The correlation between immunohistochemical expression of MMP-2 and the prognosis of epithelial ovarian cancer

Związek pomiędzy immunohistochemiczną ekspresją MMP-2 a rokowaniem w raku jajnika

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Abstract

Objectives: *The Objectives: The goal of the study was to evaluate the correlation of matrix metalloproteinase-2 (MMP-2) expression with tumor spread, metastasis, survival and recurrence in early and advanced-stage Epithelial Ovarian Cancer (EOC).*

Material and methods: *Medical records of patients, hospitalized at the Department of Obstetrics and Gynecology, İzmir Atatürk Training and Research Hospital between 2003 and 2008, were reviewed. Patient age, tumor size, localization, histologic type and tumor grade, stage, metastasis status, patient outcomes and follow-up data were obtained from the records of the obstetrics and gynecology clinic, as well as during face-to-face or telephone interviews.*

Results: *The percentage of MMP-2 staining (expression) in the epithelial cells was not significantly associated with tumor stage and grade, histologic type, tumor diameter, recurrence and overall survival ($p>0.05$). A significant correlation was found between the percentage of MMP-2 staining (expression) and metastasis status ($p<0.05$). The staining intensity of MMP-2 was not significantly associated with tumor stage and grade, diameter, recurrence, metastasis and overall survival ($p>0.05$), but was with histologic type ($p<0.05$). Total scores were not significantly associated with tumor stage and grade, histologic type, tumor diameter, recurrence, metastasis and overall survival ($p>0,05$). Stromal staining (expression) of MMP-2 was not significantly correlated with tumor stage and grade, histologic type, tumor diameter and outcomes ($p>0.05$), but was with recurrence and presence of metastasis ($p<0.05$). No significant association was found between the overall survival and percentage of MMP-2 staining ($p>0.05$), total score ($p>0.05$) and staining intensity ($p>0.05$). The association of disease-free survival with the percentage of MMP-2 staining ($p>0.05$), total score ($p>0.05$), staining intensity ($p>0.05$) and stromal staining ($p>0.05$) was not statistically significant. The survival of patients with positive stromal staining was significantly shorter compared to cases with negative stromal staining ($p<0.05$).*

Conclusions: *Large-scale, comprehensive research is needed to verify whether MMP 2 may be used as a routine prognostic factor for EOC.*

Key words: **epithelial ovarian cancer** / **immunohistochemical expression** / / **mmp-2** / **prognosis** /

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Streszczenie

Celem pracy była ocena związku pomiędzy ekspresją macierzowej metalloproteinazy-2 (MMP-2) a rozsiewem nowotworowym, przerzutami, przeżyciem i nawrotem we wczesnym i zaawansowanym raku jajnika (EOC).

Materiał i metoda: *Przeanalizowano dane medyczne pacjentek leczonych w Klinice Położnictwa i Ginekologii w Izmir Ataturk Training and Research Hospital w latach 2003 – 2008. Wiek pacjentek, rozmiar guza i jego lokalizację, typ histologiczny i stopień zróżnicowania, stopień zaawansowania i status menopauzalny, obecność przerzutów, wynik leczenia pacjenta i dane z kontroli po leczeniu uzyskano z dokumentacji Kliniki Położnictwa i Ginekologii jak również podczas wywiadu bezpośredniego i telefonicznego.*

Wyniki: *Odsetek ekspresji MMP-2 w komórkach nabłonkowych nie był istotnie związany ze stopniem zaawansowania i zróżnicowania nowotworu, typem histologicznym, rozmiarem guza, nawrotem choroby i całkowitym przeżyciem (p>0,05). Znaleziono istotny związek pomiędzy odsetkiem ekspresji MMP-2 a obecnością przerzutów (p<0,05). Nasilenie zabarwienia MMP-2 nie było istotnie związane ze stopniem zaawansowania i zróżnicowania nowotworu, rozmiarem guza, nawrotem choroby, obecnością przerzutów i całkowitym przeżyciem (p>0,05), ale było związane z typem histologicznym (p<0,05). Całkowita ilość punktów za odsetek i nasilenie ekspresji nie była związana ze stopniem zaawansowania i zróżnicowania nowotworu, typem histologicznym, rozmiarem guza, nawrotem choroby, obecnością przerzutów i całkowitym przeżyciem (p>0,05). Ekspresja MMP-2 w zrębie nie była istotnie związana ze stopniem zaawansowania i zróżnicowania nowotworu, typem histologicznym, rozmiarem guza i wynikiem leczenia (p>0,05), ale była związana z nawrotem choroby i z obecnością przerzutów (p<0,05). Nie znaleziono istotnych związków pomiędzy całkowitym przeżyciem a odsetkiem ekspresji MMP-2 (p>0,05), całkowitą ilością punktów (p>0,05) i nasileniem reakcji (p>0,05). Związek pomiędzy czasem wolnym od wznowy a odsetkiem ekspresji MMP-2 (p>0,05), całkowitą ilością punktów (p>0,05), nasileniem reakcji (p>0,05) i zabarwieniem zrębu (p>0,05) nie był istotny statystycznie. Czas przeżycia pacjentek z dodatnim zabarwieniem zrębu był istotnie krótszy w porównaniu do przypadków z negatywnym zabarwieniem zrębu (p<0,05).*

Wnioski: *Konieczne są dalsze badania na większej liczbie pacjentek celem zweryfikowania czy MMP-2 może być powszechnym czynnikiem prognostycznym w raku jajnika.*

Słowa kluczowe: **nabłonkowy rak jajnika** / **ekspresja immunohistochemiczna** / / **MMP-2** / **rokowanie** /

Introduction

Ovarian cancer ranks sixth in cancer-related deaths among women. In Western developed countries it is the fourth most common cause of cancer-related deaths [1]. Epithelial ovarian cancer (EOC) is the most commonly encountered ovarian malignancy. One percent of ovarian cancers occur before the age of 20 and 85% occur in women over 50. The average age for the onset of ovarian cancer is 63 years. The risk of developing ovarian cancer is lower under the age of 30 [2]. Lack of specific and early symptoms, as well as reliable screening tests, are the reasons why most patients are diagnosed in the advanced stages of the disease [3].

