

Association between the ratio of matrix metallo proteinase-2 (MMP-2) to tissue inhibitor of MMP-2 (TIMP-2) level and the expression of collagen type-III in endopelvic fascia of menopausal women with pelvic organ prolapse

M.R.Z. TALA^{1*}, M.F.G. SIREGAR^{2**}, R. AKBARI GANIE³, I. BUDI PUTRA⁴, B. HASAN PURWA⁵, M. IQBAL PAHLEVI⁶, E. MUTIARA⁷

SUMMARY: Association between the ratio of matrix metallo proteinase-2 (MMP-2) to tissue inhibitor of MMP-2 (TIMP-2) level and the expression of collagen type-III in endopelvic fascia of menopausal women with pelvic organ prolapse.

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Objective. Pelvic organ prolapse (POP) is one of the most disturbing conditions reported by menopausal women. It has been understood that the balance of MMP-2 and TIMP-2 level play an important role in degradation of collagen type-III in endopelvic fascia which is known as underlying mechanism of POP. This study aimed to investigate the association between ratio of MMP-2 to TIMP-2 level and the expression of collagen type-III in endopelvic fascia of menopausal women with POP.

Method. This was a case-control study involved menopausal women who visited Urogynecology unit of Department of Obstetric and Gynecology, Adam Malik General Hospital from July 2017 to the date till the number of minimal sample was achieved. MMP-2 and TIMP-2 level and the expression of collagen type-III were measured from biopsy specimen taken during hysterectomy and also from blood serum.

Result. We recruited 45 menopausal women with POP as case group and 47 menopausal women without POP as control group. The mean of ratio of MMP-2 to TIMP-2 level in case group (1,53) was higher than control group (1,02). There was negative correlation between the ratio of MMP-2 to TIMP-2 level and the expression of collagen type-III with r value = 0,638 (close to 0).

Conclusion. This study found that higher ratio of MMP-2 to TIMP-2 level was associated with lower expression of collagen type-III in fascia endopelvic of menopausal women.

KEY WORDS: Pelvic organ prolapse - Matrix metallo proteinase-2 - Tissue inhibitor of MMP-2 - Collagen type-III.

Introduction

The increase in life expectancy of women results to bigger population of women with advanced age. In 2015, life expectancy for Indonesian women was 71 years (1). Women with longer life will experience menopause period and some problems related to it.

There are some physical and psychological disturbances affected the quality of life of menopausal women, one of them is pelvic organ prolapse (POP).

Pelvic organ prolapse (POP) is a common condition found among parous women. Eventhough it is not considered as life threatening condition, POP causes some problems both for individual and society.

¹ Department of Obstetrics and Gynecology, Adam Malik General Hospital, Faculty of Medicine, Universitas Sumatera Utara, Indonesia

² Professor at Fertility and Reproduction Endocrinology Division, Department of Obstetrics and Gynecology, Adam Malik General Hospital, Faculty of Medicine, Universitas Sumatera Utara, Indonesia; President of Indonesian Menopause Association (Perkumpulan Menopause Indonesia)

³ Professor at Pathology Clinic Department, Adam Malik General Hospital, Faculty of Medicine, Universitas Sumatera Utara, Indonesia

⁴ Dermatology and Venerology Department, Adam Malik General Hospital, Faculty of Medicine, Universitas Sumatera Utara, Indonesia

⁵ Department of Obstetrics and Gynecology, Universitas Padjajaran, Hasan Sadikin Hospital, Indonesia

⁶ Pediatric Surgery Department, Adam Malik General Hospital, Faculty of Medicine, Universitas Sumatera Utara, Indonesia

⁷ Public Health Faculty, Universitas Sumatera Utara, Indonesia

* Author in Research work

** Corresponding Author in Research work

In USA, POP was the reason for more than 226.000 gynecologic surgery every year, with estimated cost was more than 1 million USD. The rate of reoperation as high as 30% despite adequate prior surgery (2-5). POP frequently presents as sensation of fullness in vaginal area, due to vaginal protrusion. POP (including uterine prolapse) is a manifestation of the weakened supporting organ (6-8).

Recently, some molecular biology studies about mechanism involved in POP have been revealed that there is a specific molecular mechanism which is assumed as underlying etiology of POP (9-11).

