Original Article

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Pathology Section

Matrix Metalloproteinase-9 Expression in Surface Epithelial Ovarian Carcinoma and its Association with Clinicopathological Parameters: A Cross-sectional Study

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ABSTRACT

Introduction: Ovarian cancer is one of the most lethal gynaecological malignancies worldwide, largely due to its late-stage diagnosis and high metastatic potential. The tumour microenvironment plays a crucial role in disease progression, with Extracellular Matrix (ECM) remodelling being a key process in tumour invasion and metastasis. Among the mediators of ECM degradation, Matrix Metalloproteinase-9 (MMP-9) has garnered significant attention due to its ability to degrade type IV collagen, a major component of the basement membrane. MMP-9, a member of the gelatinase subfamily of MMP, is involved in multiple oncogenic processes, including tumour growth, angiogenesis, and immune evasion. Overexpression of MMP-9 has been observed in various malignancies, including ovarian cancer, where it is associated with poor prognosis, increased tumour aggressiveness, and resistance to therapy.

Aim: To analyse the expression of MMP-9 and its association with different clinicopathological parameters in surface epithelial ovarian carcinoma.

Materials and Methods: This observational, cross-sectional descriptive study included 80 cases of epithelial ovarian carcinoma diagnosed at Nil Ratan Sircar Medical College and Hospital, Kolkata, West Bengal, India. Tumour samples were collected and processed for histopathological examination, followed by immunohistochemical analysis to assess MMP-9 expression. Clinicopathological parameters, including histological type, grade, and stage, were recorded. The intensity of MMP-9 expression was categorised into two groups—high and low expression—based on a predetermined scoring system. The association between MMP-9 expression and various clinicopathological features (such as tumour laterality, histological type, tumour grade, tumour stage, and presence of malignant cells in ascitic fluid) was analysed using IBM Statistical Package for the Social Sciences (SPSS) for Windows, Version 10.0, Armonk, NY: IBM Corp.). Descriptive statistics were calculated in terms of frequency and percentages for the qualitative variables. Clinical features were evaluated by descriptive analysis; mean and median values were calculated. Differences between variables were assessed using the chi-square (χ^2) test. A p-value <0.05 was considered statistically significant.

Results: Among the 80 samples analysed, high epithelial MMP-9 expression (moderate or strong) was observed in 63 (78.75%) cases, while 17 (21.25%) showed negative or weak expression. Stromal MMP-9 expression was detected in 76 (95.00%) out of 80 specimens, with 48 (60.00%) tumours demonstrating high stromal MMP-9 levels. High stromal MMP-9 expression was significantly associated with advanced stage (p-value=0.001), high grade (p-value=0.01), and serous histology (p-value=0.026). However, epithelial MMP-9 expression showed no statistically significant association with any clinicopathological parameters.

Conclusion: High stromal MMP-9 expression is associated with poor prognosis in ovarian cancer patients. Down-regulation of MMP-9 may be an important therapeutic strategy to reduce cancer-related mortality.

Keywords: Ovary, Tumour, Collagenase, Stroma

INTRODUCTION

The most common lethal malignancy among all gynaecological cancers is ovarian cancer [1]. It causes the death of approximately 185,000 women every year worldwide [1], primarily due to late diagnosis [2]. About 95% of ovarian malignancies are of epithelial origin [3], and High-Grade Serous Carcinoma (HGSC) is the most frequent histopathological type. Several risk factors have been identified, including increasing age, nulliparity, and hormone replacement therapy [4]. The prognosis of ovarian cancer is usually poor, with a 5-year survival rate of only 17% for patients diagnosed at an advanced stage [1]. There are several prognostic factors in ovarian cancer, such as histological subtype, tumour grade, tumour stage, performance status, and disease-free interval after first-line treatment. Among these, staging is considered very informative, although certain biases are associated with it. Therefore, numerous markers have been studied using immunohistochemical and cytogenetic methods to identify prognostic and predictive factors in ovarian cancer, including MMP-2, MMP-9, survivin, and Ki-67 index. MMP-9 is among the most widely studied markers in this context [5,6].

MMPs are zinc-dependent endopeptidases that cleave one or more constituents of the extracellular matrix, such as fibrillar collagen types I, II, and III. The ECM not only provides structural support for organs and tissues but also actively participates in various functions, including regulation of the cell cycle, cell motility, survival, and apoptosis. The ECM is composed of hundreds of molecules, including proteoglycans, glycosaminoglycans, structural proteins (such as collagen and elastin), adhesion proteins (such as fibronectin and laminin), and proteases known as MMPs. The MMP family consists of 23 members, which are zinc-containing, calcium-

dependent enzymes capable of degrading and remodelling ECM proteins [1].