Pathogenesis of ovarian cancer has not been fully elucidated. Various hypotheses to explain the transformation of normal ovarian tissue into dysplastic or in situ precursor lesions, or to invasive, metastatic cancer, have been proposed [4]. Degradation of extracellular matrix is one of the important steps of metastatic cascade in which active proteolytic enzymes, including serine proteases, cysteine proteases and matrix metalloproteinases, are involved. Matrix metalloproteinases (MMPs) exhibit broad-spectrum proteolytic activity. MMPs belong to a zinc-dependent enzyme family and are capable of degrading extracellular matrix (ECM) and basal membrane components. They are also one of the important molecules that play a role in tumor invasion, angiogenesis and metastasis. More than 25 members of MMPs have been reported. MMP-2 is a 72 kDa enzyme that belongs to the MMP family. MMP-2 is capable of degrading type IV collagen, fibronectin and all basal membrane components, and therefore facilitates stromal and vascular invasion of tumor cells [5, 6].

Numerous prognostic factors for the ovarian cancer have been identified. However, routine clinical practice lacks a molecular marker to be used in the diagnosis. Better understanding of the molecular basis might lead to an earlier diagnosis, better prediction of the survival and recurrence. Awareness of the molecular mechanisms of metastasis is essential for a more effective use and selection of therapeutic agents.

Objectives

The goal of the study was to evaluate the correlation between MMP-2 expression in epithelial cells and stroma with tumor spread, metastasis, survival and recurrence in EOC.

Materials and Methods

Medical records of patients operated at the Department of Obstetrics and Gynecology, İzmir Atatürk Training and Research Hospital, between 2003 and 2008, were retrospectively reviewed. Fifty patients diagnosed with epithelial carcinoma of the ovary were included in the study. Patient age, tumor size, localization, histologic type and grade, stage, metastasis status, follow-up data and patient outcomes were obtained from patient records as well as during face-to-face or telephone interviews.

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All cases underwent surgical staging in accordance with the International Federation of Obstetrics and Gynecology (FIGO) recommendations, followed by optimal cytoreductive surgery. Pelvic and paraaortic lymphadenectomy were performed in all cases. Gynecologic Oncology Group (GOG) grading system and FIGO staging system were used to determine postoperative tumor grade and stage, respectively. Patients received chemotherapy if required.

Hematoxylin-eosin-stained tissue sections were retrieved from the archives for reassessment. Slides were examined in a light microscope. For each patient, the most representative block was selected for immunohistochemical (IHC) studies. 4-5 micron-thick fresh sections were prepared from the selected paraffin blocks and mounted on poly-L-lysine-coated slides. Tissue sections, transferred onto poly-L-lysine-coated slides, were dried at 60˚C for half an hour and then sections were immersed in the Target Retrieval Solution (High pH (50x)) in Dako PT Link device and incubated at 97°C for 25 minutes. Next, the samples were aligned horizontally and prepared for antibody application. Mouse monoclonal MMP-2 antibody was used as the primary antibody (Leica, Lot: 6008926, Clon: NCL-MMP-2-507). Tissue sections were incubated for 60 minutes with primary antibody diluted 1:50 with Thermo large volume UltrAb Dilvent. The following procedure was followed: Sections were rinsed for 5 minutes with Dako peroxidase blocking agent with a volume of 100 microliters per slide. Sections were then incubated with diluted (1:50) MMP-2 antibody for 60 minutes. At the third step, rabbit FLEX+Linker (Dako) was added and samples were incubated for 15 minutes. At the fourth step, samples were incubated with rabbit EnVision FLEX+Linker for 20 minutes. Samples were then incubated with substance buffer + DAB (chromogen) solution for 10 minutes and stained with EnVision FLEX Hematoxylin for 5 minutes. Tissue samples were dehydrated through 3 changes of xylene alcohol (80%, 96%, 99%) for 20 minutes each and air-dried. Tissue samples were rehydrated by dipping the slides sequentially in 100%, 95% and 90% ethanol for 5 minutes each. Next, samples were washed with distilled water and then with PBS buffer solution (pH 7.6) for 5 minutes. Sections were washed with 3% hydrogen peroxide solution for 5 minutes to block endogenous peroxidase and incubated in retrieval solution (pH 9) in microwave oven 3 times for 5 minutes each. Samples were then allowed to cool for 20 minutes at room temperature. All sections were washed with distilled water 3 times and left in protein blocking serum for 5 minutes. The slides were aligned horizontally and primary antibody was added.

Inflamed small bowel was used as the positive control for MMP-2 expression.

