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Immunohistochemical Expression of Matrix Metalloproteinase-2 (MMP-2) in Benign Ovarian Cyst at Adam Malik General Hospital in 2017

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Abstract:
Objective*: To determine the immunohistochemical expression of matrix metalloproteinase-2 (MMP-2) in benign ovarian cyst tissue at Adam Malik General Hospital in 2017*
Material and Method*: This study was an retrospective observational analytic study with a cross sectional design in 22 cases of benign ovarian cysts undergoing gynecological surgery at Adam Malik General Hospital Medan in 2017. Subjects were recruited based on inclusion criteria. Data were collected from medical records and immunohistochemical results which showed tissue MMP-2 expression, then analyzed using descriptive statistics and chi-square test with a significance value of P<0.05.*
Result*: Most subjects with benign ovarian cysts at the age of 20-50 years, non- menopausal, had children ≥1 and normal body mass index. The highest histopathological distribution of benign ovarian cysts with epithelium types, namely mucinouscystadenoma. There was no significant relationship between age, age of menarche, menopause status and body mass index with immunohistochemical expression of MMP-2 in benign ovarian cyst. There was no significant relationship between histopathological subtypes of benign ovarian cysts with MMP-2 immunohistochemical expression. Immunohistochemical expression of MMP-2 in benign ovarian cyst tissue mostly showed negative results (59%).*
Conclusion*: Immunohistochemical expression of MMP-2 was negative in benign ovarian cyst tissue*
Keywords*: Benign ovarian cyst,matrix metalloproteinase-2, immunohistochemical expression*

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I. Introduction

Ovarian cysts are a bag containing fluid or semi-liquid originating from ovarian tissue. Ovarian cysts are gynecological problems that women often encounter during their reproductive period, can be physiological cysts, some are benign, while others can be malignant cysts.[1,2] In premenopausal women, almost all ovarian cysts and masses are benign. The overall incidence of asymptomatic ovarian cysts in premenopausal women who developed malignancy was 1:1000 and increased to 3:1000 at age 50.[3] According to Smeltzer et al, benign ovarian cysts were found in 10-20% of cases of all ovaries. This type of cyst most often occurs in women aged between 20-50 years and rarely occurs in prepubertal periods.[4]

The etiology of the formation of an ovarian cyst is unknown and influenced by many factors. Some factors that cause changes in the ovarian epithelium are age, genetics, age of menarche, menopause status, body mass index and parity, where there will be an imbalance of estrogen and progesterone hormones, ovarian degeneration and uncontrolled follicular growth.[5,6,7] Other factors that support the formation of an ovarian cyst are infectious / inflammatory factors that originate from infection / inflammation in the pelvic region and unhealthy lifestyles such as smoking, alcohol consumption, consumption of high-fat diets and lack of fiber diets, lack of exercise and pollution that result in occurrence of changes at the genetic / epigenetic level which results in uncontrolled epithelial growth, then ovarian degeneration resulting in ovarian cysts.[5,6,7] Genetic and epigenetic changes appear in pathways that stimulate cell proliferation, inhibition of cell death and stimulate neoangiogenesis and accelerate growth.[8] However, the precursor lesions of the cyst are not known with certainty[9], which causes the majority of cysts to be late detected.[10,11]

Matrix metalloproteinase (MMP), also called matrixins, is a group of enzymes that use zinc as a catalytic mechanism that functions to degrade matrix and non-matrix proteins, including proteoglycans.[12] MMP plays a major role for collagen degradation, but also functions in tissue repair and remodeling, wound healing, and morphogenesis. Its function is not only limited to the degradation of the extracellular matrix (ECM) because MMP has an important role in surface cells and protein activation in ECM.[13] The matrix