MMP-2 and MMP-9 have been extensively studied in cancer, including ovarian cancer, with a considerable body of literature documenting their expression patterns and roles in tumour progression. Moreover, the expression levels of these MMPs vary across different tumour subtypes, with MMP-2 being higher in benign tumours compared to borderline and malignant tumours, while MMP-9 is higher in malignant tumours compared to borderline tumours [2]. These variations are observed both in tumour cells and in the associated stromal tissue. Discrepancies may arise from differences in sample analysis methods and arbitrary thresholds used by different research groups to determine staining intensity.

MMP-2 and MMP-9 have also been investigated for their roles in ovarian cancer cell migration and invasion. Both are secreted and activated in ovarian cancer, and are closely correlated with invasion, metastasis, and poor survival [2]. Some studies suggest that the absence of MMP-9 results in markedly reduced tumour incidence, growth, and microvessel density, highlighting the importance of host-derived MMP-9 in tumour angiogenesis and progression [3]. It has been demonstrated that weak stromal MMP-9 staining is associated with significantly longer survival compared to moderate or intense staining, suggesting that stromal MMP-9 is an independent prognostic factor [4].

MMP-9 has been implicated in the release of Vascular Endothelial Growth Factor (VEGF) from tumour cells, contributing to ascites formation in ovarian cancer. Interestingly, MMP-9 may play dual roles in tumour development, acting as a tumour promoter in the stroma while inhibiting tumour progression when expressed in the epithelium [3]. Platelet-Derived Growth Factor-D (PDGF-D) has also been implicated in promoting ovarian cancer invasion by increasing the expression of MMP-2 and MMP-9 [3]. Finally, a meta-analysis of 30 published studies on MMP-9 and its prognostic value in ovarian cancer revealed an overall positive correlation between MMP-9 expression and poor prognosis [3].

The novelty of this study lies in the observation that careful evaluation of staining patterns may help in predicting the prognosis of different surface epithelial carcinomas.

MATERIALS AND METHODS

This institution-based observational cross-sectional study was conducted in the Department of Pathology, in collaboration with the Department of Gynaecology and Obstetrics, at Nil Ratan Sircar Medical College and Hospital (NRSMCH), Kolkata, West Bengal, India, over a period of 12 months (October 2022 to September 2023), following approval from the Institutional Ethical Committee (Memo No. NRSMC/IEC/143/2022, dated 16/11/2022). A total of 80 cases of ovarian cancer were included. Written informed consent was obtained from each participant, and patient confidentiality was strictly maintained throughout the study.

Inclusion criteria:

1. Histopathologically confirmed cases of epithelial ovarian carcinoma.
2. Patients who underwent primary surgical treatment (TAH-BSO or ovarian cystectomy) at Nil Ratan Sircar Medical College and Hospital.

Exclusion criteria:

1. Benign and borderline epithelial tumours.
2. Non epithelial ovarian tumours (e.g., germ cell tumours, sex cord-stromal tumours, etc.).
3. Secondary (metastatic) malignancies involving the ovary.
4. Patients who had received neoadjuvant chemotherapy prior to surgery.
5. Insufficient clinical or pathological data.
6. Biopsy sections showing autolysed tissue.
7. Patients who did not consent to biopsy.

Sample size: The total number of study participants was 80. As this was an observational descriptive cross-sectional study, the data were assumed to be normally distributed. The population (N) was very large (> 250,000), and n/N was <0.05. The sample size (n) was calculated using a standard formula for cross-sectional studies with categorical variables, where the outcome of interest was the expression of MMP-9 in surface epithelial ovarian cancer, represented as P (proportion) = P (prevalence).

Formula was: $n=Z^2\ pq/d^2$, where, Z=1.96 (two tail) at 95% Confidence interval

P=proportion of patients sustaining the expression of genetic of interest.

q (complement of p)=(100-p)

d=allowable error according to reported p value

As per existing literature, 76.70% of specimens are showing MMP-9 positivity

Hence, P=76.70%, so q=23.30%

Considering d=10 (absolute) and putting the value in formula

$n=(1.96)2\times76.7\times23.3/10^2$

$=3.8416\times76.7\times23.3/100$

$=7005.47/100=70.05$

Considering 10% non response rate of cases, the revised sample size will be

=70+(10% of 70)

=70+7=77

Hence, total sample size=80

Study Procedure

Tissue specimens were obtained from Total Abdominal Hysterectomy with Bilateral Salpingo-Oophorectomy (TAH-BSO) and ovarian cystectomy. Relevant clinicopathological information, including patient age, menopausal status, smoking history, family history of ovarian cancer, tumour laterality, histological type, tumour grade, tumour stage, presence of malignant cells in ascitic fluid, and any history of chemotherapy or radiotherapy, was documented. The surgical specimens were immediately placed in 10% neutral buffered formalin for 24-48 hours to ensure proper fixation and preservation of tissue morphology. After adequate fixation, the specimens were grossly examined according to the protocol of the College of American Pathologists (CAP) [1] for ovarian carcinoma. Tumour size, consistency, presence of solid or cystic components, haemorrhage, necrosis, and capsular invasion were assessed. A meticulous search was performed for lymph nodes submitted separately. Representative sections were carefully selected from the tumour, ovarian capsule, and adjacent normal tissue for further histopathological and immunohistochemical analysis.