Evaluation of Immunohistochemical Staining

All tumor fields were examined under light microscopy at low magnification (x10). The proportion of all stained cells to all tumor cells in the epithelial area for each antibody at x20 magnification (fraction of stained cells in percentage) and subjective assessment of the intensity of tumor cells at high magnification (x40) were evaluated. Cytoplasmic staining was considered significant for MMP-2. Percentages of staining cells, staining intensity and total scores were determined in accordance with the study by Kamat and colleagues [7].

Accordingly:

- Percentage of staining for MMP-2 is ranked as: 0-5%=0, 6-50%=2, 50%< 3.
- Staining intensity for MMP-2 is ranked as: weak=1, moderate=2, strong=3.

Total score is derived from the sum of percentage of staining and staining intensity. Total score is ranked as the following:

- Score 0 = negative (regardless of staining intensity, if no staining or <5%);
- Score 1 = weak staining (total score 1-2);
- Score 2 = moderate staining (total score 3-4);
- Score 3 = strong staining (total score 5-6) ;

Scores of 0-1 represent weak expression and scores of 2-3 represent strong expression.

Results

Fifty patients were included in the study. Mean age was 54.5 years (38-80 years). The patients were divided into two groups for statistical purposes: 42 patients (70%) ≤ 60 and 18 patients (30%) > 60 years of age. The patients were also grouped according to tumor diameter: 8 patients (16%) < 5 cm, 23 patients (56%) 5-10 cm, and 10 patients (20%) > 15 cm. In our study, 35 patients (70%) had serous adenocarcinoma, 10 (20%) had endometrioid adenocarcinoma, 3 (6%) had serous + endometrioid adenocarcinoma, 1 (3.3%) had serous + clear cell adenocarcinoma and 1 (3.3%) had undifferentiated adenocarcinoma. When the patients were grouped according to tumor grade, 9 (18%) had well-differentiated, 18 (36%) had moderately differentiated and 23 (51.7%) had poorly differentiated tumor. The patients were grouped as early (IA-IIC) and advanced (late) (IIIA-IV) stage according to the FIGO classification system. Seventeen patients (34%) had early stage and 33 patients (66%) had advanced-stage disease. Distant metastasis was found in 10 , liver metastasis in 5 (10%), pleural effusion in 3 (6%), metastasis to pancreatic tail in 1 (2%) and lung metastasis was found in 1 patient (2%).

Percentage of MMP-2 staining (expression) in epithelial cells was below 5% in 37 patients, between 6-50% in 12 patients and above 50% in 1 patient. In terms of staining intensity, no staining was observed in 37 patients, whereas 3 patients had weak, 6 patients had moderate and 4 patients had strong staining. 37 patients had the total score of 0, while 9 patients had the score of 2 and 4 patients had the score of 3, whereas no patient had the score of 1. In statistical analysis, patients were divided into two groups: scores of 0 and 1 constituted the ‘weak expression’ group and scores of 2 and 3 comprised the ‘strong expression’ group. Stromal MMP-2 staining was not found in 37 patients (74%).

The percentage of MMP-2 staining (expression) in epithelial cells was not significantly associated with tumor stage ($p=0.647$ and $p<0.05$) and grade ($p=0.408$ and $p<0.05$), histologic type ($p=0.445$ and $p<0.05$), tumor diameter ($p=0.887$,$p=0.711$ and $p<0.05$), recurrence ($p=0.381$ and $p<0.05$) and overall survival ($p=0.319$ and $p<0.05$). However, significant association was found between the percentage of MMP-2 staining (expression) and metastasis status ($p=0.040$ and $p<0.05$). (Table I).

The staining intensity of MMP-2 in the epithelial cells was not significantly associated with tumor stage ($p=0.833$ and $p<0.05$) and grade ($p=0.140$ and $p<0.05$), tumor diameter ($p=0.632$, $p=0.319$ and $p<0.05$), recurrence ($p=0.308$ and $p<0.05$), metastasis ($p=0.166$ and $p<0.05$) and overall survival

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(p=0.733 and p<0.05). However, the staining intensity of MMP-2 was significantly associated with histologic type (p=0.035 and p<0.05). (Table II).

Total scores were not significantly associated with tumor stage (p=0.733 and p<0.05) and grade (p=0.424 and p<0.05), histologic type (p=0.126 and p<0.05), tumor diameter (p=0.508, p=0.194 and p<0.05), recurrence (p=0.408 and p<0.05), metastasis (p=0.092 and p<0.05) and overall survival (p=0.589 and p<0.05). (Table III).

Table I. Association between percentage of MMP-2 staining (expression) and other parameters.

		Below 5%	6-50%	Above 50%	P
		n / %	n / %	n / %	
Stage	Early stage	12 / 24	5 / 10	0 / 0	0.647
	Late stage	25 / 50	7 / 14	1 / 2	
Grade	Well-differentiated	6 / 12	3 / 6	0 / 0	0.408
	Moderately differentiated	16 / 32	2 / 4	0 / 0	
	Poorly differentiated	15 / 30	7 / 14	1 / 2	
Histologic type	Serous	28 / 56	6 / 12	1 / 2	0.445
	Endometrioid	7 / 14	3 / 6	0 / 0	
	Serous + endometrioid	2 / 4	1 / 2	0 / 0	
	Undifferentiated	0 / 0	1 / 2	0 / 0	
	Serous + clear cell	0 / 0	1 / 2	0 / 0	
Tumor diameter	< 5 cm	7 / 14	1 / 2	0 / 0	0.877
	5-10 cm	16 / 32	6 / 12	1 / 2	
	11-15 cm	6 / 12	3 / 6	0 / 0	
	> 15 cm	8 / 16	2 / 4	0 / 0	
Tumor diameter	≤ 10 cm	23 / 46	7 / 14	1 / 2	0.711
	> 10 cm	14 / 28	5 / 10	0 / 0	
Recurrence	Absent	21 / 42	5 / 10	0 / 0	0.381
	Present	16 / 32	7 / 14	1 / 2	
Metastasis	Absent	31 / 62	7 / 14	0 / 0	**0.040**
	Present	6 / 12	5 / 10	1 / 2	
Survival	Yes	18 / 36	8 / 16	0 / 0	0.319
	No	19 / 38	4 / 8	1 / 2	

