or hysterectomy for non-malignant lesions (fibroids) showed endometriotic implants. Control group included the other 28 patients, which were normal and fertile. Specimens were prepared and MMPs-9 was immunohistochemically detected and assessed in tissue sections.

Results

The results of the current study showed a significant increase in MMP-9 expression in the ectopic endometrial tissue of women with endometriosis.

Conclusion

MMP-9 could be considered a good marker for endometriosis and might play a significant role in the progression of endometriosis.

Keywords: Ectopic endometrial tissue, endometriosis, matrix metalloproteinase-9

Introduction

Endometriosis is an estrogen-dependent benign common gynecologic disease characterized by the survival of endometrial tissue outside the uterine cavity and is manifested as abdominal pain, infertility, and other symptoms [1]. Endometriosis poses a considerable burden on affected women, their families, and the healthcare system; it affects 6–10% of women of reproductive age and, according to different sources, approximately 40 to 50% of women with infertility [2,3].

The American Fertility Society considers laparoscopy the gold standard for the diagnosis and staging of endometriosis. Staging is performed on the basis of the location, diameter, and depth of lesions, and density of adhesions. Stages range from minimal to severe disease. Despite this standardization, the correlation between stage and extent of disease remains controversial [4].

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Correspondence to: Ashraf M. Kansouh, MD, Department of Obstetrics and Gynecology, Shibeen Elkom Teaching Hospital, Berket El Sabaa Berket El Sabaa Menofia, Egypt, Tel: 01001050611. E-mail: enasmaths1974@yahoo.com

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Several theories have attempted to explain the development of this disease, but the definitive cause is still unclear [5]. The most commonly accepted mechanism for the development of peritoneal endometriotic lesions is Sampson's theory, claiming the adhesion and growth of endometrial fragments deposited into the peritoneal cavity by retrograde menstruation [6]. Sampson's theory does not account for the existence of endometriosis in areas far removed from the pelvis. It is reported to occur in locations that do not communicate with the peritoneal cavity, such as the lung and brain, where retrograde menstruation cannot account for the presence of this tissue which suggests an alternative theory for etiology of endometriosis [7].

In patients with endometriosis, increased viability, hyperplasia of proliferative endometrium, and invasiveness of endometrial cells beyond the uterus are considered to play an important role in the development of endometriosis [8,9]. To clarify the mechanisms of enhanced invasiveness of endometrial cells, special attention is paid to the assessment of expression of integrin molecules and matrix metalloproteinases (MMPs) in the endometrium. The current data are contradictory [9,10] and have not yielded a clear conclusion on the role of these factors in the pathogenesis of endometriosis [9].

MMPs belong to the family of Zn2+-dependent and Ca2+-dependent endopeptidases, participating in remodeling of the connective tissue by destruction of its organic components at physiological pH levels. The activity of MMPs regulated by tissue or cellular metalloproteinase inhibitors. MMP-9 increases in many conditions such as breast cancer, ovarian carcinoma, endometriosis, osteoarthritis, myocardial infarction, and rheumatoid arthritis [11].

The aim of this research was to determine the expression of MMP-9 in the ectopic endometrial tissue of women with endometriosis in an attempt to assess their role in the invasiveness of ectopic endometrium.

Patients and methods

This prospective case–control study was carried out in the Obstetrics and Gynecology Department of Shibeen El-Kom Teaching Hospital and in collaboration with the Pathology Department, Faculty of Medicine, Benha University, and Menoufia University, Egypt. The study was carried out during the period from May 2013 till December 2015. The study was approved by the institutional ethics committee and informed consent was obtained from the patients.

The study was carried in 56 patients (aged, 20–45 years), who were divided into two groups (study and control). The study group included 28 patients, who were infertile, and in whom diagnostic laparoscopy, open laparotomy, and hysterectomy for nonmalignant lesions (fibroids) showed endometriotic implants. The control group included the other 28 fertile patients, who had undergone laparoscopy for tubal ligation, removal of ovarian cyst, or for drilling in cases of polycystic ovary, and there was no visible evidence of endometriosis upon laparoscopy, and had vaginal bleeding.

