



## ASSOCIATION BETWEEN THE EXPRESSION OF MATRIX METALLO PROTEINASE-2 AND COLLAGEN TYPE-III THICKNESS IN FASCIA ENDOPELVIC OF MENOPAUSAL WOMEN WITH PELVIC ORGAN PROLAPSE

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### ABSTRACT

Pelvic organ prolapse (POP) is a disturbing conditions in menopausal women. MMP-2 break collagen type-III in endopelvic fascia. This study investigate the association between the expression of MMP-2 and collagen type-III thickness in endopelvic fascia of menopausal women with POP. 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 until the number of minimal sample was achieved. MMP-2 level and collagen type-III thickness were measured from biopsy specimen taken during hysterectomy and blood serum. 45 menopausal women with POP as case group and 47 menopausal women without POP as control group. Negative correlation between the expression of MMP-2 and collagen type-III thickness with  $r$  value = 0,638 (close to 0). This study found that higher expression of MMP-2 was associated with lower collagen type-III thickness in fascia endopelvic of menopausal women.

**KEYWORDS** : 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 (WHO, 2017). 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).

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. 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 (Chow, 2013 ; Alrbrich, 2015 ; Barber, 2013 ; Lowenstein, 2009). 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 (Lagana, 2017 ; Vitale, 2016 ; Jelovsek, 2007).

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 (Ahmed, 2011 ; Altman, 2011 ; Braekken, 2010).

The weakened endopelvic fascia has strong association with POP. Endopelvic fascia is collagen contained-connective tissue which covered uterosacral ligament (Wu, 2010). 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 (Page-McCaw, 2007 ; Bai, 2007 ; Alperin, 2006 ; Petros, 2007).

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 (Sun, 2016 ; Dviri, 2011 ; Kerkhof, 2009).

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. There are some evidences about

the role of MMP-2 and its inhibitor in POP cases. Jackson in 1996 found that there were increased pro MMP-2, active MMP-2 and MMP-9 in 8 POP patients compared to 10 control patients. In Indonesia, Asniar et all in 2013 found that the expression of MMP-2 level in uterosacral ligament of POP patients was higher than general population.

There is some data that records the involvement of MMP-2 and its inhibitors in cases of pelvic organ prolapse. Jackson in 1996, saw an increase in pro MMP-2, active MMP-2 and MMP-9 in 8 POP patients compared with 10 control patients. In Indonesia alone, Asniar, et al in 2013, found an increase in protein expression of matrix metalloproteinase degradation -2 (MMP-2) of the sacrouterine ligament is higher in women with pelvic organ prolapse (Siri, 2015). However, the association between the expression of MMP-2 and and the thickness of collagen type-III in POP have 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 asses 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 5cc blood specimens obtained from veins on mediana cubiti regio, used 5cc syringe and needles number 23 which is free germs and only used once by Clinicians. After that, specimens was kept in special container which is prepared by laboratory and directly send to laboratory for storing in freezer - 200C within 30 minutes. The examination done by immunoassay in ng/dl (ratio/numeric variable scale).

Research subject 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 4.1.

**Table 4.1 Characteristics of Sample**

Characteristics	prolaps		non-prolaps		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	

The average percentage of the expression of MMP-2 and collagen type-III thickness in POP and normal menopausal women is presented in table 4.2 and 4.3.

**Table 4.2. 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 4.3 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

Based on calculations with Kolmogorov Smirnov test, it was showed that the data was not normally distributed, therefore bivariate analysis of correlation between the expression of MMP-2 and collagen type-III thickness was performed with rank spearman rho. The results is presented in table 4.6.:

**Table 4.6 Correlation between the expression of MMP-2 and collagen type-III thickness**

Correlation	r Value	p Value
Expression of MMP-2 and collagen type-III thickness	-0.638	0.000

**\*Spearman Correlation Test**

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 expression of MMP-2 was associated with lower collagen type-III thickness. 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 (Kuncharapu 2010 ; Haylen, 2010). 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), constanta (-1,679). The conclusion is parity, menopause state, age is predictor factor for deciding POP development probability (Nizomy, 2011).

**Matrics 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 (Bortolini, 2011; Sun, 2011 ; Klein, 2011 ; Kritzkova, 2011).

**Collagen Type III Thickness**

This research found that average percentage of collagen type III thickness was lower in POP group (249,29 m) compared to non POP group (447,56 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 %.28 Liapis and partners 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.28 Abnormal collagen metabolism has been identified for having relationship POP and stress incontinence incidence (Phillips, 2006).

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

Previous study conducted by Hu Y et al showed similar finding that the expression of MMP-2 was higher in POP group compared to control, and conversely, the collagen type-III thickness was lower in POP group compared to control (Hu, 2017).

**CONCLUSION**

Our study found that there was negative association between

expression of MMP-2 and collagen type-III thickness in endopelvic fascia of menopausal women with POP. However, our finding is needed to be confirmed in further research.

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