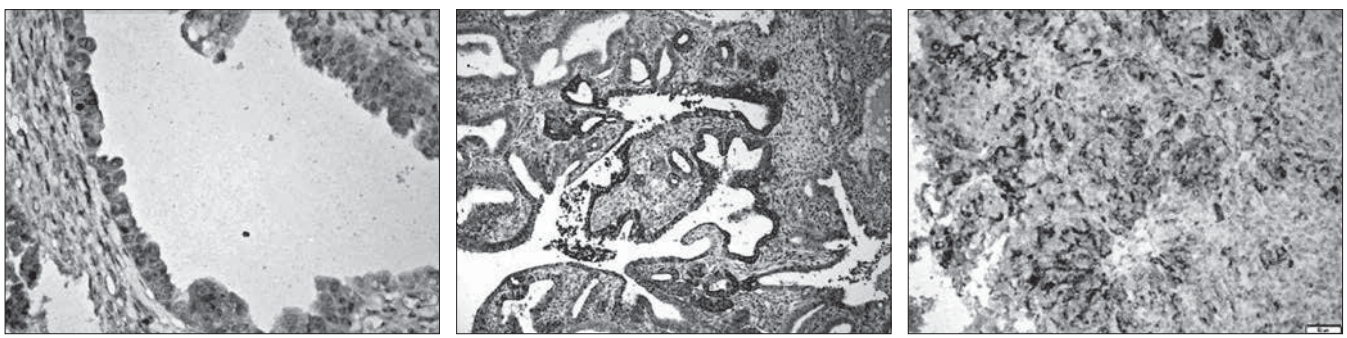

Figure 1. Fields with MMP-2 score 1 at x20 magnification; fields with MMP-2 score 2 at x10 magnification; fields with MMP-2 score 3 at x40 magnification, respectively..

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Table II. Association between staining intensity of MMP-2 and other parameters.

		No staining	Weak	Moderate	Strong	p
		n / %	n / %	n / %	n / %	
Stage	Early stage	12 / 24	1 / 2	3 / 6	1 / 2	0.833
	Late stage	25 / 50	2 / 4	3 / 6	3 / 6	
Grade	Well-differentiated	6 / 12	1 / 2	1 / 2	1 / 2	0.140
	Moderately differentiated	16 / 32	2 / 4	0 / 0	0 / 0	
	Poorly differentiated	15 / 30	0 / 0	5 / 10	3 / 6	
Histologic type	Serous	28 / 56	2 / 4	3 / 6	2 / 4	**0.035**
	Endometrioid	7 / 14	0 / 0	1 / 2	2 / 4	
	Serous + endometrioid	2 / 4	1 / 2	0 / 0	0 / 0	
	Undifferentiated	0 / 0	0 / 0	1 / 2	0 / 0	
	Serous + clear cell	0 / 0	0 / 0	1 / 2	0 / 0	
Tumor diameter	< 5 cm	7 / 14	0 / 0	1 / 2	0 / 0	0.632
	5-10 cm	16 / 32	2 / 4	4 / 8	1 / 2	
	11-15 cm	6 / 12	0 / 0	1 / 2	2 / 4	
	> 15 cm	8 / 16	1 / 2	0 / 0	1 / 2	
Tumor diameter	≤ 10 cm	23 / 46	2 / 4	5 / 10	1 / 2	0.319
	> 10 cm	14 / 28	1 / 2	1 / 2	3 / 6	
Recurrence	Absent	21 / 42	2 / 4	1 / 2	2 / 4	0.308
	Present	16 / 32	1 / 2	5 / 10	2 / 4	
Metastasis	Absent	31 / 62	2 / 4	3 / 6	2 / 4	0.166
	Present	6 / 12	1 / 2	3 / 6	2 / 4	
Survival	Yes	18 / 36	2 / 4	3 / 6	3 / 6	0.733
	No	19 / 38	1 / 2	3 / 6	1 / 2	

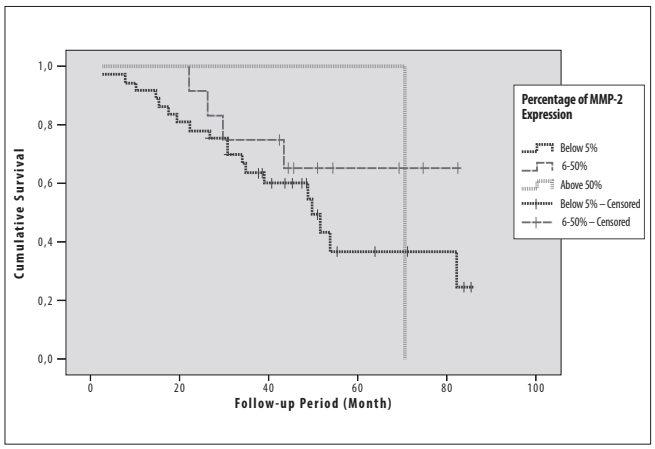

Figure 2. Kaplan-Meier survival curve for MMP-2 expression.