Patients with a history of pelvic inflammatory disease or malignancy, adenomyosis uteri, intake of GnRH agonists, or exposure to steroids within the last 6 months before surgery were excluded.

All patients were subjected to a detailed assessment of personal, present, and previous medical history, general, clinical examinations, and full laboratory investigations. Vaginal ultrasound was performed for all patients to identify any pelvic lesions, for example, endometrioma (chocolate cyst). Specimen collection and preparation of fragments of ectopic endometrium (endometriotic tissue) were performed by laparoscopic (8–12 days of menstrual cycle), open surgical procedures for severe endometriosis or during hysterectomy for nonmalignant lesions such as fibroids. Control specimens were obtained from patients who were fertile and healthy (confirmed by laparoscopic surgery). Eutopic endometrial biopsies were obtained by either curettage because of vaginal bleeding immediately before the laparoscopic procedure or by the pathologist immediately after removal of the organ in patients undergoing hysterectomy for reasons unrelated to endometrial pathology.

Immunohistochemical staining

Paraffin-embedded tissue sections, 4 μm thick, were mounted on positively charged slides and fixed in a hot-air oven at 60° centigrade for 30 min, and then deparaffinized and rehydrated through a series of xylene and descending grades of alcohol before staining. After antigen retrieval with microwave pretreatment in 10 mM citrate buffer (Neo-Markers, Cat. # AP-9003), pH 6, endogenous peroxidase was blocked with 3% hydrogen peroxide for 20 min. The sections were washed three times with cold 0.01 M PBS. After blocking with 10% normal rabbit serum, the sections were incubated overnight with ready-to-use Epitope Specific Rabbit MMP-9 Antibody (Neo-Markers, Cat. #RB-9234-R7). The DAB–substrate–chromogen solution prepared was added and incubation was performed for 5–15 min until color intensity was reached. Counterstaining was performed using Mayer's hematoxylin solution, followed by a wash in tap water for 10 min. Dehydrate and clear though 95% ethyl alcohol, absolute ethyl alcohol, xylene, and two changes each for 2 min each. Mount with resinous medium. The negative control was established using normal nonimmune serum instead of the MMP-9 antibody. The positive control was established on the tissue section received with the kit.

Interpretation and evaluation of immunohistochemical staining

Immunostained tissue sections were examined in a blinded manner by two independent pathologists for interpretation of the immunohistochemical results of MMP-9. Only brown cytoplasmic staining was considered positive. The MMP-9 expression was categorized into three grades according to the intensity of staining: mild positivity: grade 1+, moderate positivity: grade 2+, and strong positivity: grade 3+.

Statistical analysis

(1) Data were analyzed using IBM SPSS Statistics, version 23 (IBM Corp., Armonk, New York, USA). Normality of numerical data distribution was examined using the Shapiro–Wilk test. Normally distributed numerical variables were presented as mean ± SD, and intergroup differences were compared using an unpaired Student's *t* test for two-group comparison, or one-way analysis of variance for multiple-group comparison.
(2) Categorical variables were presented as number (%) and differences between groups were compared using Fisher's exact test. Ordinal data were compared using the χ^2 test for trend.
(3) Correlations were tested using the Spearman rank correlation.
(4) *P* value less than 0.05 was considered statistically significant.

Results

The mean age of included women was 29.06 ± 4.51 years (range: 20 – 45 years). There was no significant differences between women of both groups regarding age . There were no significant differences between women of both groups regarding body mass index and residence (Table 1).

