Correlation between the expression of matrix metalloproteinase 9 and the expression of tissue inhibitor of metalloproteinase-1 of uterosacral ligament in uterine prolapse

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Objective

This study aimed to analyze the correlation between the immunoexpression of matrix metalloproteinase 9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TiMP1) in the uterosacral ligaments in patients with uterine prolapse.

Methods

This analytic-correlative cross-sectional study included 32 patients who were admitted at the Hasan Sadikin General Hospital from July to December 2013. Sixteen of the patients had uterine prolapse, while the rest did not. The patients underwent total hysterectomy, radical hysterectomy, or staging laparotomy. MMP-9 and TiMP1 expression in the uterosacral ligaments was measured via immunohistochemical staining. The median expression per field of view was calculated using a histoscore.

Results

MMP-9 expression in patients with uterine prolapse was found to be higher than that in the control group. Meanwhile, TiMP1 expression showed no significant difference between the groups. Spearman's analysis showed a moderate correlation between the expression of MMP-9 and uterine prolapse incidence (P=0.02), with a correlation coefficient of 0.574.

Conclusion

There is a moderate correlation between MMP-9 expression and the incidence of uterine prolapse. It can be considered one of the primary etiologies of uterine prolapse.

Keywords: Matrix metalloproteinase 9; TiMP1; Correlation; Uterosacral ligament; Uterine prolapse

Introduction

Uterine prolapse generally results from poor cardinal or uterosacral ligament (USL) apical support, which allows downward protrusion of the cervix and uterus toward the introitus [1]. Incidence of uterine prolapse increases with age, and it usually occurs in post-menopausal women. It does not increase mortality, but does result in relatively high morbidity [2].

Uterine prolapse is associated with decreased quality of life, loss of productivity, and increased use of healthcare resources [3].

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The incidence of uterine prolapse varies with race [4,5]. In a population-based study, data collection to assess the prevalence and incidence of uterine prolapse is complex due to the asymptomatic nature of early stage uterine prolapse (stage I and II). Patients with more severe cases (stage III or IV) generally visit the pelvic organ prolapse clinic.

In the USA, 11% of women aged up to 80 years undergo surgery for pelvic organ prolapse, and nearly one-third of the procedures are repeat surgeries [1]. Epidemiologic studies have shown that women have an 11-19% risk of undergoing operation for POP during their lifetime, and up to one third of them undergo additional operation for disease recurrence. Data from the Women's Health Initiative revealed anterior pelvic organ prolapse in 34.3%, posterior wall prolapse in 18.6%, and uterine prolapse in 14.3% of women included in the study [1].

However, the prevalence of uterine prolapse in Indonesia has not been comprehensively reported. During the last decade, a report from Hasan Sadikin Hospital in Bandung revealed an increase in the number of total hysterectomies, from 85 cases in 1999 to 121 cases in 2010, performed for uterine prolapse.

The pelvic organs are anatomically supported by the pelvic floor muscles and pelvic fascia consisting of connective tissue. The fascia is a loosely woven network of collagen, elastin, and fat, within which the blood vessels, lymphatic vessels, and nerves are present. Condensation of pelvic fascia in specific areas gives rise to ligaments [6].

The USL is the primary structure that supports a large part of the uterus [6]. The mechanical strength is maintained by type I and III collagen, constituting 70% of the extracellular matrix [7]. Uterine prolapse is clinically considered a consequence of decreased strength of the pelvic organ supporting tissue [4]. Prolapse occurs due to the alterations in collagen fiber content, which reduces the elasticity and durability of USL in supporting the anatomical integrity of the pelvic organs. Disorders of remodeling damaging type I and type III collagen fibers in the USL matrix after trauma may affect the mechanical function of the normal anatomic USL to support the pelvic organs [4,8].

The balance between the synthesis and degradation of collagen is essential for maintaining tissue integrity and tensile strength during tissue remodeling. Degradation depends on a combination of matrix metalloproteinase (MMP) activity, regulation, release, and activation. Growth factor-binding proteins, cell surface receptors, and cell adhesion molecules also affect collagen degradation.

Tissue inhibitor of metalloproteinase-1 (TiMP1) is a protein that inhibits the metalloproteinase enzyme. TiMP1 plays a role in the activation of MMP-9. TiMP1 inhibits the active forms of MMP-2 and MMP-9. The role of collagen type III degradation in uterine prolapse remains controversial. Furthermore, the lack of remodeling of the extracellular matrix remains a question that cannot be ignored.

This study aimed to investigate the correlation between the expression of MMP-9 and TiMP1 in USL in patients with uterine prolapse. It is expected that the results of this study will provide a foundation for understanding the etiology of uterine prolapse.

Materials and methods

A cross-sectional design was used in this correlative analytic study that assessed the correlation between the expression of MMP-9 and the expression of TiMP1 in USL in uterine prolapse.