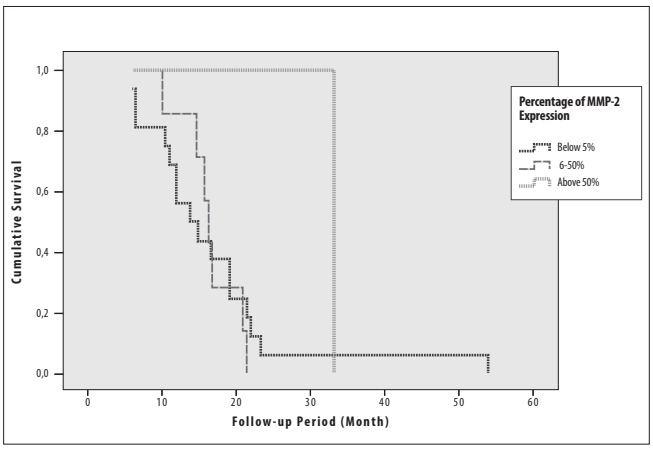

Figure 3. Kaplan-Meier disease-free survival curve for percentage of MMP-2 expression.

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Table III. Association between total scores and other parameters.

		Negative	Moderate expression: 3-4	Strong expression: 5-6	P
		n / %	n / %	n / %	
Stage	Early stage	12 / 24	4 / 8	1 / 2	0.733
	Late stage	25 / 50	5 / 10	3 / 6	
Grade	Well-differentiated	6 / 12	2 / 4	1 / 2	0.424
	Moderately differentiated	16 / 32	2 / 4	0 / 0	
	Poorly differentiated	15 / 30	5 / 10	3 / 6	
Histologic type	Serous	28 / 56	5 / 10	2 / 4	0.126
	Endometrioid	7 / 14	1 / 2	2 / 4	
	Serous + endometrioid	2 / 4	1 / 2	0 / 0	
	Undifferentiated	0 / 0	1 / 2	0 / 0	
	Serous + clear cell	0 / 0	1 / 2	0 / 0	
Tumor diameter	< 5 cm	7 / 14	1 / 2	0 / 0	0.508
	5-10 cm	16 / 32	6 / 12	1 / 2	
	11-15 cm	6 / 12	1 / 2	2 / 4	
	> 15 cm	8 / 16	1 / 2	1 / 2	
Tumor diameter	≤ 10 cm	23 / 46	7 / 14	1 / 2	0.194
	> 10 cm	14 / 28	2 / 4	3 / 6	
Recurrence	Absent	21 / 42	3 / 6	2 / 4	0.450
	Present	16 / 32	6 / 12	2 / 4	
Metastasis	Absent	31 / 62	5 / 10	2 / 4	0.092
	Present	6 / 12	4 / 8	2 / 4	
Survival	Yes	18 / 36	5 / 10	3 / 6	0.589
	No	19 / 38	4 / 8	1 / 2	

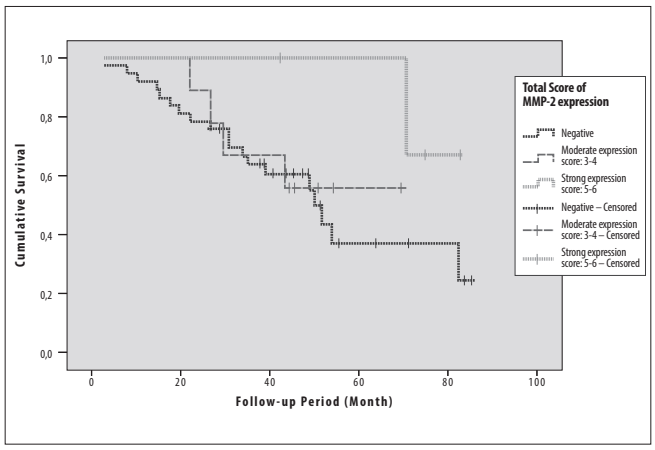

Figure 4. Kaplan-Meier survival curve for total scores of MMP-2 expression.

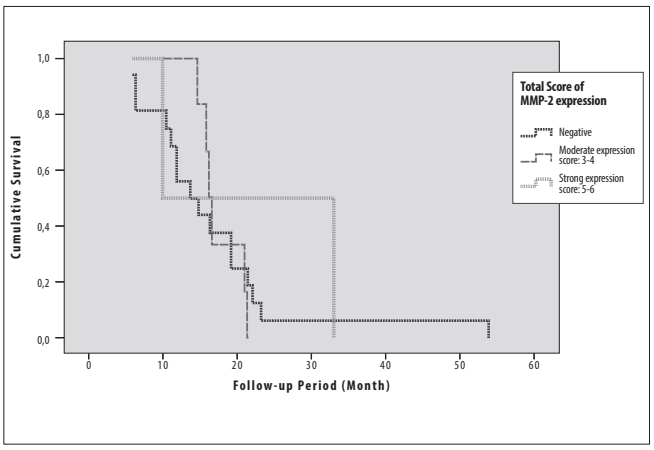

Figure 5. Kaplan-Meier disease-free survival curve for total scores of MMP-2 expression.