In terms of the clinical presentation of the patients (Table 2), there was a significant difference in the type and duration of infertility. Of the 28 women included in the study group, nine (32%) had primary infertility, whereas nine (32%) had secondary infertility, four (14%) had dysmenorrhea, four (14%) had chronic pelvic pain, and two (7%) had dyspareunia. However, all 28 women of the control group had abnormal uterine bleeding. In terms of the presence of anemia, there was no significant difference between the women in both groups. In the study group, 19 women (67%) had mild anemia, five women (17%) had moderate anemia, and four women (14%) had severe anemia. In the control group, 15 women (53%) had mild anemia, eight women (28%) had moderate anemia, and five women (17%) had severe anemia.

According to the ultrasound findings in the study group, three women (10%) were normal, but 16 women (57%) had chocolate cyst, eight women (28%) had fibroid, and only one woman (3%) had adenomyosis (Table 3). In patients with endometriosis, ovarian endometrioma was found in 15 (53%) women: unilateral in 10 (36%) women and bilateral in five (17%) women, mean size 2.2 ± 0.8 cm (range, 1–3 cm). Pelvic adhesions were identified as filmy in 11 (39%) women and dense in 17 (60%) women. Endometriotic spots were superficial in 23 (82%) women and deep in five (17%) women (Table 4).

According to the American Society of Reproductive Medicine, the patients in the current study were staged as follows: stage I: four women (14%), stage II: 13 women (46%), stage III: seven women (25%), and stage IV: four women (14%) (Table 5).

According to the immunohistochemical expression of MMP-9 in the study group, four women (14%) were in grade 1+, 13 women (46%) were in grade 2+, and 11 women (39%) were in grade 3, although all women in the control group

Table 1: Characteristics of cases with endometriosis and controls

Variables	Study group (*n*=28)	Control group (*n*=28)	*t*	*P*
Age (years)	29.0±5.5	27.6±3.2	0.91	0.251
BMI (kg/m^2)	24.6±4.6	22.2±3.9	0.97	0.040[a]
Area of residence				0.068
Urban	24 (85.7)	17 (60.7)		
Rural	4 (14.3)	11 (39.3)		

Data are presented as mean±SD or *n* (%).

Table 2: Clinical presentation in the study and control groups

	Study group	Control group	*P*
Clinical presentation			<0.001
Primary infertility	9 (32.1)	0 (0.0)	
Secondary infertility	9 (32.1)	4 (14.3)	
Dysmenorrhea	4 (14.3)	0 (0.0)	
Dyspareunia	2 (7.1)	0 (0.0)	
Chronic pelvic pain	4 (14.3)	2 (7.1)	
Abnormal vaginal bleeding	0 (0.0)	22 (78.5)	
Duration of infertility (years)	5.4±2.0	-	-
Anemia			0.380
Mild (10-10.9 g/dl)	19 (67.9)	15 (53.6)	
Moderate (9-7 g/dl)	5 (17.9)	8 (28.6)	
Severe (<7 g/dl)	4 (14.3)	5 (17.9)	

Data are presented as *n* (%) or mean±SD.

Table 3: Ultrasound findings in the study group

	Value
Normal	3 (10.7)
Chocolate cyst	16 (57.1)
Fibroid	8 (28.6)
Adenomyosis	1 (3.6)

Data are presented as *n* (%).

Table 4: Laparoscopic findings in the study group

	Value
Ovarian chocolate cyst	
None	13 (46.4)
Unilateral	10 (35.7)
Bilateral	5 (17.9)
Cyst size (cm)	2.2±0.8
Adhesions	
Filmy	11 (39.3)
Dense	17 (60.7)
Endometriotic spots	
Superficial	23 (82.1)
Deep	5 (17.9)

Data are presented as *n* (%) or mean±SD.

were negative and MMP-9 could not be detected. These data indicated a significant difference in the MMP-9 expression in both groups (Table 6). There was a very strong relation between the level of MMP-9 and the stage of endometriosis as all women with grade 1 MMP-9 had stage I endometriosis, all women with grade 2 MMP-9 had stage II endometriosis, and 11 women with grade 3 MMP-9 seven had stage III endometriosis and four women had stage IV endometriosis (Table 7 and Fig. 1). According this table, there was a very strong significant correlation between the level of MMP-9 and stages of endometriosis (0.976) as the *P* value was less than 0.0001 (Table 8 and Figs. 2, 3).