The weakened endopelvic fascia has strong association with POP. Endopelvic fascia is collagen contained-connective tissue which covered uterosacral ligament (12). Collagen is one of glycoproteins found in connective tissue which forms a net-like web in the tissue. The integrity of the connective tissue mostly depends on the number of collagen, the type of collagen (specifically collagen type-III), and the degree of crossing in the net-like web made by collagen (13-16).

Uterosacral ligament of women's pelvic is one of important elements which support the pelvic floor. It is a part of level 1 supporting system of cervix and vagina. Study about this ligament demonstrated that the keys of tissue stability are ultrastructure quantity, and extracellular matrix protein organization, such as collagen, fibronectin, and elastin, and also matrix metalloproteinase enzyme (MMPs) which plays a role in degradation of collagen and other matrix proteins (17-19).

The disturbance of extracellular matrix protein especially collagen is caused by the imbalance between MMP enzyme, subtype gelatinase (MMP-2 and MMP-9) and inhibitor endogen protein (Tissue inhibitors of metalloproteinase type-2/TIMP-2) resulted in degradation of collagen type-III. Activity of MMP-2 and MMP-9, especially MMP-2, is regulated and inhibited by inhibitor endogen protein, TIMP-2. Ratio of MMP-2 to TIMP-2 has been studied for its potency in prevention and management of POP (20-23). However, the balance between MMP-2 and TIMP-2 in POP has not researched yet.

Experimental

This was an analytical study using case-control design. Variabel analysis was performed in

univariat, bivariat, and multivariat form. This study conducted at Department of Obstetric and Gynecology, Adam Malik General Hospital/Faculty of Medicine, University of North Sumatera, Patology Anatomy Laboratorium of Faculty of Medicine, University of North Sumatera, and Prodia Laboratorium started from Juli 2017 to the date till the number of minimal sample was achieved. This study had obtained approval from Health Research Ethics Committee of Faculty of Medicine, University of North Sumatera.

Our sample consisted of menopausal women with POP as case group and menopausal women without POP as control group who visited Urogynecology unit of Department of Obstetric and Gynecology, Adam Malik General Hospital from July 2017 to the date till the number of minimal sample was achieved.

Primary data such as age, parity, body mass index and menopause status were obtained from history taking. Physical examination was performed to assess vital signs and general health status. Gynecologic examination was also performed to determine the degree of POP based on POP-Q of *The International Continence* in case group and to confirm the absence of POP in control group.

Uterosacral ligament specimens was taken during procedure of transvaginal hysterectomy due to POP or transabdominal hysterectomy due to indication that met inclusion criterias. Then, the specimens were fixated in formalin solution 10%, after that wrap in special container and then send to Pathology Anatomy laboratory medicine Faculty University of North Sumatra. Furthermore, the specimen will be examined about the expression of MMP-2 and TIMP-2 on uterosacral ligaments from uterine tissues in operation.

Then 10 cc blood specimens obtained from veins on mediana cubiti regio, used 10 cc syringe and needles number 23 which is free germs and only used once by Clinicians. Total blood volume that obtained was 10 cc then divided into 2 tube each 5 cc for MMP-2 and TIMP-2 examination. After that, specimens was kept in special container which is prepared by laboratory and directly send to laboratory for storing in freezer -20°C within 30 minutes. The examination was done by immunoassay in ng/dl (ratio/numeric variable scale).

Research subjects who have been examined, blood and tissue specimens obtained will be given analgesic per oral for 2 days.

Results

This study was performed using observational analytical method which involved 45 POP and 47 non POP menopausal women. The characteristics sample is presented in Table 1.

The average percentage of MMP-2, TIMP-2 and collagen type-III thickness expression in POP and normal menopausal women is showed in Tables 2, 3 and 4.

Then we performed analysis of the comparison of average ratio of MMP-2 to TIMP-2 expression between the two groups, and the result is showed in Table 5.

TABLE 1 - CHARACTERISTICS OF SAMPLE.