The population in this study consisted of patients admitted to Dr. Hasan Sadikin Hospital, Bandung, between July and December 2013, who had uterine prolapse, had not undergone operative treatment, and met the inclusion criteria. The inclusion criteria were as follows: patients who underwent hysterectomy for stage III and IV uterine prolapse according to the Pelvic Organ Prolapse-Quantification (POP-Q), had not received any prior hormonal therapy, and patients who were peri-menopausal or post-menopausal. The control group consisted of patients who underwent hysterectomy for indications other than uterine prolapse such as ovarian carcinoma, epithelial type cervical cancer stage I, endometrial carcinoma, ovarian cysts, or uterine myoma. The confounding factors were age, body mass index (BMI), number of vaginal deliveries, and parity. The inclusion criteria of peri-menopausal and menopausal periods were carefully determined to suppress the effects of age.

The sample size was determined from the categorical correlative numerical analytic unpaired study of the two groups, which equaled to 16 patients in each group. Samples were collected by consecutive sampling.

An immunohistochemical examination of the MMP-9 antibody and TiMP1 obtained from the USL network was

performed after vaginal or abdominal hysterectomy. The USL network sample was processed by forming paraffin blocks stored in a freezer until it reached a specific temperature. These blocks were sliced to a thickness of 4 microns using a microtome.

Statistical analysis was used to determine the difference between the expression of MMP-9 and TiMP1 in the USL of the two groups. Statistical *P*-values were derived based on the Mann-Whitney analysis. Mann-Whitney analysis was selected because the data were not normally distributed. The strength of association between the two variables was investigated using Spearman's Rho analysis as the non-parametric correlation test. Statistical significance was determined based on the value of *P*<0.05.

Results

The samples in this study were derived from 32 paraffin blocks obtained from 16 patients with POP and 16 patients without POP (those with uterine myoma, benign ovarian tumors, malignant ovarian tumors, cervical cancer, or endometrial cancer). All patients with uterine prolapse underwent vaginal hysterectomy (n=16).

Table 1 shows that the average age in the uterine prolapse group was higher than that in the control group. Older age was significantly associated with uterine prolapse (*P*=0.0001). The majority of uterine prolapse patients had a normal BMI (18.5-22.9 kg/m^2). In contrast, the majority of patients in the control group were overweight (BMI, 23-24.9 kg/m^2). This means that a normal BMI was also significantly associated with uterine prolapse (*P*=0.005). Patients with uterine prolapse also had a higher number of vaginal deliveries and parity (*P*=0.001).

On the basis of the normality test, the values of both MMP-9 and TiMP1 were non-normal. Therefore, the data are presented as median and interval values (minimal to maximal). The median MMP-9 expression in the uterine prolapse group was higher than that in the control group; however, the median TiMP expression was the same in both groups (Table 2). Statistical analysis using the Mann-Whitney test resulted in a *P*-value of 0.004. This indicated that there was a significant difference in the level of MMP-9 expression between the two groups.

Table 3 shows the correlation between MMP-9 and TiMP1 expression calculated using the Spearman correlation, which showed a moderate correlation between the diagnoses. This correlation was negatively correlated (r=-0.523, *P*=0.02), which means that the higher the diagnosis of uterine prolapse, the higher the MMP-9 score. Additionally, patient characteristics, age, BMI, parity, and number of vaginal deliveries were significantly different between the uterine pro-

Table 1. Characteristics of patients in the uterine prolapse and the control groups

Characteristic	Uterine prolapse (n=16)	Non-uterine prolapse (n=16)	*P*-value	t	Z
Age[a]	62.56±6.5 (51-59)	53.63±2.73 (49-73)	0.0001	5.070	
Body mass index[b]	21.35 (20-26.84)	23.87 (20-29.28)	0.005		2.829
Parity	6.06±2.48	3.56±1.31	0.001	3.552	
Total vaginal labor[a]	5.19±1.83	3.25±1.12	0.001	3.602	

Values are presented as mean±standard deviation (min-max) or median (interval).
[a]Normally distributed; [b]Not normally distributed.

Table 2. Comparison of histoscore of MMP-9 and TiMP1 expression between the uterine prolapse and the control groups

Variable	Group research		*P*-value	Z
	Uterine prolapse (n=16)	Non-uterine prolapse (n=16)		
MMP-9	6.00 (2-12)	2.50 (2-12)	0.004[a]	-2.912
TiMP1	0 (0-2)	0 (0-12)	0.619	-0.498

Values are presented as median (interval).
MMP-9, matrix metalloproteinase 9; TiMP1, tissue inhibitor of metalloproteinase-1.
[a]Significant difference.

lapse and the control groups. Therefore, it was necessary to perform a linear regression for these 5 X variables (diagnosis, age, BMI, parity, and vaginal delivery) to ascertain whether these characteristics affected the MMP-9 score.

Table 4 shows that the results of the linear regression were significant (*P*=0.030, r=0.603). However, only the diagnostic variable (i.e., presence of uterine prolapse) was significantly associated with MMP-9 expression (*P*=0.017). Since only one variable showed a significant relationship, multivariate regression was not needed. To ensure that age, BMI, parity,

and delivery variables did not affect the TiMP1 score, linear regression was performed for the uterine prolapse and the control groups.

