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Determine the role of Metalloproteinase MMP14 and Osteopotin OPN expression and explain its role in ovarian carcinoma tissues developmental invasion and metastasis by immunohistochemical IHC study

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Abstract

Background: Ovarian carcinomas is a varied category of neoplasms, are composed of several entities with different risk factors, precursor lesions, an etiology, patterns of spread, molecular profiles, clinical outcomes, and responses to chemotherapy. In decidualized clinical decision-making requires knowledge of the histologic subtype and the associated molecular characteristics. MMP play a number of additional roles in cancer they influence the proliferation, epithelial-to-mesenchymal transition, and intracellular activities in the nucleus of cancer cells. The MMPs are produced at the cell surface rather than being secreted. MMP14 is a member of the membrane and the Osteopotin OPN plays a significant role in carcinogenesis. The main aim of this study was elucidating the role of metalloproteinase MMP14 and Osteopotin in ovarian carcinoma tissues developmental invasion and metastasis by immunohistochemical (IHC) study. **Methods:** The study involved 65-woman patients clinically diagnosed with ovarian carcinoma, that samples tissue are collected from patients under historically surgical resection. **Results:** Immunohistochemistry detected significantly higher MMP14 expression in 64% of samples, especially High–grade serous ovarian carcinoma (30%) and MOC (10%), showing strong cytoplasmic and membrane staining. OPN expression was also elevated in most samples (82%), overwhelmingly in HGSOC (43%) displaying strong positivity. **Conclusions:** These data demonstrate MMP14 and OPN are aberrantly overexpressed in ovarian cancer compared to normal ovary, with preferential upregulation in HGSOC.

Keywords: Ovarian Carcinoma, Immunohistochemistry, MMP14, Osteopotin

1. Introduction

Ovarian carcinomas (OC) are a diverse and heterogeneous category of neoplasms, comprised of individual entities with unique molecular profiles, etiology, precursor lesions, and risk factors (1, 46). The most common epithelial ovarian cancers are serous, endometrioid, clear cell, and mucinous carcinomas. These develop from the ovarian surface epithelium or epithelial inclusions (2). Less common epithelial tumors include Brenner, squamous cell, transitional cell, and undifferentiated carcinomas (3). While germ cell tumors originate from

primordial germ cells and include dysgerminomas, yolk sac tumors, and immature teratomas (4). Benign ovarian tumors include serous and mucinous cystadenomas, Brenner tumors, fibromas, and dermoid cysts (5). Borderline tumors exhibit some malignant features but low metastatic potential (6). Of the malignant types, high-grade serous carcinoma is the most frequent and deadly (7).

Epithelial ovarian carcinoma (EOC) divided into five basic types based on its histology, immunological, and molecular profile (7, 8). High–grade serous ovarian carcinoma (HGSOC), is the most prevalent and aggressive subtype of ovarian cancer and it's a type of heterogeneous cancer with unstable chromosomes with the most genomically complex (9, 10)**.** Endometrioid carcinoma (EC) is either glandular or maze-like with frequent squamous differentiation, according to FIGO grading (11). Clear cell ovarian carcinoma (CCC) is rare type of ovarian carcinoma with distinctive histo-types of epithelial ovarian carcinomas (12). Only 3-4% of ovarian carcinomas are mucinous carcinomas (MOC), which makes up 10-15% of all primary ovarian neoplasms (13). Finally, low-grade serous ovarian carcinomas are less common epithelial ovarian cancers that originate from serous borderline tumors (14). Ovarian cancer represents a cancerous growth. 80% of ovarian cancers originate from epithelial cells in one or both ovaries. The most frequent epithelial type cancers are serious ones, with mucinous or clear cell tumours making up around 5% of cases (45).

Matrix metalloproteinase-14 (MMP14) is a member of the matrix metalloproteinase (MMP) family, which plays an important role in the breakdown of extracellular matrix (ECM) components. MMP14 has been shown to be involved in various processes related to cancer progression, including proliferation, invasion, angiogenesis, and metastasis (15). Overexpression of MMP14 which promotes angiogenesis, inflammation, cancer cell invasion, and metastasis (16). The development of advanced tumor is mediated through several pathways, MMP-14 expression occurs in most advanced-stage ovarian cancers and not in all early-stage tumor (17). MMP-14 as a member of the membrane-type MMP (MT-MMP) subfamily, which is responsible for the MMPs' expression at the cell surface as opposed to their secretion and animal models have shown its impact on invasion and metastasis (18).