Table 5: Staging of the study group

	Value
Stage I	4 (14.3)
Stage II	13 (46.4)
Stage III	7 (25.0)
Stage IV	4 (14.3)

Data are presented as *n* (%).

Table 6: Matrix metalloproteinase-9 assay in the study and control groups

	Study group	Control group	χ^2	*P*
Specimen				<0.001[a]
Ovarian cyst	19 (67.9)	0 (0.0)		
Uterus after hysterectomy	9 (32.1)	0 (0.0)		
Endometrial tissue by D&C	0 (0.0)	28 (100.0)		
MMP-9 level			46.337	<0.001
Negative	0 (0.0)	28 (100.0)		
Grade 1+	4 (14.3)	0 (0.0)		
Grade 2+	13 (46.4)	0 (0.0)		
Grade 3+	11 (39.3)	0 (0.0)		

Data are presented as *n* (%). χ^2, χ^2 statistic; MMP-9, matrix metalloproteinase-9. [a]χ^2 test for trend.

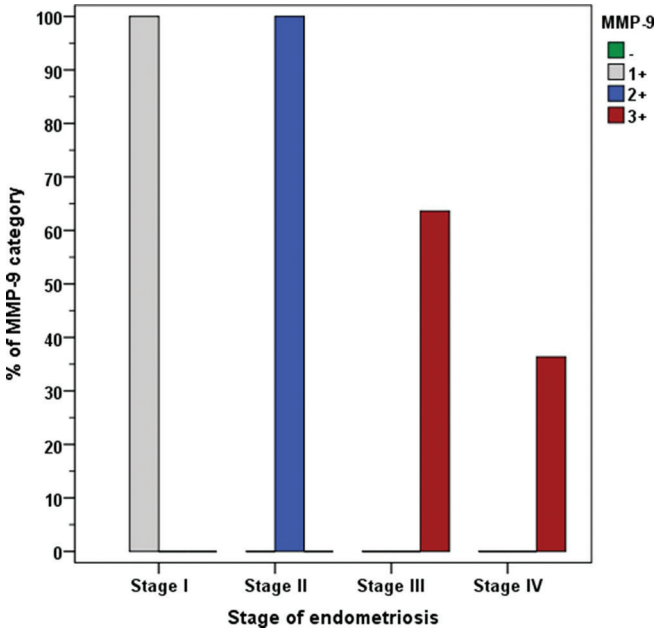

Figure 1: Relation between the stage of endometriosis and MMP-9 expression. MMP-9, matrix metalloproteinase-9.

Discussion

Endometriosis is a benign gynecological condition that affects up to 10–15% of reproductive-aged women. The disease is characterized by the growth of lesions resembling the endometrium in sites outside the uterus [12]. However, the presence of endometrial cells in the abdominal cavity is observed frequently in women during menses and this theory therefore fails to explain why some women develop endometriosis while others do not. It is likely that additional factors(MMPs and integrines) determine the ability of these endometrial deposits to implant, proliferate and persist [13].

One of the most prominent phenotypic features of endometriotic lesions is the expression of specific MMP. Constituting a group of matrix-degrading zinc enzymes, MMP are not only known to play a pivotal role in the initiation of menstrual bleeding but have also been shown to contribute to implantation and further invasion of seeded endometriotic explants [14].

The current study attempted to clarify the implications of MMP-9 in the evaluation of different stages of endometriosis. It was concluded that an increased level of MMP-9 is associated with endometriosis; this was in agreement with Marion *et al*. [15], whose study concluded that MMP-9 is a useful marker to evaluate the aggressiveness and invasiveness of endometriosis in different localizations.

