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The correlation of matrix metalloproteinase-9 serum levels with clinicopathological factor in epithelial type ovarian cancer patients

M. RUSDA, D. NURVITA, M.R. YAZNIL, D. ALDIANSYAH, E. ARDIANSYAH, R. RIVANY

SUMMARY: **The correlation of matrix metalloproteinase-9 serum levels with clinicopathological factor in epithelial type ovarian cancer patients.**

M. RUSDA, D. NURVITA, M.R. YAZNIL, D. ALDIANSYAH, E. ARDIANSYAH, R. RIVANY

Objective. *To determine correlation of serum matrix metalloprotein-9 level with peritoneal cancer index in epithelial type ovarian cancer patients.*

Methods. *This study is a descriptive study with case series design on 26 subjects with epithelial type ovarian cancer at H. Adam Malik Hospital Medan in March to May 2018. Subjects were selected by consecutive sampling and blood samples was obtained from patients and serum MMP-9 levels was measured by ELISA. Data were analyzed using statistics Mann-Whitney test, t-test independent and Spearman correlation test. A p-value less than 0.05 is applied to each statistical test as significant.*

Results. *From 26 patients. Most of the subject of study was above 50 years, have ≥ 1 children with a kind of histopathologic type adenocarcinoma serosum, stage III-IV and optimal cytoreduction. Histopathology type of 16 patients (61.5%) was serous adenocarcinoma, 5 patients (19.2%) were mucinous adenocarcinoma, and 5 patients (19.2%) were others. The median MMP-9 serum levels was highest with serous adenocarcinoma type (1002.6 ng/mL). Patients with CA-125> 500 U/mL, suboptimal cytoreduction, PCI score >10 had high MMP-9 serum levels with median 1530.2 ng/mL 2157.6 ng/mL and 1942.4 ng/mL respectively. Considerably higher than those with CA-125 ≤500 IU/mL optimal cytoreduction and PCI score ≤10. Menopausal status showed no significant difference with p-value 0.311.*

Conclusion. *There is significant difference in serum MMP-9 level based on CA-125 level, cytoreduction and PCI. But serum MMP-9 level based on histopathology, menopausal state, and stage is not statistically different. PCI score is not statistically different in stage II and stage III-IV patients.*

KEY WORDS: Epithelial ovarian carcinoma - Matrix metalloproteinase-9 - Peritoneal cancer index.

Background

Ovarian cancer is the fifth frequent cancer type of gynecological tumor by the National Cancer Institute in 2014 (1, 2). This condition is caused by the difficulty of diagnosing ovarian cancer at an early stage. Approximately 70% of cases are found at an advanced stage, with a 5-year survival rate 30%. About 90% of ovarian cancers originate from the epithelial cells and are referred to as the "lady silent killer" because it is often diagnosed at an advanced stage (3, 4).

Matrix metalloproteinase (MMP) is an enzyme that plays a role in physiological and pathological processes. Pathologically this enzyme shows for tumor cell invasion and metastasis. MMP-9 serum levels in patients with different ovarian cancer were statistically significant compared with healthy women or benign ovarian tumors. The expression of MMP-9 is increased according to tumor invasion and progression of ovarian cancer (5-7).

The first-line management of ovarian cancer is surgery, which is performed by cytoreduction (7). The Peritoneal Cancer Index (PCI) is an objective assessment to assess the extent of peritoneal spread in an intra-abdominal and intrapelvic malignancy. This

Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

Corresponding author: Muhammad Rusda, e-mail: mrusdaharahap@yahoo.com

assessment can provide an overview of the degree of malignancy and extent of metastasis as the basis for optimal cytoreduction. PCI is performed at the time of surgery (8).

Calculation of protein levels in the circulation is a less-invasive method and potentially applicable to all patients. Increased MMP-9 levels in cases of ovarian malignant tumors may be a presurgical predictor in assessing the degree of malignancy and extent of metastasis. The purpose of this study was to assess the correlation between serum MMP-9 serum marker levels and PCI in epithelial ovarian tumor epithelial tumors.