Table 5 shows the linear regression analysis to determine the effect of patient characteristics on uterine prolapse. It can be seen that none of the characteristics met the requirements for multivariate analysis, and therefore, it was not performed (*P*=0.51, r=0.488).

Discussion

The characteristics of the patients in this study included age, BMI, parity, and the number of vaginal deliveries. These characteristics act as risk factors for uterine prolapse [4] and potentially confounding variables that may affect the study's validity.

A wide range of age among the 32 patients with and without uterine prolapse can also lead to bias. Therefore, age homogeneity was difficult to achieve in this study. The youngest

Table 3. Correlation between the expression of MMP-9 and TiMP1 in the uterine prolapse and the control groups

	Correlation coefficieny r	Significancy *P*-value
Uterine prolapse	0.523[a]	0.02[b]
Non-uterine prolapse	0.362[a]	0.168

MMP-9, matrix metalloproteinase 9; TiMP1, tissue inhibitor of metalloproteinase-1.
[a]Moderate correlation; [b]Significant difference.

Table 4. Simple linear regression analysis for determining the association between patient characteristics (age, BMI, vaginal delivery, and parity) and MMP-9 expression in the uterine prolapse group

Model	Unstandardized coefficients	Standard error coefficient B	Standardized coefficients	t	*P*-value	Regression	Linear regression *P*-value
Constant	29.665	9.753	Beta	3.042	0.005	0.603	0.03
Diagnosis	-5.211	2.034	-0.711	-2.562	0.017		
Age	-0.208	0.128	-0.374	-1.629	0.115		
BMI	-0.184	0.354	-0.101	-0.519	0.608		
Parity	-0.224	0.595	-0.141	-0.377	0.709		
Total vaginal labor	0.325	0.778	0.157	0.418	0.679		

BMI, body mass index; MMP-9, matrix metalloproteinase 9.

Table 5. Simple linear regression analysis to determine the association between patient characteristics (age, BMI, vaginal delivery, and parity) and uterine prolapse

Model	Unstandardized coefficients	Standar error coefficient B	Standardized coefficients	t	*P*-value	Regression	Linear regression *P*-value
Constant	29.665	9.753	Beta	3.042	0.005	0.488	0.51
Age	-0.208	0.128	-0.374	-1.629	0.115		
BMI	-0.184	0.354	-0.101	-0.519	0.608		
Parity	-0.224	0.595	-0.141	-0.377	0.709		
Total vaginal labor	0.325	0.778	0.157	0.418	0.679		

BMI, body mass index.

patient was 49 years old, and the oldest was 73 years old. Age is a risk factor for POP, which can affect the validity of the research results.

Age is closely associated with menopause, which directly affects collagen synthesis. Low estrogen receptors make MMP-9 associated with increased flexural strength of the USL [8]. Although postmenopausal women have been reported to have a higher risk of uterine prolapse than younger women, factors such as the number of parity and a high BMI also increase the risk of uterine prolapse [5,9]. Although BMI was not significantly correlated with POP stage (P=0.271) [10].

In this study, patients in the uterine prolapse group had more vaginal deliveries (P=0.001). According to Strinic et al. [11], uterine prolapse is more common, especially in women who gave birth vaginally more than thrice or gave birth to a macrosomic baby.

The median MMP-9 expression in the uterine prolapse group was higher than that in the control group. The results of the analysis of MMP-9 expression (Table 2) in this study are consistent with the findings of a previous research conducted by Moalli et al. [12]. They found an increase in MMP-9 expression in patients with uterine prolapse [12]. Our results are also consistent with previous studies showing that the increased expression levels of MMP-9 and the reduced expression levels of TiMP1 are directly associated with the presence of uterine prolapse by Wang et al. [13]. This indicates that the differential expression levels of MMP-9 and TiMP1 are correlated with the occurrence and development of uterine prolapse.

The excessive state of degradation is also regulated by endogenous inhibitors, as well as by TiMP1. The reduction in TiMP1 levels in the uterine prolapse group (Table 2) is also consistent with that reported by Chen et al. [14].

Based on these results, it can be assumed that the decrease in the USL tensile strength and flexibility in uterine prolapse in the patients of this study was related to the state of excess degradation.

These findings demonstrate that the differential expression of MMPs and TiMPs is involved in the onset and development of uterine prolapse. A practical method to treat uterine prolapse may be to restrict MMP-9 expression and increase TiMP expression levels. Therefore, decreasing collagen degradation and increasing the level of the local binding protein, thus enhancing the tissue elasticity of the uterus.

Nevertheless, this research has several limitations. First, the sample size was relatively small. The second limitation concerns the variability of cases, since, ideally, multiple stages of uterine prolapse should be included in the study. This was not possible because of the unavailability of patients with stage I or II prolapse for sampling.

Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical approval

The Research Ethics Committees of both Universitas Padjajaran and Dr. Hasan Sadikin Hospital, Bandung approved this study.

Patient consent

Informed consent was not required because this was a retrospective study.

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