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Table IV. Association between stromal staining (expression) of MMP-2 and other parameters.

		negative stromal staining	positive stromal staining	p
		n / %	n / %	
Stage	Early stage	14 / 28	3 / 6	0.499
	Late stage	23 / 46	10 / 20	
Grade	Well-differentiated	4 / 8	5 / 10	0.077
	Moderately differentiated	15 / 30	3 / 6	
	Poorly differentiated	18 / 36	5 / 10	
Histologic type	Serous	25 / 50	10 / 20	0.085
	Endometrioid	9 / 18	1 / 2	
	Serous + endometrioid	3 / 6	0 / 0	
	Undifferentiated	0 / 0	1 / 2	
	Serous + clear cell	0 / 0	1 / 2	
Tumor diameter	< 5 cm	4 / 8	4 / 8	0.113
	5-10 cm	16 / 32	7 / 14	
	11-15 cm	9 / 18	0 / 0	
	> 15 cm	8 / 16	2 / 4	
Tumor diameter	≤ 10 cm	20 / 40	11 / 22	0.095
	> 10 cm	17 / 34	2 / 4	
Recurrence	Absent	23 / 46	3 / 6	**0.015**
	Present	14 / 28	10 / 20	
Metastasis	Absent	32 / 64	6 / 12	**0.007**
	Present	5 / 10	7 / 14	
Survival	Yes	22 / 44	4 / 8	0.075
	No	15 / 30	9 / 18	

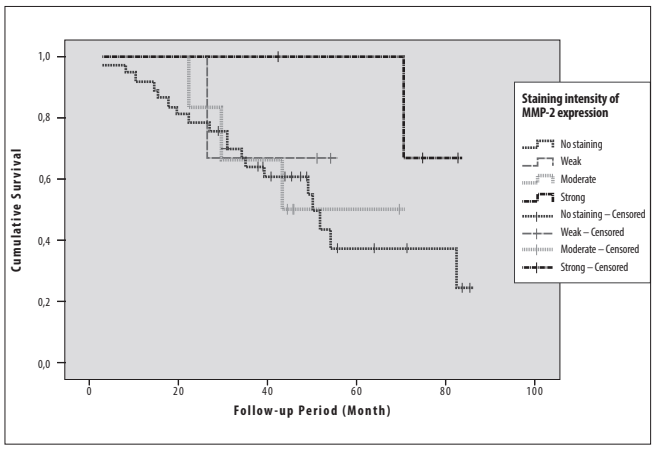

Figure 6. Kaplan-Meier survival curve for staining intensity of MMP-2 expression.

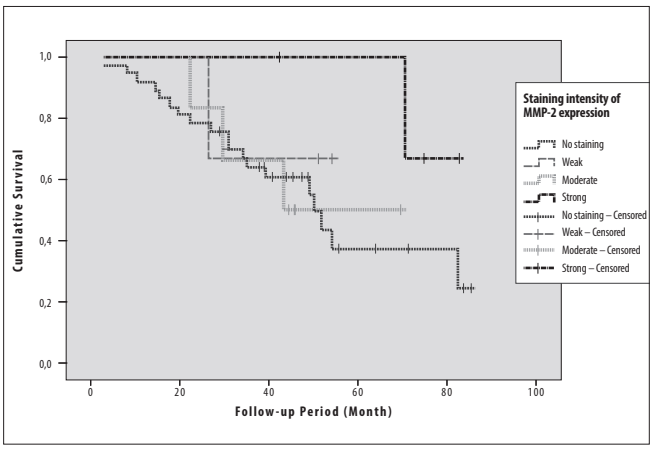

Figure 7. Kaplan-Meier disease-free survival curve for staining intensity of MMP-2 expression.

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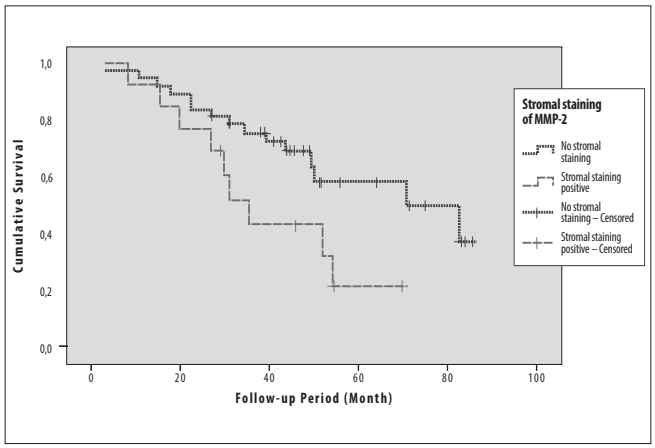

Figure 8. Kaplan-Meier survival curve for stromal staining of MMP-2.