Also, Collette *et al*. [16] reported, in their study, evidence for an increased release of proteolytic activity by the ectopic endometrial tissue in women with endometriosis and for the involvement of MMP-9. They concluded that there was a significant increase in MMP-9 protein expression in the ectopic endometrium in women with endometriosis compared with healthy women.

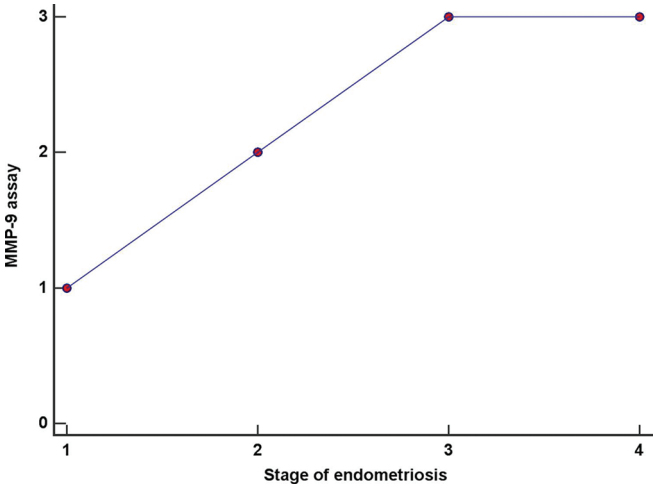

Figure 2: Correlation between stages of endometriosis and MMP-9 expression. MMP-9, matrix metalloproteinase-9.

Table 7: Relation between the level of matrix metalloproteinase-9 expression and clinicopathological variants of endometriosis

	1+ (*n*=4)	2+ (*n*=13)	3+ (*n*=11)	*F*/χ^2	*P*
Age (years)	30.0±3.6	27.5±5.1	30.4±6.3	0.748	0.534
BMI (kg/m^2)	27.5±2.4	24.0±4.6	24.3±5.1	1.159	0.346
Duration of infertility (years)	5.7±1.5	5.5±2.4	5.3±1.3	0.186	0.904
Ultrasound findings				1.624	0.202[b]
Normal	0 (0.0)	2 (15.4)	1 (9.1)		
Chocolate cyst	4 (100)	8 (61.5)	4 (36.4)		
Fibroid	0 (0.0)	2 (15.4)	6 (54.5)		
Adenomyosis	0 (0.0)	1 (7.7)	0 (0.0)		
Affected side				1.167	0.280[b]
None	3 (75.0)	5 (38.5)	5 (45.5)		
Unilateral	1 (25.0)	6 (46.2)	3 (27.3)		
Bilateral	0 (0.0)	2 (15.4)	3 (27.3)		
Size of cyst (cm)	2.0±1.2	2.1±0.8	2.4±0.7	0.803	0.504[b]
Adhesions				0.477	0.490[b]
Filmy	1 (25.0)	5 (38.5)	5 (45.5)		
Dense	3 (75.0)	8 (61.5)	6 (54.5)		
Endometriotic spots				1.519	0.218[b]
Superficial	4 (100.0)	11 (84.6)	8 (72.7)		
Deep	0 (0.0)	2 (15.4)	3 (27.3)		
Stage of endometriosis				23.727	<0.001[b]
Stage I	4 (100)	0 (0.0)	0 (0.0)		
Stage II	0 (0.0)	13 (100.0)	0 (0.0)		
Stage III	0 (0.0)	0 (0.0)	7 (63.6)		
Stage IV	0 (0.0)	0 (0.0)	4 (36.4)		

Data are presented as mean±SD or *n* (%). *F*, *F* statistic; χ^2, χ^2 statistic. One-way analysis of variance. [b]χ^2 test for trend.

Table 8: Correlation between the level of matrix metalloproteinase-9 P4 and quantitative variables in endometriosis

	Correlation coefficient (rho)	*P*
Age (years)	0.135	0.494
BMI (kg/m^2)	−0.148	0.452
Duration of infertility (years)	−0.081	0.750
Size of cyst (cm)	0.173	0.377
Stage of endometriosis	0.976	<0.0001

Matrix metalloproteinase-9 level. [a]Spearman's rank correlation.