metalloproteinase-2 (MMP-2) is an MMP which has the ability to degrade gelatin. MMP-2 is expressed as a 72kDa proenzyme and activated by the MMP-2 inhibitor complex under stoichiometric conditions.[13] MMP-2 can break type IV gelatin, elastin, and fibronectin and has the ability to degrade collagen in the basement membrane of blood vessels. MMP-2 becomes involved in the process of neovascularization under physiological conditions as well as pathological conditions as in tumor metastasis. This proves that MMP-2 plays a role in the process of angiogenesis where it plays a role in inducing endothelial cell apoptosis.[13.14]MMP-2 also plays a role in the repair and rupture of the ovarian follicle wall at the time of ovulation. Luteinizing Hormone (LH) surge activates pro-MMP-2 to become the active MMP-2 form to degrade follicle walls. Inadequate LH levels will cause ovarian cysts to form, so that if LH levels decrease then the process of activating pro-MMP-2 to MMP-2 will also decrease. This is what causes the occurrence of collagen degradation which is followed by the secretion of an enzyme called LysilOxydase (LOX) where this enzyme will inhibit collagen damage in the membrane follicles so that ovulation does not occur.[14]

From the explanation above, it appears that MMP-2 plays a role in collagen degradation in the basal membrane of follicles and if there is a decrease in MMP-2 concentration there will be no degradation and cause thickening of the follicle wall and formation of ovarian cysts.[16] MMP-2 level in human blood has lower than in the tissue, so observing the presence of MMP-2 expressions will be more visible on the tissue. Decreasing the expression of this enzyme in tissue can be observed by immunohistochemical techniques, especially on stromal cells.[15] Therefore, it is necessary to assess the concentration and expression of MMP-2 in humans so that it can be used as a basis for predictive diagnostic formation of ovarian cysts.

II. Material and Method

This study was anretrospective observational analytic study with a cross sectional design, using secondary data from medical records and the results of MMP-2 immuhistochemical expression on tissues. The study was conducted between March to May 2018 in the Department of Obstetric& Gynecology and Anatomical Pathology of the Medical Faculty of the University of Sumatera Utara at Adam Malik General Hospital in Medanwith the subject of this research is the total number of paraffin blocks that are stored in the Pathology Laboratory of Anatomy whereshowed histopathological results of a benign ovarian cyst, namely mucinous cystadenoma, serouscystadenoma and dermoid cyst (mature teratoma) obtained from gynecologic surgery from January to December 2017. The 26 sample included in the inclusion criteria, but only 22 cases had paraffin blocks and immunohistochemicalof MMP-2 was carried out.

Medical records of patients who meet the inclusion criteria are recorded register numbers, age, menarche age, menopausal status, parity, body mass index (BMI) and histopathological results that show benign ovarian cysts. Then immunohistochemical examination of MMP-2 expression in tissue paraffin blocks was carried out by staining using human MMP-2 specific antibodies (Monoclonal Mouse IgG_1). Immunohistochemical interpretation of MMP-2 expression was performed by anatomical pathologist with a microscope using an Allred score (negative, weak positive, moderate positive, strong positive). All recorded data are grouped, processed, and analyzed using SPSS version 20 (SPSS Inc.,). Descriptive data to see the clinicopathological characteristics of the research subjects. The relationship between variables was carried out by Chi square test with 95% confidence interval and $p < 0.05$ was considered as significant. Ethical clearance was obtained from the Research Ethics Commission of the Faculty of Medicine, University of Sumatera Utara.

III. Result

During the period January to December 2017, there were 26 cases of benign ovarian cysts who met the inclusion criteria, but only 22 cases had paraffin blocks. The characteristics of the research subjects were divided based on age,age of menarche, menopause status, parity, and body mass index according to table 1. Most of the subjects were aged 20-50 years (81.8%), the non-menopausal status was 81.8%, had parity more than 1 times and BMI is in normoweight around 54.5%.

Table 1: Characteristics of subjects

Characteristics	Benign Ovarian Cyst		Mean ± SD
	n	%	
Age (year)			
<20	0	0	
20-50	18	81,8	41,4 ±9,82
>50	4	18,2	
Menarche age (year)			
≤ 12	11	50,0	13 ±1,54
> 12	11	50,0	
Menopause Status			
Non- menopausal	18	81,8	1,82 ±0,39
Menopausal	4	18,2	

Parity			
Nulliparous	0	0	3,14 ±1,32
≥ 1	22	100,0	
Body Mass Index			
Normoweight	12	54,5	
Overweight	6	27,3	25,8 ±3,35
Obesitas	4	18,2	

Table 2: Histopathological distribution of benign ovarian cysts

Benign ovarian cyst histopathology	**Total**		**Mean ± SD**
	n	**(%)**	
Epitel			
• Mucinous cystadenoma	9	40,9	
• Serous cystadenoma	10	45,5	1,68 ± 0,71
Non Epitel			
• Dermoid cyst	3	13,6	

Based on table 2, histopathological distribution of benign ovarian cysts, it is known that most are epithelial types equal to (86.4%), namely mucouscystadenoma (40.9%) and serous cystadenoma (45.5%), whereas non epithelial types are only 3 from 22 samples (13.6%) namely dermoid cysts.