Literature review

Ovarian cancer is the fifth leading cause of death from cancer in women (2). In Indonesia 2012 there were 10.238 new cases of ovarian cancer with a mortality rate of 7.075 cases (7.7%) (9). Risk factors found in patients with ovarian cancer are nullipara, early nullipara and late menopause age and use of fertility drugs. The cause of the malignant changes of the ovaries is unknown. Some hypotheses that cause ovarian cancer are incessant ovulation, gonadotropin, hormonal, and inflammatory (10). Based on histopathology, the World Health Organization (WHO) classified ovarian tumors based on the cell origin: epithelial cells, germ cells, stroma, and metastasis of another tumor. About 90% of ovarian malignancy originates from epithelial cells (11).

MMP is an enzyme that plays a role in the degradation of extracellular matrix (ECM) in tumor growth invasion and metastasis (12). MMP-9 is a metalloproteinase enzyme that has the highest activity in degrading collagen (13). Levels of serum MMP-9 is increased in ovarian cancer when compared with healthy ovary tissue or benign ovarian tumor (6, 7). After surgery of patients with ovarian cancer there was a significant decrease in MMP-9 levels. Positive correlation was found in tumor differentiation, staging, and lymph node metastases of ovarian cancer (14).

The success of ovarian cancer cytoreduction can be predicted by using a scoring system of PCI. Quantitatively the tumor will be divided into 13 abdominopelvic regions with tumor lesion size (1-3) used as values in PCI scores. Patients with PCI $\leq$ 10 had better survival rates compared with PCI > 10. The maximum score of PCI is 39 (8).

Methods

This research was a descriptive research with inferential analysis in case series design. Research conducted from March 2018 until the number of samples are met. Samples were taken in patients with ovarian cancer from H. Adam Malik Medan General Hospital and dr. Pirngadi Medan Regional General Hospital. Serum blood samples were examined at the Integrated Laboratory of the Faculty of Medicine, Universitas Sumatera Utara.

Subject selection was done by non-probability sampling with consecutive sampling technique until the minimum sample size (26 subject). The inclusion criteria are patients who have given consent, diagnosed with a malignant ovarian tumor, have not received adjuvant chemotherapy, did not have a non-gynecologic tumor or a history of having another tumor, were not diagnosed not only with infectious or inflammatory disease but also has no evidence to support an infection in that patient, not pregnant, and diagnosed by malignant ovarian tumors stage II, III, IV. The exclusion criteria were damaged sample preparations and histopathologic results did not show epithelial malignant ovarian tumors. This research variable are serum MMP-9 level as independent variable and PCI as dependent variable.

Patients who have scheduled oncology surgery are examined and asked for approval for the study. A total of 3cc of blood samples were taken for calculation of MMP-9 levels. The patient underwent surgery and the PCI score was assessed. The tumor mass was examined histopathologically. Laboratory and histopathology results were collected and tabulated and data analysis was performed.

Blood samples are taken to the laboratory for examination. The blood sample was centrifuged to separate the serum. Serum then diluted 100 times. This study used RD5-10 as control and normal human MMP-9 as standard. Control standard and subject solutions were added to each well on the well plate. Each well was washed four times with Wash Buffer. After that each well is given Stop Solution until the color become yellow. The optical density was read at 450 nm. The results will be averaged and analyzed.

Data were analyzed descriptively and analytically. The descriptive data were the distribution of subject frequencies by variables. Analytical data were: the mean difference of MMP-9 based on tumor stage use independent t-test for normal distributed data or with Mann Whitney U-test for not-normally

distributed data and correlation of MMP-9 level with Pearson correlation test if the data is distributed normal or Spearman correlation if data is not normally distributed. The p value is considered significant if p <0.05 with a 95% confidence degree.

Results

Twenty-six subjects were found to meet the inclusion criteria. All subjects were examined MMP-9 levels. Characteristics of the study subjects were divided by age and parity according to Table 1. A total of 42.3% subjects aged 51-60 years. Subjects with parity ≥ 1 were found to be 65.4%.