Tissue sections of 4 µm thickness were prepared using a rotary microtome and mounted on glass slides. Haematoxylin and Eosin (H&E) staining was performed for microscopic evaluation. Tumours were classified based on histological subtype, tumour grade, and stage, following the International Federation of Gynaecology and Obstetrics (FIGO) staging system [2].

Immunohistochemical staining procedure [3]: Sections reserved for immunohistochemical (IHC) analysis were first deparaffinised by immersion in xylene (two changes, 5 minutes each), followed by rehydration through a graded ethanol series (100% and 95% ethanol, 5 minutes each). Antigen retrieval was performed using Tris-EDTA buffer (pH 9.0) in a microwave oven for three cycles of five minutes each, using the EZ Antigen Retriever system. The slides were then cooled to room temperature and rinsed with Tris-buffered saline (TBS).

To minimise non specific antibody binding, the sections were incubated with a blocking solution (Power Block) for 10 minutes at room temperature. The slides were then incubated with a rabbit monoclonal anti-human MMP-9 antibody (EP127 clone, ready-to-use) for one hour at 4°C in a humidified chamber. A secondary antibody was applied after three washes with TBS. To visualise antibody binding, 3,3'-diaminobenzidine (DAB tetrahydrochloride) substrate was applied to the sections for 10 minutes.

For all immunostaining, cell counting was performed in areas where the tumour was most prominent. Normal splenic tissue was used as a positive control for MMP-9 staining. A negative control, omitting the primary antibody, was included in each staining run to confirm specificity.

Assessment of MMP-9 expression [3]: IHC assessment of MMP-9 expression was performed on formalin-fixed, paraffin-embedded tissue sections. Cytoplasmic staining in tumour epithelial and stromal cells was evaluated. The proportion of positive cells and staining intensity were scored, and an overall score was calculated to categorise expression as low or high for statistical analysis [Table/Fig-1].

IHC Parameter	Score	Criteria
Percentage of positive tumour cells	0	0%-5%
	1	6%-50%
	2	>50%
Staining intensity	1	Weak
	2	Moderate
	3	Strong
Overall Score (OS)	0	Negative expression
	1	Weak expression
	2	Moderate expression
	3	Strong expression
Expression category (for Statistical Analysis)	OS 0-1	Low expression
	OS 2-3	High expression

[Table/Fig-1]: Immunohistochemical assessment of MMP-9 Expression.

STATISTICAL ANALYSIS

Image acquisition was performed using a Nikon Eclipse E600 microscope and Lucia 5 software. ANOVA and Pearson's chi-square tests (SPSS version 10) were used for statistical analysis. Data were tabulated in Microsoft Excel and analysed with appropriate statistical methods. Numerical variables were summarised as mean and standard deviation (SD), while categorical variables were presented as percentages. A p-value <0.05 was considered statistically significant.

RESULTS

A total of 80 cases of epithelial ovarian carcinoma were included in the study. The mean age of the patients was 58.70 years (range: 28-71 years) . Serous adenocarcinoma was the most common histological subtype, accounting for 56 (70.00%) cases [29 (51.78%) cases were high-grade serous carcinoma and 27 (48.22%) cases were low-grade serous carcinoma], followed by mucinous carcinoma (12 cases, 15.00%), endometrioid carcinoma (8 cases, 10.00%), and clear cell carcinoma (4 cases, 5.00%) [Table/Fig-2].

Of the 80 tumours, 53 (66.25%) were classified as high-grade [tumours with marked cytological atypia, presence of multinucleated cells, and higher mitotic count, usually more than 12 mitoses per 10 high-power fields (HPFs)], and 27 (33.75%) were low-grade [tumours with mild to moderate cytological atypia and lower mitotic count, usually up to 12 mitoses per 10 HPFs] [2] [Table/Fig-2]. According to FIGO staging, 22 (27.50%) cases were stage I, 13 (16.25%) were stage II, 39 (48.75%) were stage III, and 6 (7.50%) were stage IV [Table/Fig-2]. Malignant cells were detected in ascitic fluid in 40 (50.00%) cases, while 36 (45.00%) cases had negative ascitic cytology, and 4 (5.00%) cases were unknown [Table/Fig-2].