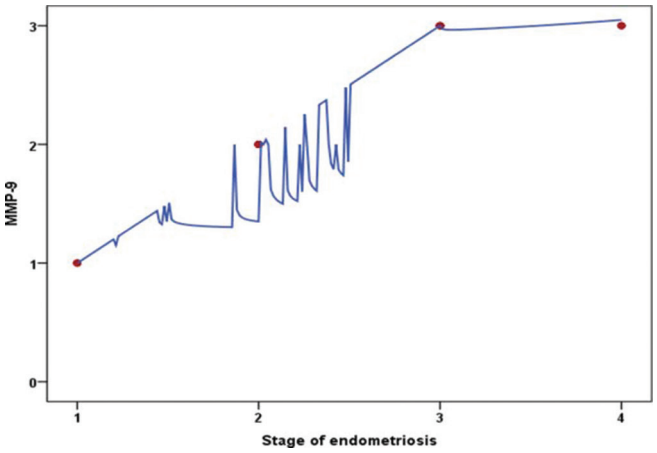

Figure 3: Correlation between the stage of endometriosis and MMP-9. Scaling for MMP-9: 0, negative; 1, 1+; 2, 2+, and 3, 3+. The fitted line represents the local regression smoothing (LOESS) trend line. MMP-9, matrix metalloproteinase-9.

The results of the current study are in agreement with those of Chung and colleagues [17–21]. Also, this may be attributed to what stated by Bergers *et al*. [22] that MMP-9 has been described to release the biologically active form of vascular endothelial growth factor, which plays an important role in angiogenesis. This process is complemented by the direct proteolytic degradation of vascular basement membrane proteins, indicating that MMP-9 may play a crucial role in the formation of new blood vessels.

Also, Dubois *et al*. [23] reported that MMP-9 is crucial during several stages of the female reproductive cycle (implantation of the embryo and remodeling of endometrial tissue that occurs during the menstrual cycle).

In terms of the role of MMP-9 in the pathogenesis of endometriosis, Saare *et al*. [24] postulated that this process involves the physiologic processes of adhesion, proliferation, and angiogenesis. In addition, complete remodeling of the extracellular matrix is necessary for the ectopic growth of endometrial implants, which requires the presence of MMPs. Endometrial stromal cells express several MMPs, including MMP-2 and MMP-9, which seem to play a key role in endometrial extracellular matrix breakdown.

Altered expression of these proteinases may lead to the establishment and progression of endometriosis because

aberrant MMP-9 gene expression has been reported in eutopic and ectopic endometrial tissue of endometriosis patients and changes in gene expression levels, and could thus be associated with a predisposition to a variety of diseases [24].

De Sanctis [25] Contradicting results were presented in the study reported by De Sanctis *et al*. [25] on matrix metalloproteinase-3 mRNA and MMP-9, he denoted that circulating mRNA for MMP-3 is significantly higher in patients with endometriosis than in control patients; regard less of the degree of severity. Conversely, the level of circulating mRNA for MMP-9 did not distinguish patients from controls.

Thus, the findings obtained are indicative of the increased MMP-9 activity in the sites of endometriotic lesion. This may lead to the consideration that their ability to exert a lytic and remodeling action on underlying connective tissue may promote the infiltration of endometrial cells into the underlying tissue (peritoneum). It may also improve capillary permeability and involvement of macrophage and lymphocyte cellular components, with further formation of infiltrates.

Conclusion

It could be concluded that; Matrix Metalloproteinase-9(MMPs-9) is a good marker for endometriosis. It may have also a significant role in the progression of endometriosis. Wide scale study on MMP-9 in endometriosis is recommended as it may open up a new era in the management of endometriosis.

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Conflicts of interest

There are no conflicts of interest.

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