On other hand, osteopontin (OPN) is a multifunctional phosphor-glycoprotein expressed in a wide range of cells, including osteoblasts, osteoclasts, neurons, myeloid, epithelial cells, B, T, NK, NK T, and innate lymphoid cells. OPN is involved in numerous disorders, including, and plays a crucial function in a variety of biological processes, such as

cardiovascular, diabetes, kidney, proinflammatory, fibrosis, nephrolithiasis, wound healing, and cancer (19). OPN is a glycoprotein that is involved in various cellular processes such as cell adhesion, migration, and survival. It has been shown to be overexpressed in several types of cancer, including ovarian cancer (20).

The study aims to determine how the expression of MMP14 and OPN contributes to the development of ovarian carcinoma tissues and their ability to invade and metastasize. The IHC technique will be used to visualize the expression of these proteins in ovarian carcinoma tissues.

2. Methods

The study comprised the collection of 65 samples from female clinically diagnosed with ovarian carcinoma in different ages. Patients were attended Al-Moannaa General Hospital in Basra City from March 2022 to March 2023. Approval for conducting the study was obtained from the Ethical Committee of Al-Moannaa General Hospital and informed consent was obtained from the patients. The tissue samples were collected from patients with ovarian cancer who under historically surgical resection (oophorectomy). The 65 tissue samples were classified into: HGSOC (31 samples), EC (8), MOC (7), MOC borderline (4), CCC (5), BSBT (5), synchronous (2) and benign tumor (3).

2.1 Sample Collection Steps

Clinical data and tissue samples for the patients were collected on the ovarian carcinoma study. The patient tissue samples of ovarian carcinoma were kept in 10% buffered neutral formalin to prepare for pathological and immunohistochemical (IHC) diagnosis.

2.2 Tissue Preparation

Ovarian tissue samples were prepared as paraffin embedded tissue according to the protocol described previously (21). Sections of 3 - 5 µm thickness were cut from paraffin blocks. Sections were submerged in water heated to 45°C for one minute. Sections were mounted on positively charged slides and allowed to cure at room temperature before being dewaxed in an oven for 30 min. at 80°C. To remove the paraffin, the portions were submerged in xylol for five minutes. The sections were submerged for two minutes in alcohol with a graded concentration of 95%, 70%, and 50% to rehydrate them. They were then washed with dH2O. All slides were put through the antigen retrieval stage by being submerged in a container of citrate buffer pH 6 at 98°C for 20 minutes. The slides were then dried at room temperature after being cleaned with dH_2O and chilled.

2.3 Preparation of Monoclonal Antibody

A human MMP-14 and rabbit recombinant OPN monoclonal antibody IgG isotype was diluted by mixing 10 µL of monoclonal antibody stock solution with 490 µL of blocking serum solution (supplied with 2-step Plus Poly-HRP Anti Rabbit/Mouse IgG detection system).

2.4 Immunohistochemistry Protocol

Tissue sections were deparaffinized in xylene and rehydrated with graded alcohol followed by staining with hematoxylin and eosin. After that the samples were prepared for immunohistochemical analysis to detect the expression of MMP14 by using anti-MMP14 antibody, RDEFNab5236 (MMP14 Antibody), recommended dilution: IHC: 1:100, isotype: IgG. While for osteopontin expression, anti- osteopontin antibody was used, RDEFNab06033 (OPN Antibody), recommended dilution: IHC: 1:50-1:200, isotype: IgG. The immunohistochemical reaction for MMP-14 and OPN was evaluated semi quantitatively according to the criteria in Table (1). The cell staining is distinguished with the formation of brown product and staining intensity was described as weak, moderate and strong positivity (22, 23).

Table 1: Scoring criteria for the expression of MMP14 and OPN in ovarian tissue by IHC analysis.