Characteristics		prolapse		non-prolapse		total		Nilai p
		n	%	n	%	n	%	
Age	40-49 y.o.	1	2.2	36	76.6	37	40.2	0.000
	50-59 y.o.	17	37.8	11	23.4	28	30.4	
	60-69 y.o.	16	35.6	0	0	16	17.4	
	>= 70 y.o.	11	24.4	0	0	11	12.0	
BMI	low	3	6.7	8	12.8	9	9.8	0.018
	normal	18	40.0	30	63.8	48	52.2	
	obese risk	8	17.8	6	12.8	14	15.2	
	obese I	16	35.6	5	10.6	21	22.8	
Parity	primipara	0	0	9	19.1	9	9.8	0.003
	multipara	45	100	38	80.9	83	90.2	
Menopause states	haven't menopause	0	0	25	53.2	25	27.2	0.000
	menopause	45	100	22	46.8	67	72.8	

TABLE 2 - THE AVERAGE PERCENTAGE OF MMP-2 EXPRESSION IN POP AND NORMAL MENOPAUSAL WOMEN.

Group	Mean	Median	SD	Min	Max
Prolapse	4.77	4.00	1.47	1	7.00
Non-Prolapse	2.63	3.00	0.87	1.00	4.00

TABLE 3 - THE AVERAGE PERCENTAGE OF TIMP-2 EXPRESSION IN POP AND NORMAL MENOPAUSAL WOMEN.

Group	Mean	Median	SD	Min	Max
Prolapse	3.04	3.00	0.47	1.00	4.00
Non-Prolapse	2.57	3.00	0.80	1.00	4.00

TABLE 4 - THE DIFFERENCES OF AVERAGE PERCENTAGES OF COLLAGEN TYPE III THICKNESS IN POP AND NORMAL MENOPAUSAL WOMEN.

Group	Mean	Median	SD	Min	Max	Nilai p*
Prolapse	249.29	245.60	33.28	190.05	357.30	0.000
Non-Prolapse	447.56	459.80	77.86	245.60	580.70	

*Mann Whitney Test

TABLE 5 - THE DIFFERENCES OF AVERAGE PERCENTAGES OF RATIO OF MMP-2 TO TIMP-2 EXPRESSION BETWEEN POP AND NORMAL MENOPAUSAL WOMEN (WITH TOTAL SCORE).

Group	Mean	Median	SD	Min	Max	p Value
Prolapse	1.53	1.33	0.43	1.00	2.33	0.000
Non-Prolapse	1.02	1.00	0.82	1.00	1.33	

TABLE 6 - CORRELATION BETWEEN RATIO OF MMP-2 TO TIMP-2 EXPRESSION AND COLLAGEN TYPE-III EXPRESSION.

Correlation	r Value	p Value
Ratio of MMP-2 and TIMP-2 enzymes expression with collagen type III expression	-0.638	0.000

*Spearman Correlation Test

Based on calculations with Kolmogorov Smirnov test, it was showed that the data was not normally distributed, therefore bivariate analysis of correlation between the ratio of MMP-2 to TIMP-2 enzymes expression ratio and collagen type-III expression was performed with rank spearman rho. The results of this comparison are presented in Table 6.

Based on result of rank spearman rho analytical correlation test, (r) value = -0,638, and p value =0,000, with negative correlation value (close to 0), so we concluded that higher ratio of MMP-2 and TIMP-2 expression was associated with lower collagen type-III expression. The r value was negative (close to 0), with p value <0,005, which was showed that the association was statistically significant.

Discussion

It is estimated that 50% of parous women will experience POP and about 20% of gynecology cases which ended up in surgery is POP cases (24, 25). POP cases become higher as women life expectancy become longer. Besides age and parity, there is another risk factor which is known take part cause POP.

This research obtained that there was correlation between age, BMI, parity and menopause state with p value <0,05 (p=0,000, p=0,018, p=0,003, p=0,000, consecutively), which means there was statistically

significant correlation between those factors and POP. The results of risk factor in this research in accordance with research conducted by Nizomy et al. which obtained coefficient correlation value between risk factor and POP occurrence as 0,702 (p < 0.05) with just three risk factors as significant predictor for POP, such as parity, age and menopause state. Further analysis with equation regression model states that three factors with predictor coefficient is parity (1,357), menopause state (1,023), age (0,785), constant (-1,679). The conclusion is parity, menopause state, age is predictor factor for deciding POP development probability (26).