Clinicopathological parameters	Number (%)
Laterality	
Right	34 (42.50)
Left	15 (18.75)
Bilateral	31 (38.75)
Histological type	
Serous adenocarcinoma	56 (70.00)
Mucinous adenocarcinoma	12 (15.00)
Endometroid adenocarcinoma	08 (10.00)
Clear cell carcinoma	04 (05.00)
Histologic grade (based on differentiation)	
Low (grade 1 or grade 2) (low grade serous carcinoma)	27 (33.75)
High (grade 3) (high grade serous carcinoma, mucinous carcinoma, endometrioid carcinoma, clear cell carcinoma)	53 (66.25)
Stage (FIGO staging)	
I	22 (27.50)
II	13 (16.25)
III	39 (48.75)
IV	06 (07.50)
Ascites (Positive/negative for M- cell)	
Positive	40 (50.00)
Negative	36 (45.00)
Unknown	04 (05.00)

[Table/Fig-2]: Clinical and histopathological parameters of the patients.

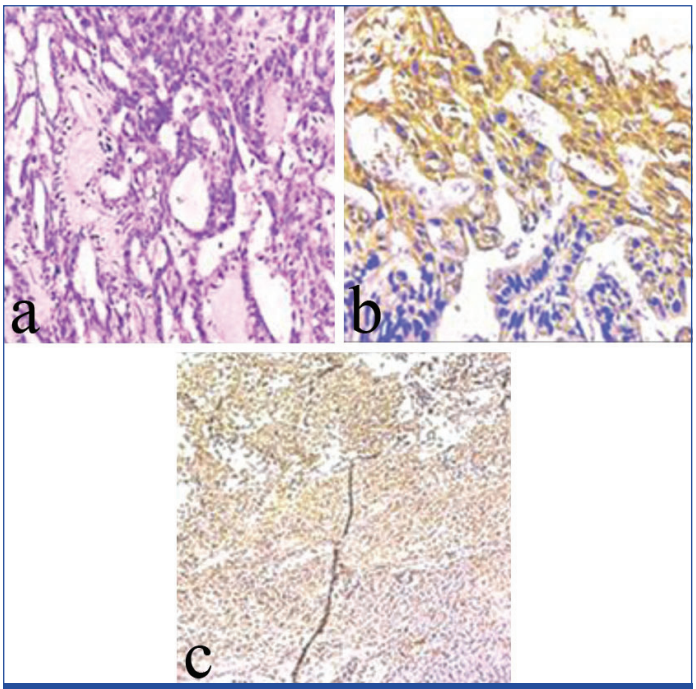
Immunohistochemical analysis revealed detectable stromal MMP-9 expression in 76 (95.00%) of the 80 specimens. Among these, 48 (60.00%) tumours exhibited high stromal MMP-9 expression. The association of stromal MMP-9 expression with histological subtype, tumour grade, FIGO stage, and ascitic fluid cytology was significant (p-values 0.02, 0.01, 0.001, and 0.001, respectively) [Table/Fig-3].

Histological type	MMP-9 epithelial Expression		MMP-9 stromal expression	
	High (overall staining intensity score-=2-3)	Low (overall staining intensity score-=0-1)	High (overall staining intensity score-=2-3)	Low (overall staining intensity score-=0-1)
Clear cell carcinoma	0	04 (100.00%)	0	04 (100.00%)
Endometrioid carcinoma	07 (88.00%)	01 (12.00%)	05 (63.00%)	03 (37.00%)
Mucinous adenocarcinoma	06 (50.00%)	06 (50.00%)	06 (50.00%)	06(50.00%)
Serous carcinoma	47 (84.00%)	09 (16.00%)	36 (64.00%)	20 (36.00%)
Chi-square value	219		136.8	
p-value	0.51 (not significant)		0.026 (significant)	
Association of Histological type with MMP-9 epithelial and stromal expression				
Histological grading	**High**	**Low**	**High**	**Low**
High grade	40 (75.00%)	13 (25.00%)	35 (66.00%)	18 (34.00%)
Low grade	23 (85.00%)	04 (15.00%)	13 (48.00%)	14 (52.00%)
Chi-square value	3.125		6.61	
p-value	0.07 non significant		0.01 significant	
Association of Histological grading with MMP-9 epithelial and stromal expression				
FIGO staging	**High**	**Low**	**High**	**Low**
Stage 1	14 (63.63%)	08 (36.37%)	06 (27.27%)	16 (72.73%)
Stage 2	04 (31.00%)	09 (69.00%)	03 (23.07%)	10 (76.93%)
Stage 3	36 (92.30%)	03 (07.70%)	34 (87.17%)	05 (12.83%)
Stage 4	06 (100.00%)	0	06 (100.00%)	0
Chi-square value	144.8		200.5	