Staining description	Levels	Cell expression
-Staining of cells 0 to 5%. - No cytoplasmic staining	0	Negative
-Staining of cells 5 to 25%. - Weak cytoplasmic expression without membrane expression	+1	Weak positive or moderate
-staining of cells 25 to 75%. - Moderate cytoplasmic expression or incomplete membrane expression	+2	Moderate positive
-staining cells >75%. - Strong cytoplasmic expression or complete membrane expression	+3	Strong positive

2.5 Statistical Analysis

Variation scoring level for MMP14 and OPN expression was calculated using Chi-square test. Probability (*p*) value considered significant at level < 0.01 (**). Statistical analyses were performed using SPSS (version 19).

3. Results

3.1 Expression of MMP14

The expression of MMP14 in tissue sections collected from ovarian carcinoma was immunohistochemically detected using designated anti-MMP14 monoclonal antibodies. Out

of 65 samples, 5 (7.68%) samples exhibited negative expressions for MMP14 (3 HGSOC and 2 Synchronous). All the remaining samples showed MMP14 expressions at different levels. The majority, 43 samples (64.29%), showed strong positive immune staining with high intensity for anti-MMP14 (Table 2, Fig. 1) indicating strong cytoplasmic expression or complete membrane expression. Out of 43 strong positive samples, 20 (30%) belong to HGSOC, 7 (10%) belong to MOC, 5 (7.69%) belong to BSBT and the remaining 11 samples were distributed among other ovarian types. Only synchronous showed no strong positive MMP14 expression. Seventeen (25.84%) samples were indicated as moderate positive expression for MMP14 in the cytoplasm with incomplete membrane expression, 8 (12%) HGSOC, 6 (9.23%) EC and 3 (4.61%) CCC. No sample showed weak expression. The expression of MMP14 in the ovarian epithelium and stroma varied significantly ($p < 0.0001$) between different types of ovarian cancer.

Table 2: The MMP14 immunohistochemical staining levels and expression.

	Histopathology EOC	n	MMP14 Expression Negative	%	MMP14 Expression Positive						
					Week +1	%	Moderate +2	%	Strong +3	%	Positive %
1	HGSOC	**31**	3	4.61	0	0	8	12	20	30	42
2	EC	**8**	0	0	0	0	6	9.23	2	3	12.2
3	MOC	**7**	0	0	0	0	0	0	7	10	10
4	MOC borderline	**4**	0	0	0	0	0	0	4	6	6
5	CCC	**5**	0	0	0	0	3	4.61	2	3	7.61
6	BSBT	**5**	0	0	0	0	0	0	5	7.69	7.69
7	Synchronous	**2**	2	3.07	0	0	0	0	0	0	0
8	Benign tumour	**3**	0	0	0	0	0	0	3	4.6	4.6
	Total sample	**65**									