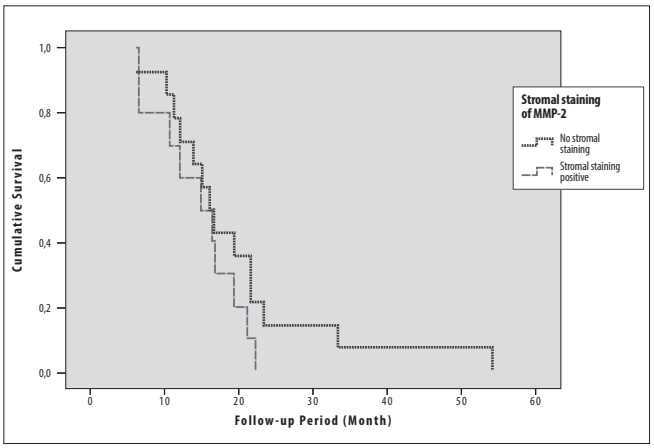

Figure 9. Kaplan-Meier disease-free survival curve for stromal staining of MMP-2.

Stromal staining (expression) of MMP-2 was not significantly associated with tumor stage (p=0.499 and p<0.05) and grade (p=0.077 and p<0.05), histologic type (p=0.085 and p<0.05), tumor diameter (p=0.113, p=0.095 and p<0.05) and overall survival (p=0.075 and p<0.05). However, stromal staining of MMP-2 was found to be significantly associated with recurrence (p=0.015 and p<0.05) and presence of metastasis (p=0.007 and p<0.05). (Table IV).

No significant association was found between overall survival and percentage of MMP-2 staining (p=0.492 and p<0.05) , total score (p=0.348 and p<0.05) and staining intensity (p=0.498 and p<0.05) (Figures 3, 4, 6). The association of disease-free survival with the percentage of MMP-2 staining (p=0.462 and p<0.05), total score (p=0.831 and p<0.05), staining intensity (p=0.946 and p<0.05) and stromal staining (p=0.236 and p<0.05) was not statistically significant (Figures 3, 5, 7, 9).

The survival of patients with positive stromal staining was significantly shorter compared to those with negative stromal staining (p=0.048 and p<0.05) (Figure 8).

Discussion

Breakdown of the extracellular matrix is a very important step in invasive growth of cancers and MMPs are the key elements of that process. The role and the contribution of the tumor and stromal cell compartments to the increased levels of MMPs in carcinoma tissue are still controversial. Some investigators suggest an almost exclusive stromal origin of MMPs detected in cancer tissue [8].

According to another theory, tumor associated macrophages (TAMs) are a major component of most, if not all, tumor microenvironments [9], and are recruited to tumors by a number of growth and chemotactic factors. In the presence of a proinflammatory tumor microenvironment, macrophages are induced to switch from their phagocytic phenotype to one that promotes production of factors that stimulate angiogenesis, breakdown of matrix, and tumor cell motility [10]. Activated macrophages influence angiogenesis in several ways, including production of matrix metalloproteinases 2 and 9 (MMP-2 and MMP-9) [11].

To date, numerous studies have been conducted on the treatment, patient follow-up after the primary treatment and prognostic factors in the course of EOC, that takes an important place among female cancers. However, apart from the obvious lack of validated test of EOC for community screening, there is also no defined indicator that would serve as a prognostic factor after primary treatment, with the exception of optimal surgery and residual tumor volume. MMPs have been previously investigated for their association with disease-free survival, overall survival and recurrence rate. Similar to our study, Kamel et al., studied 30 patients with malignant epithelial ovarian tumor. Of their patients, 12 had serous carcinoma, 9 had mucinous carcinoma, 5 had endometrioid carcinoma, 1 had malignant Brenner, 1 had mixed cell cancer and 2 had clear-cell carcinoma. In histologic examination, 22 patients appeared to have grade 1-2 and 6 patients had grade 3 tumor. Of the cases, 9 had stage I, 2 had stage II, 13 had stage III and 6 had stage IV cancer [11]. Although the number of cases in both studies is similar and patients with serous type EOC constitute the largest group , there is, however, a marked difference between the studies with respect to tumor stage and grade [11]. In the study of 100 patients conducted by Périgny et al., mean age was 64 years (28-88 years). Of these 100 cases, 41 were histologically classified as grade 1 or 2 and 59 were classified as grade 3, which was similar to our study [12].

The results of MMP-2 expression are contradictory. In the studies conducted by Määttа et al., and De Nictolis et al., MMP-2 expression was found to be higher in ovarian carcinomas with low potential of metastasis compared to tumors with high potential of metastasis [13, 14]. In an immunohistochemical study, Campo et al., demonstrated that MMP-2 expression is negative or minimal in benign ovarian disease. The expression was intense in case of invasive malignant ovarian cancer and in the presence of metastasis [15]. In a comprehensive study by Sakata et al., MMP-2 expression was found to be increased in ovarian carcinomas compared to benign and borderline tumors [16].

Considering the contradictory results in the literature, general MMP-2 expression in the tissue (percentage of staining), staining intensity and the intensity of stromal staining were examined individually for their association with the tumor stage and grade, tumor diameter, recurrence, presence of metastasis and overall survival in this present study. We achieved the total score of staining by combining general percentage of staining and the intensity of staining of MMP-2, and examined its association with

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tumor stage and grade, tumor diameter, recurrence, presence of metastasis and overall survival. The association of the percentage of MMP-2 expression, intensity of staining, combined total score of staining and stromal staining with overall survival was evaluated by the Kaplan-Meier survival curves. Differently from the subjective evaluation of the MMP-2 expression on microscopy and analysis of the MMP-2 expression in tissue cultures using conventional PCR techniques, we conducted a comprehensive evaluation of the MMP-2 expression in tissue samples of EOC. In our study, the percentage of MMP-2 staining in the epithelial cells was below 5% in 37 patients, between 6-50% in 12 patients and above 50% in 1 patient. In terms of staining intensity, no staining was observed in 37 patients, whereas 3 patients had weak, 6 had moderate and 4 had strong staining. 37 patients had the total score of 0, while 9 patients had the score of 2 and 4 patients had the score of 3, whereas no patients had the score of 1.