According to Table 2 the most common type of cancer is serous adenocarcinoma (61.5%). The MMP-9 levels in this group also had the highest median of 1002.6. There was no statistical significance of this type of cancer with MMP-9 levels. Levels of CA-125 was statistically significant with MMP-9 levels. A total of 19 subjects with optimal cytoreduction had lower MMP-9 levels compared with the suboptimal cytoreduced group with p <0.05. Patients

TABLE 1 - SUBJECT CHARACTERISTIC.

Characteristic		**n**	**%**
Age	< 40 years	2	7.7
	40-50 years	7	26.9
	51-60 years	11	42.3
	> 60 years	6	23.1
Parity	Virgin	5	19.2
	Nullipara	4	15.4
	Parity ≥1	17	65.4

TABLE 2 - MMP-9 LEVELS AND CLINICOPATHOLOGICAL FACTOR.

Factor	**MMP-9 levels (ng/ml)**			**p-value**
	n	**Median**	**Min-Max**	
Histopathology				
- Serous Adenocarcinoma	16	1002.6	274 - 2584.8	0.547
- Mucinous Adenocarcinoma	5	604.7	587 - 1803.2	
- Other	5	707.5	503 - 2463.3	
CA-125				
- ≤ 500 IU/ml	13	604.7	313.2 - 2375.5	0.043
- > 500 IU/ml	13	1530.2	274.4 - 2584.8	
Cytoreduction				
- Optimal	19	707.5	274.4 - 2463.3	0.042
- Suboptimal	7	2157.6	422.3 - 2375.5	
PCI				
- ≤ 10	16	615.4	274.4 - 2463.3	0.014
- > 10	10	1942.4	422.3 - 2584.8	
Menopausal Status				
- Before Menopause	13	913.6	274.4 - 2463.3	0.311
- Menopause	13	626.1	313.2 - 2584.8	

with PCI ≤ 10 had lower levels of MMP-9 compared with the PCI > 10 group with significant differences. The menopausal status did not make a significant difference to MMP-9 levels.

Discussion

Increased age is one of the risk factors for ovarian cancer. In this study the largest number of subjects was in age group above 50 years. This condition is in accordance with research by Siegel and Reis. The average age of ovarian cancer patients is 63 years (15). High parity should reduce the risk of ovarian cancer incidence but in this study multiparous subjects have a higher incidence of ovarian cancer. This condition can be caused by consecutive sampling which cannot describe the incidence of the case as a whole (2).

The levels of CA-125 were significant from the MMP-9 levels according to Gehrstein et al. which showed a significant correlation (16). The incidence of ovarian cancer increased at the perimenopausal age (60%) (15). This study showed the same number of subject between menopausal and non-menopausal women with ovarian cancer. Few samples might cause this condition.

A total of 73.1% of the subjects of this study were ovarian cancer stage III-IV. Zhang et al. stated that patients with ovarian cancer seek medical attention on advanced stage. Their study found 64% of patients with stage III-IV and 35% of patients with stage I-II (7). MMP-9 levels are increasing with stage found in this study (stage III-IV mean 1266 ng/mL and stage II mean 903. 0 ng/mL) and in accordance with Zhang et al. (7).

Positive correlations were found between MMP-9 levels and PCI in malignant epithelial ovarian cancer in this study. This study is the first study to conduct a correlation between MMP-9 with PCI in cases of ovarian cancer. Zhang et al. studied comparing MMP-9 levels with invasion and peritoneal metastasis. Their study obtained a sensitivity of 72.2% and a specificity of 68.9% of MMP-9 to ovarian cancer (7).

Conclusion

Most of the subjects in this study were 50-60 years old and had ≥ 1 parity. There were significant differences between MMP-9 with CA-125, cytoreduction, and PCI levels. There was no significant difference between MMP-9 levels and histopathologic type and menopausal status. Staging of cancer with MMP-9 levels and PCI scores did not find significant differences. A statistically significant correlation between MMP-9 serum levels and PCI scores was found in this study.

Recommendation

The use of MMP-9 serum levels may be recommended as prognostic criteria for malignant epithelial ovarian tumor optimal cytoreduction.

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