Chi-Square=46.95 DF=14 *P* Value<0.0001**

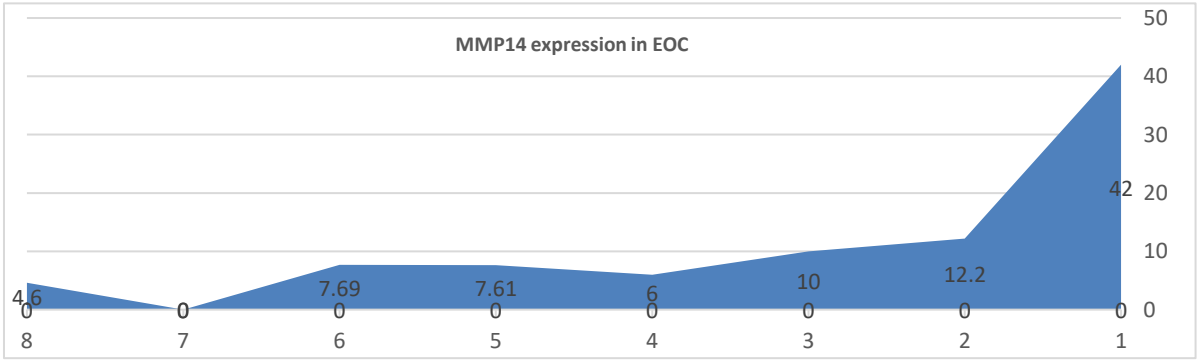
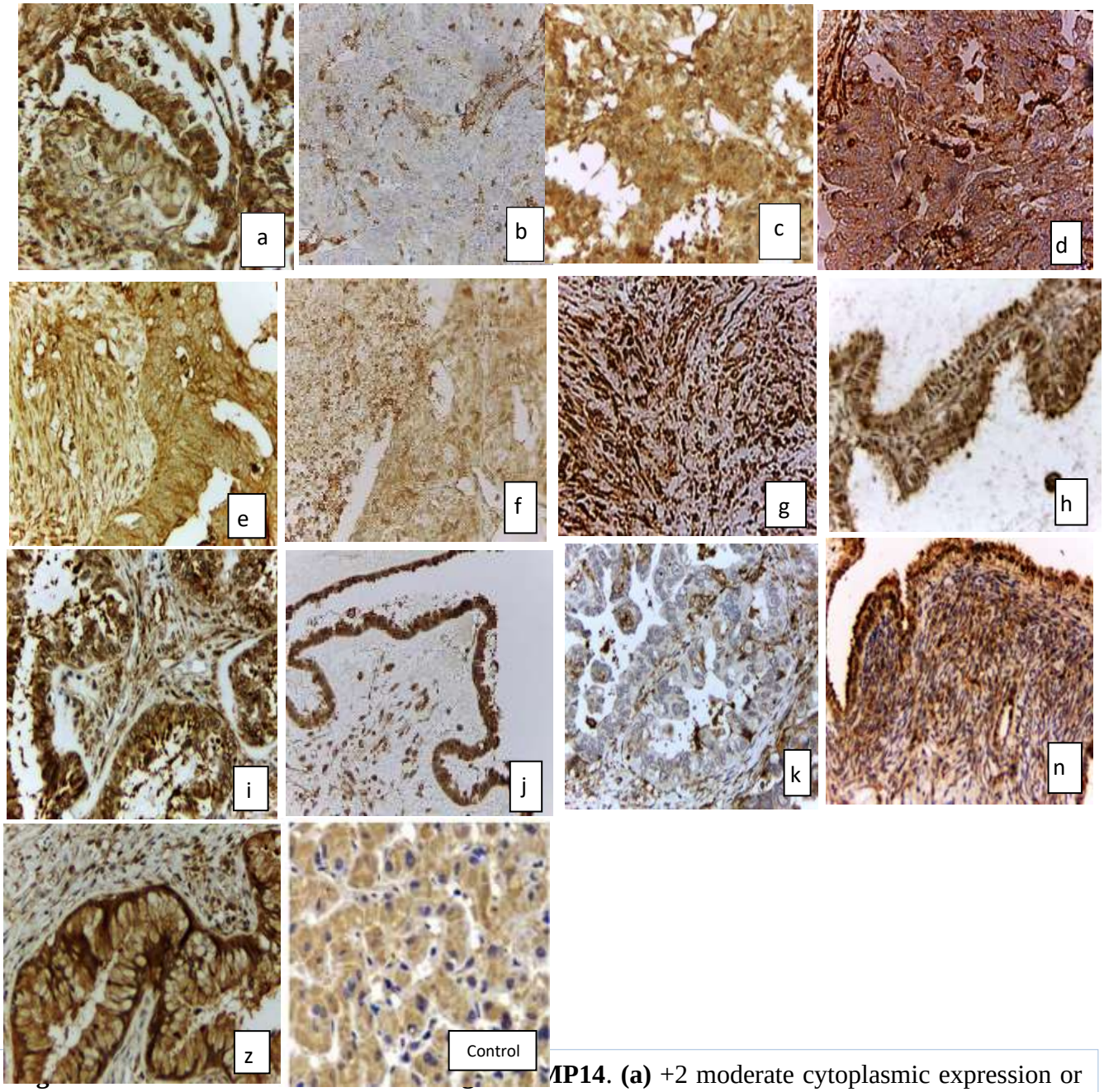


FIGURE 1: The positive expression of MMP14 in EOC area.



MP14. **(a)** +2 moderate cytoplasmic expression or incomplete membrane expression of mmp14 in HGSOC. **(b)** negative for staining in HGSOC. **(c, d**) +3 strong cytoplasmic expression or complete membrane expression. **(e)** +3 strong cytoplasmic expression or complete membrane expression in ECO. **(f)** +2 moderate cytoplasmic expression or incomplete membrane expression of mmp14 in ECO. **(g**) +2 moderate cytoplasmic expression or incomplete membrane expression of mmp14 in CCC. **(h)** +3 strong cytoplasmic expression or complete membrane expression in MOC. **(i)** +3 strong cytoplasmic expression or complete membrane expression in BSBT. **(j)** 2 moderate cytoplasmic expression or incomplete membrane expression of mmp14 in BSBT. **(k**) negative immune stain in synchronous**. (n-z)** +3 strong cytoplasmic expression or complete membrane expression in benign tumour. (magnification power ×40).