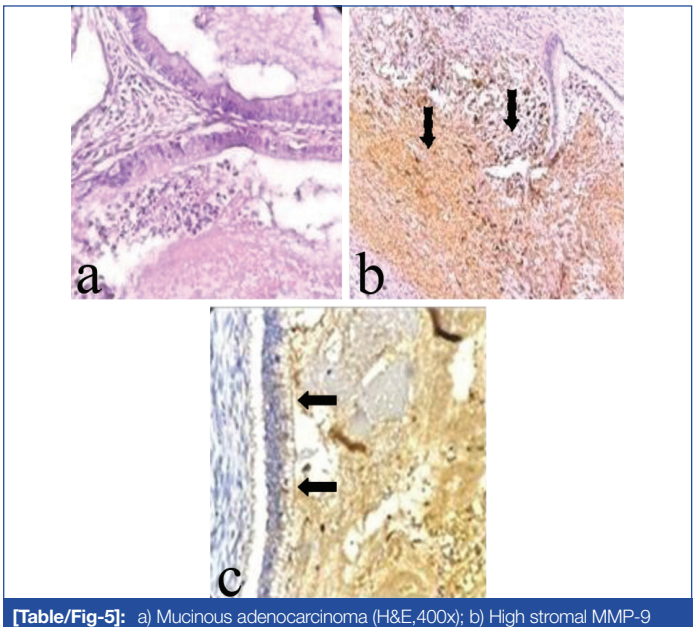
p-value	0.07 (not significant)		0.001 (significant)	
Association of FIGO staging with MMP-9 epithelial and stromal expression				
Ascites status	**High**	**Low**	**High**	**Low**
Positive	37 (92.50%)	03 (07.50%)	38 (95.00%)	02 (05.00%)
Negative	25 (69.44%)	11 (30.56%)	10 (27.78%)	26 (72.22%)
Chi-square value	03.30%		55.16%	
p-value	0.068 (not significant)		0.001 (significant)	
Association of Ascites status (Positive/negative for M- cell) with MMP-9 epithelial and stromal expression				

[Table/Fig-3]: Association of histology, grade, stage and ascitic fluid cytology with MMP-9 expression in the epithelium and stroma.

High stromal MMP-9 expression was found in 36 (64.00%) serous carcinomas [Table/Fig-4], 06 (50.00%) of 12 mucinous carcinomas [Table/Fig-5], 05 (63.00%) of 08 endometrioid carcinomas, and 00 of 04 clear cell carcinomas [Table/Fig-6a].

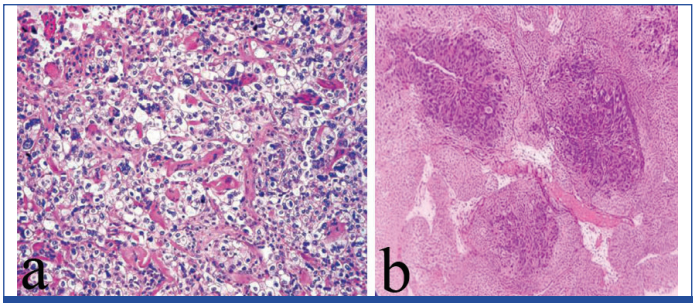


[Table/Fig-4]: a) High grade serous carcinoma (H&E,400x); b) High epithelial MMP-9 expression (H&E,400x); c) High stromal MMP-9 expression (IHC,400x).



[Table/Fig-5]: a) Mucinous adenocarcinoma (H&E,400x); b) High stromal MMP-9 expression (IHC,400x); c) High epithelial MMP-9 expression (Block arrows) (IHC,400x).

Among the 80 samples analysed, high epithelial MMP-9 expression (moderate or strong) was observed in 63 (78.75%) cases, while 17 (21.25%) showed negative or weak expression. However,



[Table/Fig-6]: a) Clear cell carcinoma (H&E,400x); b) Transitional cell carcinoma (H&E,400x).

epithelial MMP-9 expression showed no significant association with clinicopathological parameters (histological type, grade, stage, and ascites) compared to stromal expression (p-values 0.51, 0.07, 0.07, and 0.06, respectively). High epithelial MMP-9 expression was seen in 47 (84.00%) serous carcinomas [Table/Fig-4], 06 of 12 (50.00%) mucinous carcinomas [Table/Fig-5], 7 of 8 (88.00%) endometrioid carcinomas, and 00 of 4 clear cell carcinomas (p-value=0.51).

Regarding tumour grade, 35 (66.03%) of 53 high-grade tumours had high stromal MMP-9 expression, compared to 13 (48.00%) of 27 low-grade tumours (p-value=0.01) [Table/Fig-3]. With respect to FIGO stage, 40 (34+06) of 45 (88.88%) advanced-stage tumours (stages III and IV) demonstrated high stromal MMP-9 expression, compared to 09 (06+03) of 35 (25.71%) early-stage tumours (stages I and II) (p-value=0.001) [Table/Fig-3].

Malignant ascitic fluid was significantly associated with high stromal MMP-9 expression, with 38 of 40 (95.00%) cases showing elevated expression, in contrast to 10 of 36 (27.78%) cases without malignant ascites (p-value=0.001) [Table/Fig-3].