Table3: Correlation of age andimmunohistochemical expression of MMP-2 in benign ovarian cyst

Age (yo)	**MMP-2 Intensity Score**				**P***
	Negative	**Weak positive**	**Moderate positive**	**Total**	
	n (%)	**n (%)**	**n (%)**	**n (%)**	
< 20	0	0	0	0	
20 – 50	11 (61)	3 (17)	4 (22)	18 (100)	0,903
> 50	2 (50)	1 (25)	1 (25)	4 (100)	
Total	**13 (59)**	**4 (18)**	**5 (23)**	**22 (100)**	

*Pearson *chi-square test*

Table 4: Correlationof menopausal status and immunohistochemical expression of MMP-2 in benign ovarian cyst

Menopause Status	**MMP-2 Intensity Score**				**P***
	Negative	**Weak positive**	**Moderate positive**	**Total**	
	n (%)	**n (%)**	**n (%)**	**n (%)**	
Non-menopausal	11 (61)	3 (17)	4 (22)	18 (100)	0,903
Menopausal	2 (50)	1 (25)	1 (25)	4 (100)	
Total	**13 (59)**	**4 (18)**	**5 (23)**	**22 (100)**	

*Pearson *chi-square test*

Table5: Correlation body mass index andimmunohistochemical expression of MMP-2 in benign ovarian cyst

Body mass index	**MMP-2 Intensity Score**				**P***
	Negative	**Weak positive**	**Moderate positive**	**Total**	
	n (%)	**n (%)**	**n (%)**	**n (%)**	
Normoweight	1 (33)	1 (33)	1 (34)	3 (100)	
Overweight	8 (89)	0	1 (11)	9 (100)	0,199
Obesitas	4 (40)	3 (30)	3 (30)	10 (100)	
Total	**13 (59)**	**4 (18)**	**5 (23)**	**22 (100)**	

*Pearson *chi-square test*

Table 3, 4 and 5 showedthe correlation of age, menopause status and body mass index of subjects with immunohistochemical expression of MMP-2 in benign ovarian cyst tissue. In the age group of 20-50 years, most showed negative MMP-2 immunohistochemical expressions as many as 11 samples (61%). In the age group> 50 years, negative MMP-2 immunohistochemical expressions were 2 samples (50%), positively weak and moderate each with 1 sample (25%). Based on table 4, most of the non-menopausal groups showed negative MMP-2 immunohistochemical expressions as many as 11 samples (61%) while in the menopause group, negative MMP-2 immunohistochemical expression was 2 samples (50%). From table 5, major of the overweight group showed negative immunohistochemical MMP-2 expression which was 8 samples (89%)whereas in the obese group, negative MMP-2 immunohistochemical expressions were 4 samples (40%), positively weak and moderate, each

with 3 samples (30%). Statistically the value of p> 0.05 for three groups showed that there was no significant correlation between the age, menopausal status and body mass index of the subjects with immunohistochemical expression of MMP-2 in the group of benign ovarian cysts. The staining intensity based by figure 1.