Matrices metalloproteinase (MMP-2) enzymes expression

This research showed that MMP-2 expression was positive in POP group with average percentage as 4,77 based on allred score (positive expression value is 3-8), but in non POP group, the MMP-2 enzyme expression was negative which average percentage as 2,63 based on allred score (negative expression value is 0-2). This result is consistent with previous research that found there was increased MMP-1, MMP-2, and MMP-9 enzymes expression in uterosacral ligament and vaginal tissue in patients with prolapse compared to control (27-30).

Tissue inhibitor of metalloproteinase-2 (TIMP-2) enzymes expression

In this research found that there is enhancement TIMP-2 expression in menopausal women with pelvic organ prolapse with average expression is 3,04 based on allred score (positive expression value is 3-8). Average expression of TIMP-2 enzymes is lower than average expression of last MMP enzymes. Besides that, in menopausal women without prolapse group has negative expression value which is 2,57 based on allred score (negative expression value is 0-2). There is meaningful differences between TIMP-2 enzymes expression in menopausal women who experienced prolapse and the normal one's that normally statically with p value $p < 0,005$). The odds ratio value is 8,221 that means the risk for pelvix organ prolapse of menopausal women with positive TIMP-2 expression is 8 times higher than women who has negative MMP-2 enzymes expression.

Research by Huang also showed that uterosacral ligaments of women with pelvic organ prolapse have significantly enhancement of MMP-2 mRNA expression and decrease TIMP-2 mRNA expression compared with control (31).

Collagen type III thickness

This research found that average percentage of collagen type III thickness was lower in POP group (249,29 η m) compared to non POP group (447,56 η m). There was statistically significant difference of collagen type III thickness between the two groups with p value $< 0,005$.

Jackson et al. found that women with POP had decreased total collagen for about 25% (32). Liapis et al. found slightly reduction of collagen type-III of women with prolapse and more significantly reduction in women with incontinence and pelvic floor dysfunction. But some studies did not find any differences in collagen ratio between case and control group (32). Abnormal collagen metabolism has been identified for having relationship POP and stress incontinence incidence (33).

Correlation between the ratio of MMP-2 to TIMP-2 expression ratio and collagen type-III expression

This research found that average ratio of MMP-2 and TIMP-2 expression in women with pelvic organ prolapse which is mean 1,53 (ratio more than 1). This result accordance with data in menopausal women without prolapse which is mean 1,02 (ratio more than 1). There is statistically meaningful differences between this two groups.

Besides that, in this research found that negative correlation ratio between MMP-2 and TIMP-2 expression with Collagen type III expression with r value = 0,638 (close to 0), so we concluded that higher ratio of MMP-2 and TIMP-2 expression was associated with lower collagen type-III expression. The r value was negative (close to 0), with p value $< 0,005$, which was showed that the association was statistically significant.

Some researchers have been analyzed expression from collagen regulator like MM0 and TIMP. Expression of MMP-1, -2, and -9 higher in women with prolapse, that shows enhancement in decomposition of collagen with loss of tissues integrity. In some literature that has been published, controversial arguments have been found. But unluckily, researchers didn't study all subtype MMP consistently and biopsy sites also variate according to each group. Overall, actually all MMP generally have similar function (34).

Firstly, collagen degraded became fragments by MMP-1, -8 and -13 before dissolved by MMP-2 and -9. Enhancement of MMP expression shows accelerating remodelling and collagen degradation. MMP inhibits by TIMP, and decrease expression of TIMP accordance with enhancement of MMP, like conclusions found by Liang et al. in 2010 and Budatha et al. in 2011 (34).

Imbalance between MMP and TIMP in ECM has correlation with collagen metabolism, MMP and TIMP cooperates in enhancement of collagen degradation in pelvic floor connective tissue, decreases of collagen and relaxation of pelvix floor caused pelvic organ prolapse (35).

Conclusion

In this research found that negative correlation ratio between MMP-2 and TIMP-2 expression with

Endopelvic fascia Collagen type III in menopausal women with pelvic organ prolapse. But this result has been confirmed with further research and review that includes more samples.

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