For epithelial MMP-9 expression, 40 of 53 (75.47%) high-grade tumours exhibited high expression, while 23 (85.00%) of 27+1 low-grade tumours showed high expression (p-value=0.07) [Table/Fig-3]. Similarly, 42 (36+06) of 45 (93.33%) advanced-stage tumours demonstrated high epithelial expression, compared to 18 (14+04) of 35 (51.42%) early-stage tumours (p-value=0.07) [Table/Fig-3]. In cases with malignant ascites, 37 of 40 (92.50%) showed high epithelial MMP-9 expression, compared to 25 of 36 (69.44%) cases without malignant ascitic fluid (p-value=0.068) [Table/Fig-3].

DISCUSSIONS

Over the last 20-30 years, remarkable improvements have been made in the understanding and management of ovarian cancer; however, there has been no significant decrease in mortality due to the frequent presence of widely metastatic disease at the time of diagnosis. The pathological stage is still considered the most important prognostic factor for ovarian tumours [7,8]. Local invasion and distant spread of malignant tumours involve degradation of subepithelial and subendothelial basement membranes, which act as barriers between tissue compartments. Cancer cells also modify the ECM. Disruption of basement membrane integrity, a hallmark of invasive tumours, facilitates both local spread and distant metastasis. This process depends on interactions among tumour cells, endothelial cells, and host-derived stromal cells.

These critical processes are regulated by various factors including proteolytic enzymes, transcription factors, adhesion molecules, and ECM components [2]. MMPs belong to a family of calcium- and zinc-dependent proteolytic enzymes. They are produced by tumour cells or surrounding stromal cells (e.g., fibroblasts, endothelial cells, and infiltrating macrophages). MMP-2 and MMP-9 contribute to active neovascularisation and degrade major structural components of the basement membrane (type IV collagen). This step is important for tumour progression and is negatively correlated with overall survival in ovarian cancer [9,10]. They are also involved in tumour invasion, angiogenesis, and metastasis [11,12].

Several studies have shown that MMP-9 is highly expressed in breast cancer, colorectal cancer, and certain leukaemias [13,14]. The expression of MMP-9 has been detected at a higher rate in ovarian cancers compared with normal ovarian tissue, benign tumours, and borderline tumours [15]. Most studies have focused on the role of tumour cell-derived MMP expression; however, there are limited data regarding the clinical importance of stromal MMPs in ovarian carcinoma [2]. Therefore, this study aims to determine the prognostic significance of epithelial and stromal MMP-9 expression in ovarian cancers. In the present study, a cohort of 80 epithelial ovarian carcinoma cases was evaluated, with a mean age of 58.70 years and a standard deviation (SD) of 10.67, similar to Yoshida H et al., [1] (N=254, mean age 60.0 years, SD 11.4).

Serous adenocarcinoma was the predominant histological subtype, accounting for 56 (70.00%) cases. A significant proportion of tumours were high grade (53, 66.25%), and 45 (56.25%) patients presented at advanced stages (FIGO III/IV), findings comparable to those reported by Alshenawy HA, [2] [n=62, serous carcinoma 40 (64.51%), high grade 33 (53.22%)].

Immunohistochemical analysis revealed high epithelial MMP-9 expression in 63 (78.75%) cases. However, this expression did not correlate significantly with prognostic parameters such as age, histological subtype, tumour grade, FIGO stage, or the presence of malignant ascitic fluid (p-values 0.06, 0.51, 0.07, 0.07, and 0.06, respectively), similar to the findings of Ozalp S et al., [3] (p-values 0.66, 0.56, 0.08, 0.65, and 0.07, respectively).

In contrast, high stromal MMP-9 expression was observed in 76 of 80 cases (95.00%). Notably, high stromal MMP-9 expression was significantly associated with serous, mucinous, and endometrioid carcinoma subtypes (p-value=0.026), high-grade tumours (p-value=0.01), advanced-stage disease (p-value=0.001), and the presence of malignant cells in ascitic fluid (p-value=0.001), but was not associated with age (p-value=0.66). Sillanpää S et al., [16] also reported that high stromal MMP-9 positivity was significantly linked to advanced tumour stage (p-value=0.01) and shorter disease-related survival (p-value=0.04), although their study did not show significant correlations with tumour grade (p-value=0.055), histological type (p-value=0.560), or patient age (p-value=0.566).

These findings highlight the importance of the tumour microenvironment in ovarian cancer progression. The significance of stromal MMP-9 expression is supported by several previous studies. Sakata K et al., [15], Sillanpää S et al., [16], Kamat AA et al., [17], and Cowden Dahl KD et al., [18] found that both epithelial and stromal cells of ovarian tumours expressed MMP-9, consistent with the results of this study. Kamat AA et al., [17] and Cowden Dahl KD et al., [18] concluded that both epithelial and stromal staining were associated with poor prognosis. In particular, stromal staining has been associated with advanced stage, metastasis, the presence of ascites, and shorter survival times compared to tumours without stromal expression [18,19].