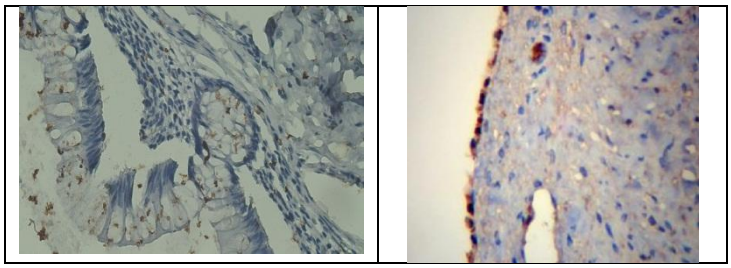

Figure 1. Immunochemical expression of MMP-2 in benign ovarian cyst (serous & mucinous cystadenoma, dermoid cyst). (A) Negative staining (B) Positive staining

Table 6: Immunohistochemical expression of MMP-2 in benign ovarian cyst

Histopathological Type	MMP-2 Intensity Score (Allred Score)				P value
	Negative	Weak positive	Moderate positive	Total	
	n (%)	n (%)	n (%)	n (%)	
• Mucinous cystadenoma	5 (56)	2 (22)	2 (22)	9 (100)	
• Serous cystadenoma	5 (50)	2 (20)	3 (30)	10 (100)	0,632
• Dermoid cyst	3 (100)	0	0	3 (100)	
Total	**13 (59)**	**4 (18)**	**5 (23)**	**22 (100)**	

*Pearson *chi-square test*

From the table above it show that the immunohistochemical expression of MMP-2 in benign ovarian cyst tissue mostly showed negative results in the mucinouscystadenomasof 5 samples (56%), serouscystadenomas in 5 samples (50%) and dermoid cysts in 3 samples (100 %). Serouscystadenomas in MMP-2 immunohistochemical expression were found with a moderate positive intensity of3 samples (30%). Statistically the value of p> 0.05 showed that there was no significant relationship between the type of histopathology of benign ovarian cysts with MMP-2 immunohistochemical expression.

IV. Discussion

Ovarian cysts are gynecological problems that women often encounter during their reproductive period, can be physiological cysts, some are benign, while others can be malignant cysts.[1,2] In premenopausal women, almost all ovarian cysts and masses are benign. According toSmeltzer et al., benign ovarian cysts were found in 10-20% of cases of all ovaries. This type of cyst is most common in women between the ages of 20-50 years and rarely occurs in prepubertal periods.[4]Pudasaini and Azhar state that most ovarian cysts are found in women aged 21-30 years (46%) [16,17] conducted by Abduljabbar, it was found out of 244 ovarian cyst patients found that the mean age was 23-47 years with an average age of 35 years.[18]Gameraddin study found that ovarian cyst patients with 36-49 years age group were the most age group.[19] In this study, the highest age distribution at the age of 20-50 years was 18 people (81.8%) and at the age of> 50 years there were 4 people (18.2%). Some studies of ovarian cysts suggest that the incidence of ovarian cysts is more common in patients with reproductive age.

Based from the characteristics of menopausal status, most of the study subjects had non-menopausal as many as 18 people (81.8%) and those who had menopause as many as 4 people (18.2%) (Table 4). The results of this study are similar with the theory which states that the risk of ovarian cyst formation will decrease after a woman experiences menopause due to reduced ovarian activity. Only 10-15% of cases of benign ovarian cysts occur after menopause. Moszynski et al's study of 167 ovarian cyst patients, it was found that about 131 ovarian cyst patients (78%) were premenopausal. Most ovarian cysts are found in non-menopausal women.[2,20,21] The results of the this study are in accordance with the study by Gafur et al. Where the highest group of benign ovarian cysts were found at the age of 20-50 years (70%), early menarche (85%), parity ≥1 (70%) and normoweight (70%).[22] This is slightly different from the study by Ninong et al where the majority of patients with benign ovarian cysts were aged 36-50 years (43.6%), non-menopausal (79.3%), obese I (49.1%), and nulliparous (42.9%).[23]

Histopathologicaltype of benign ovarian cysts, where most epithelium types (86.4%), aremucinouscystadenoma(40.9%) and serous cystadenoma (45.5%). While the non-epithelial type was only 3 of 22 samples (13.6%), isdermoid cysts (Table 6). This is based the theory that the highest incidence of benign

ovarian cysts in order includes ovarian simplex cysts, mucous ovarian cystadenoma, serous ovarian cystadenoma, endometroid cysts and dermoid cysts.[2,24]Shivaji study showed serous cystadenoma is the most ovarian cyst type (18,69%) followed by mucinouscystadenoma(9.75%).[25] This is similar to the study conducted by Pudasaini which showed that the most ovarian cyst types were serouscystadenoma (40.2%) followed by mature cystic teratoma (15.7%) [16]and a study by Rizwana which showed that the incidence of serouscystadenoma (22.5%) was followed by mucinouscystadenoma(19.4%).[26] But this result was different from the study conducted by Gafur et al where the highest histopathological distribution of benign ovarian cysts aremucinouscystadenoma (40%) followed by serouscystadenoma (35%).[22]