In our study, the percentage of MMP-2 staining was not found to be significantly associated with tumor stage and grade, histologic type, tumor diameter, recurrence and overall survival ($p>0.05$). However, significant association was found between the percentage of MMP-2 staining and presence of metastasis ($p<0.05$). The staining intensity of MMP-2 was not significantly associated with tumor stage and grade, tumor size, recurrence, metastasis and overall survival ($p>0.05$), but it was significantly associated with histologic type ($p<0.05$). We evaluated the association of the combined total score of MMP-2 staining with the tumor stage and grade, histologic type, tumor diameter, recurrence, presence of metastasis and overall survival. No significant association was found ($p>0.05$). Stromal staining of MMP-2 was not significantly associated with the tumor stage and grade, histologic type, tumor diameter and outcomes ($p>0.05$). However, stromal staining of MMP-2 was found to be significantly associated with the recurrence and presence of metastasis ($p<0.05$). In our study, no significant association was found between the overall survival and percentage of MMP-2 staining ($p>0.05$), total score ($p>0.05$) and staining intensity ($p>0.05$). We observed that the survival of patients with positive stromal staining was significantly shorter compared to those with negative stromal staining ($p<0.05$).

Kamel et al., evaluated the immunohistochemical expression of MMP-2 and found significant association with tumor grade and disease stage [11]. They also showed in the same study that MMP-2 plays an important role in tumor proliferation. Torng PL et al., showed that the expression of stromal MMP-2 was significantly associated with higher grade ($p = 0.005$) [17]. Sakata et al., found a significant correlation between surgical staging and MMP-2 expression. They found increased MMP-2 expression in patients with advanced-stage disease (stages 3 and 4) [16]. Schmalfeldt et al., and Davidson et al., found that as the expression of matrix metalloproteinases increased, the stage of the disease increased as well [18, 19]. On the other hand, another study did not find a significant difference between the MMP-2 expressions in benign disease, tumor with low malignant potential and advanced-stage malignant ovarian disease [20]. In our study, the percentage of MMP-2 staining, staining intensity, combined total score and stromal staining were not significantly associated with the stage and grade of EOC and tumor diameter. In addition, we showed that staining intensity in EOC differs significantly, depending on the histologic type.

In their study, Wu et al., found a precise correlation between MMP-2 expression and omental metastasis. They also found correlations between MMP-2 expression and metastasis to the lungs and liver and to other sites [21]. The association between MMP-2 expression and metastasis risk of EOC has been shown in numerous similar studies [11, 18, 19]. The association between the percentage of staining and the presence of metastasis ($p<0.05$) was also demonstrated in our study.

Garzetti et al., conducted an immunohistochemical evaluation of the MMP-2 expression. In their study, the authors found a positive correlation between the MMP-2 expression and disease recurrence [22]. Other studies also reported statistical correlation of MMP-2 expression and progression of ovarian carcinoma [19, 21, 22]. In a study by Périgny et al., a clear association between the MMP-2 expression in cancer cells and peritoneal implants and an increased risk of death was demonstrated. No clear association with survival between an increased stromal expression of MMP-2 in ovarian tumors, expression in cancer cells and increased expression in peritoneal implants was demonstrated. However, multivariate analysis suggested MMP-2 expression to be a prognostic factor in peritoneal implants. An increased expression of MMP-2 in cancer cells of peritoneal implants is accompanied by an increased risk of death [12]. In another study, an increased MMP-2 expression showed correlation with poor survival [19]. Despite these data, a phase 3 trial found that MMP inhibitor has no effect on preventing disease progression and prolonging life expectancy [23, 24]. In our study, no significant correlation was found between overall survival and the percentage of MMP-2 staining, total score of staining and staining intensity ($p<0.05$). The association of disease-free survival with the percentage of MMP-2 staining, total score of staining, staining intensity and stromal staining was not statistically significant ($p<0.05$). We also found that the survival of patients with positive stromal staining was significantly shorter compared to those with negative stromal staining ($p<0.05$).

Conclusions

Although the number of patients included in our study was relatively small, we were nevertheless still able to demonstrate an association between the percentage of MMP-2 staining (expression) in the epithelial cells and metastasis status. The staining intensity of MMP-2 in the epithelial cells was significantly associated with histologic type. Also, the stromal staining of MMP-2 was found to be significantly associated with the recurrence and presence of metastasis . Nevertheless, large-scale and comprehensive investigations in homogenous groups are needed to verify whether MMP 2 may be used as a routine prognostic factor for EOC.

Authors' Contribution

1. Tekin Ekinci – he played role in study, design, acquisition of data, interpretation of data, analysis and writing the text.
2. Pelin Ozun Ozbay – she played role in acquisition of data and interpretation of data.
3. Seyran Yigit – she played role in material and methods, she made immunohistochemical analysis and revised article critically.
4. Ali Yavuzcan – he played role in writing the text and revising article critically – corressponding author.
5. Selda Uysal – she played role in revising article critically.
6. Ferit Soylu – he played role in revising article critically.

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- There is no conflict of interests in this article.

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