Lengyel E et al., [20] analysed MMP-9 expression in 84 patients with advanced ovarian cancer (FIGO stage III) and 19 benign ovarian tumours using gelatin zymography. They found significantly higher MMP-9 activity in ovarian cancer tissues than in benign ovarian tissues (p-value=0.001), which correlated with short overall survival (p-value=0.019). The results were evaluated with a median follow-up period of 55 months.

Although the expression of MMP-9 has been associated with poor survival in several cancers, ovarian cancer-specific data remain limited. Li Li-Na et al., [21] investigated the prognostic significance of MMP-9 using hazard ratios (HRs) or odds ratios (ORs) with 95% confidence intervals (95% CIs) in fixed- or random-effects models. Increased expression of MMP-9 was associated with poor prognosis in ovarian cancer (HR=1.68, 95% CI 1.09-2.59, p-value=0.02). Moreover, increased MMP-9 expression was significantly associated with higher FIGO stage (OR=4.85, 95% CI 2.60-9.04, p-value <0.00001), high-grade tumours (OR=3.34, 95% CI 2.46-4.54, p-value <0.00001), and lymph node metastasis (OR=5.75, 95% CI 3.71-8.92, p-value <0.00001), but showed no association with histological type (p-value=0.066). They concluded that down-regulation of MMP-9 could be an attractive therapeutic approach to improve ovarian cancer outcomes. Kleinberg L et al., [22] demonstrated in a transgenic mouse model that the absence of MMP-9 (using MMP-9 knockout mice) resulted in markedly reduced tumour incidence, growth, microvessel density, and macrophage infiltration compared to wild-type mice, highlighting the importance of host-derived MMP-9 in tumour angiogenesis and progression. Davidson B et al., [23] found that patients with weak stromal MMP-9 staining had significantly longer survival compared to those with moderate or intense staining, suggesting that stromal MMP-9 is an independent prognostic factor. Athanassiadou P et al., [24] extended these observations by showing that high expression levels of MMP-2, MMP-9, and MT1-MMP in both the tumour epithelium and stroma were associated with aggressive tumour features and shorter disease-specific survival, with high stromal MMP-9 emerging as an independent predictor of poor outcome in multivariate analysis.

Limitations(s)

Despite sincere efforts, this study had certain limitations. It was based on a single centre and conducted at a tertiary-care hospital; therefore, the possibility of institutional or selection bias cannot be ruled out. The duration of the study was only one year. Disease-free survival and tumour recurrence were not evaluated. The association between MMP-2 (another important MMP) and clinicopathological parameters was not studied due to financial constraints. Serum MMP-9 levels were also not assessed.

CONCLUSIONS(S)

The present study demonstrates that while epithelial MMP-9 expression does not show a significant correlation with adverse prognostic variables, stromal MMP-9 expression is markedly associated with high tumour grade, advanced FIGO stage, specific histological subtypes (serous, mucinous, endometrioid, and clear cell), and the presence of malignant cells in ascitic fluid. These results suggest that MMP-9 produced by stromal cells plays a more critical role in promoting tumour aggressiveness than tumour cell-derived MMP-9. Therefore, stromal MMP-9 expression may serve as a valuable prognostic biomarker and represents a potential therapeutic target. Further research involving larger, multicentric, and prospective studies is warranted to validate these findings and elucidate underlying molecular mechanisms, which may ultimately improve management strategies for patients with epithelial ovarian carcinoma.

REFERENCES

[1] Yoshida H, Ishiko O, Sumi T, Matsumoto Y, Ogita S. Survivin,bcl-2 and matrix metalloproteinase-2 enhance progression of clear cell- and serous-type ovarian carcinomas. Int J Oncol. 2021;19:537-42.
[2] Alshenawy HA. Immunohistochemical expression of epidermal growth factor receptor, E-cadherin, and matrix metalloproteinase-9 in ovarian epithelial cancer and relation to patient deaths. Ann Diagn Pathol. 2022; 14:387-95.
[3] Ozalp S, Tanir HM, Yalcin OT, Kabukcuoglu S, Oner U, Uray M. Prognostic value of matrix metalloproteinase- 9 (gelatinase-B)expression in epithelial ovarian tumors. Eur J Gynaecol Oncol. 2023;24:417-20.
[4] Tringler B, Lehner R, Shroyer AL, Shroyer KR. Immunohistochemical localisation of MMP-9 in serous tumors of the ovary. Appl Immunohistochem Mol Morphol. 2024;12:40-3.
[5] Cohen C, Lohmann CM, Cotsonis J, Lawson D, Santoianni R. MMP-9 expression in ovarian carcinoma: Correlation with apoptotic markers and prognosis. Mod Pathol. 2023;16: 574-83.
[6] Ryan BM, O'Donovan N, Duffy MJ. MMP-9: A new target for anti-cancer therapy. Cancer Treatment Reviews. 2022;35:553-62.
[7] Seidman JD, Russel P, Kurman RJ. Surface epithelial tumors of the ovary. In: Kurman RJ, editor. Blaustein's Pathology of the Female Genital Tract. 5th ed, New York: Springer; 2022;791-904.