In this study,in benign ovarian cyst specimens, the majority showed negative MMP-2 immunohistochemical expression (59%), which in the serouscystadenoma was the highest type of 50% (5 of 10 samples), followed by 56% of mucinouscystadenoma (5 of 9 samples), and dermoid cyst types by 100%. The weak and moderate positive immunohistochemical expression of MMP-2 was found in the mucinouscystadenomatype of 22% respectively, whereas in the serous cystadenoma it was 20% and 30%. No weakand moderate positive immunohistochemical MMP-2 expression was found in dermoid cyst types (Table 6). The results of this study are appropriate with the research of Budi Santoso et al.who stated that MMP-2 expression was not found in benign ovarian cysts. If MMP-2 is not expressed, there will be no degradation of collagen and that will trigger the secretion of the lysiloxidase enzyme, resulting in thickening of the follicular wall fibrous and cyst formation.[27,28] In a study by Sakata there was no positive staining for MMP-2 in benign ovarian tumors.[29]Campo stated that in ovarian cystadenoma, type IV immunohistochemical (MMP-2) expression in the epithelial basement membrane was negative or positively weak, whereas in invasive and metastatic ovarian cancer is found to be moderate and strong positive expression caused by a significant change in the epithelial basement membrane so that the expression of this enzyme increases. This enzyme plays an important role in the mechanism of degradation of the basement membrane and then will assist the process of invasion and metastasis.[30] In this study there were moderate positive immunohistochemical expression of MMP-2 in 5 cases of benign ovarian cysts (23%), 2 cases of mucinous cystadenoma (22%) and 3 cases of serous cystadenoma(30%). This can be influenced by various things that can be confounding factors such as the presence of infection or inflammation that is not visible in the clinical and laboratory or the process of cell changes that have led to a malignant process where it is known that ovarian cysts are low malignant potential (LMP) around the serous 20% can be transformed malignantly.[31] The study by Barbara et al. showed that the expression of pro-MMP-2, pro-MMP-9, uPA and PAI-1 proteins increased from benign ovarian tumors to malignancy and that active MMP-2 was only detected in ovarian cancer and increased if there is metastase. This finding illustrates that the conversion of pro-MMP-2 to active MMP-2 is an important stage in the transition of a benign ovarian tumor that will develop into malignancy. The inhibition of MMP-2 activation will be an interesting approach in biological therapy of ovarian cancer because MMP-2 has never turned into an active form in benign ovarian tumors or low malignant potential but has been found to be as active as 66% of advanced ovarian cancers and 93 % of malignant ovarian tumors with metastases.[32]

In this study, immunohistochemical expression of MMP-2 with moderate positive intensity was higher in the type of serous cystadenoma compared to the type of mucinous (3 samples: 2 samples). This is appropriate with the study by Jean-luc et al. who showed that immunohistochemical expression of epithelial and stromal MMP-2 was higher in serous benign ovarian tumors (94.9 ± 49.5) compared to the mucinous type (52.5 ± 42.2) with a value of $p < 0.001$.[29] Study de Nictolis et al concluded that the relationship between increased MMP-2 expression and basement membrane changes supports the hypothesis of the direct role of matrix metalloproteinase in the process of stromal invasion so that evaluation of serum MMP-2 levels can be used as a prognosis predictor in cases of serous ovarian tumors.[33]

V. Conclusionsn

From the results of this study, there was no significant relationship between age, age of menarche, menopause status and body mass index with immunohistochemical expression of MMP-2 in benign ovarian cyst tissue. There was no significant relationship between histopathological subtypes of benign ovarian cysts with MMP-2 immunohistochemical expression. Immunohistochemical expression of MMP-2 in benign ovarian cyst tissue mostly showed negative results (59%).

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