[8] Denny L, Hacker NF, Gori J, Jones III HW, Ngan HYS, Pecorelli S. Staging classifications and clinical practice guidelines of gynaecologic cancers. In: Pecorelli S, YSN Hextan, Hacker NF, editors. Elsevier; 2000. 95- 115. (reprinted from Int J Gynecol Obstet 70:207-312, 2000).
[9] Lin CK, Chao TK, Yu CP, Yu MH, Jin JS. The expression of six biomarkers in the four most common ovarian cancers: Correlation with clinicopathological parameters. APMIS. 2021;117:162-75.
[10] MacKeigan JP, Murphy LO, Blenis J. Sensitized RNA screen of human kinases and phosphatases identifies new regulators of apoptosis and chemoresistance. Nat Cell Biol. 2021;7:591-600.
[11] Davidson B, Goldberg I, Berner A, Kristensen GB, Reich R. Emmprin (extracellular matrix metalloproteinase inducer) is a novel marker of poor outcome in serous ovarian carcinoma. Clin Exp Metastasis. 2021;20:161-69.
[12] Carey P, Low E, Harper E, Stack MS. Metalloproteinases in ovarian cancer. Int J Mol Sci. 2021;22(7):3403.
[13] Pellikainen JM, Ropponen KM, Kataja VV. Expression of matrix metalloproteinase (MMP)-2 and MMP-9 in breast cancer with a special reference to activator protein-2, HER2, and prognosis. Clin Canc Res. 2024;10:7621.
[14] Liabakk NB, Talbot I, Smith RA. Matrix metalloproteinase 2 (MMP-2) and matrix metalloproteinase 9 (MMP-9) type IV collagenases in colorectal cancer. Canc Res. 2021;56:190-96.
[15] Sakata K, Shigemasa K, Nagai N, Ohama K. Expression of matrix metalloproteinases (MMP-2, MMP-9, MT1-MMP) and their inhibitors (TIMP-1, TIMP-2) in common epithelial tumors of the ovary. Int J Oncol. 2020;17:673-81.
[16] Sillanpaa S, Anttila M, Voutilainen K, Ropponen K, Turpeenniemi-Hujanen T, Puistola U, et al. Prognostic significance of matrix metalloproteinase-9 (MMP-9) in epithelial ovarian cancer. Gynecologic Oncology. 2020;104:296-303.
[17] Kamat AA, Fletcher M, Gruman LM, Mueller P, Lopez A, Landen CN Jr, et al. The clinical relevance of stromal matrix metalloproteinase expression in ovarian cancer. Clin Cancer Res. 2021;12:1707-14.
[18] Cowden Dahl KD, Symowicz J, Ning Y, Gutierrez E, Fishman DA, Adley BP, et al. Matrix metalloproteinase 9 is a mediator of epidermal growth factor-dependent e-cadherin loss in ovarian carcinoma cells. Cancer Res. 2021;68:4606-13.
[19] Takai N, Miyazaki T, Nishida M, Nasu K, Miyakawa I. Expression of MMP-9 is associated with malignant potential in epithelial ovarian carcinoma. Int J Mol Med. 2022;10: 211-16.
[20] Lengyel E, Schmalfeldt B, Konik E, Späthe K, Härting K, Fenn A, et al. Expression of latent Matrix Metalloproteinase 9 (MMP-9) predicts survival in advanced ovarian cancer. Gynecologic Oncology. 2021;82(2):291-98.
[21] Li Li-Na, Zhou X, Gu Y, Yan J. Prognostic Value of MMP-9 in Ovarian Cancer: A Meta-analysis. Asian Pac J Cancer Prev. 2023;14 (7):4107-13.
[22] Kleinberg L, Florenes VA, Silins I, Haug K, Trope CG, Nesland JM, et al. Nuclear expression of MMP-9 is associated with improved survival in metastatic ovarian carcinoma. Cancer. 2020;109:228-38.
[23] Davidson B, Goldberg I, Gotlieb WH, Kopolovic J, Ben-Baruch G, Nesland JM, et al. High levels of MMP-2, MMP-9, MT1-MMP and TIMP-2 mRNA correlate with poor survival in ovarian carcinoma. Clin Exp Metastasis. 2021;17:799-808.
[24] Athanassiadou P, Grapsa D, Athanassiades P, Gonidi M, Athanassiadou AM, Tsipis A, et al. The prognostic significance of COX-2 and survivin expression in ovarian cancer. Pathol Res Pract. 2008;204:219-41.

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