MMP14 is a protein that is involved in the breakdown of extracellular matrix proteins and plays a role in cancer progression and metastasis (24). It has been shown that MMP14 is expressed in ovarian, significance of MMP-14 as determined by immunohistochemistry. Poor prognosis and high MMP14 expression were both linked to aggressive ovarian cancer features (25). The immunohistochemical staining of MMP14 can provide insights into its expression levels and localization within different types of cancer cells (26). Higher expression of MMP14 correlates with increased ovarian cancer cell migration, invasion, and angiogenesis (27). The proteolytic activity of MMP14 on the extracellular matrix promotes tumor progression (28). HGSOC is the most common and aggressive subtype. Several studies have reported that MMP14 is more highly expressed in HGSOC compared to other subtypes at both the mRNA and protein levels (29). Immunohistochemistry analysis of clinical ovarian tumor samples demonstrated stronger MMP14 staining intensity in HGSOC versus endometrioid, clear cell and mucinous carcinomas (30). The increased expression of MMP14 in HGSOC positively correlated with advanced tumor stage, higher grade, and worse prognosis. *In vitro* experiments revealed MMP14 drives HGSOC cell invasion and proliferation more robustly than in other subtypes (31). In endometrioid ovarian carcinoma, high MMP14 expression is associated with advanced stage disease and correlates with poor progression-free and overall survival (32). Clear cell and mucinous ovarian carcinomas demonstrate variable MMP14 expression, with some evidence linking higher levels to increased invasiveness (33). The expression of MMP14 in benign ovarian tumors has not been extensively studied. However, it was indicated that MMP14 levels are low to absent in benign epithelial ovarian tumors. This contrasts with malignant ovarian cancers where MMP14 is often upregulated (33). Taken together, these findings suggest MMP14 is a key mediator of the aggressive clinical behavior of mostly all types of ovarian carcinoma types and represents a potential therapeutic target and prognostic biomarker specifically for this high-risk ovarian cancer subtype.

3.2 Expression of OPN

IHC results of ovarian cancer sections were indicated in Table (3) for the OPN expression using anti-OPN antibodies. Results showed that no sample exhibited negative staining or weak OPN expression. Only 12 samples (18.4%) showed moderate OPN cytoplasmic expression or incomplete membrane expression (Fig. 2), in which 3 (4.61%) samples belonged to HGSOC, 1 (1.53%) was EC, 5 (7.65%) were CCC and 3 (4.61%) were benign tumor. As results indicated, most of HGSOC samples (28) which represent 43% of total

samples displayed a strong positive OPN expression with high intensity in stained cells that showed cytoplasmic and complete membrane expression. EC type showed 7 (10.76%) strong positive, while both MOC and MOC border line had 4 (6.15%) strong positive samples. The type BSBT and synchronous, had 5 (7.69%) and 2 (3%) strong positive samples, respectively. The distribution of different OPN expression levels in ovarian carcinoma types showed significant variation ($p < 0.0001$).

Table 2: The OPN immunohistochemical staining levels and expression.

					OPN Expression Positive						
1	**Histopathology EOC**	**n**	**OPN Expression Negative**	**%**	**Week +1**	**%**	**Moderate +2**	**%**	**Strong +3**	**%**	**Positive %**
2	HGSOC	**31**	0	0	0	0	3	4.61	28	43	47
3	EC	**8**	0	0	0	0	1	1.53	7	10.76	12.7
4	MOC	**7**	3	4.61	0	0	0	0	4	6.15	6.15
5	MOC borderline	**4**	0	0	0	0	0	0	4	6.15	6.15
6	CCC	**5**	0	0	0	0	5	0	0	0	0
7	BSBT	**5**	0	0	0	0	0	0	5	7.69	7.69
8	Synchronous	**2**	0	0	0	0	0	0	2	3	3
9	Benign tumour	**3**	0	0	0	0	3	4.61	0	0	4.61
	Total samples	**65**									

Chi-Square=39.03 DF=7 *P* Value = <0.0001**

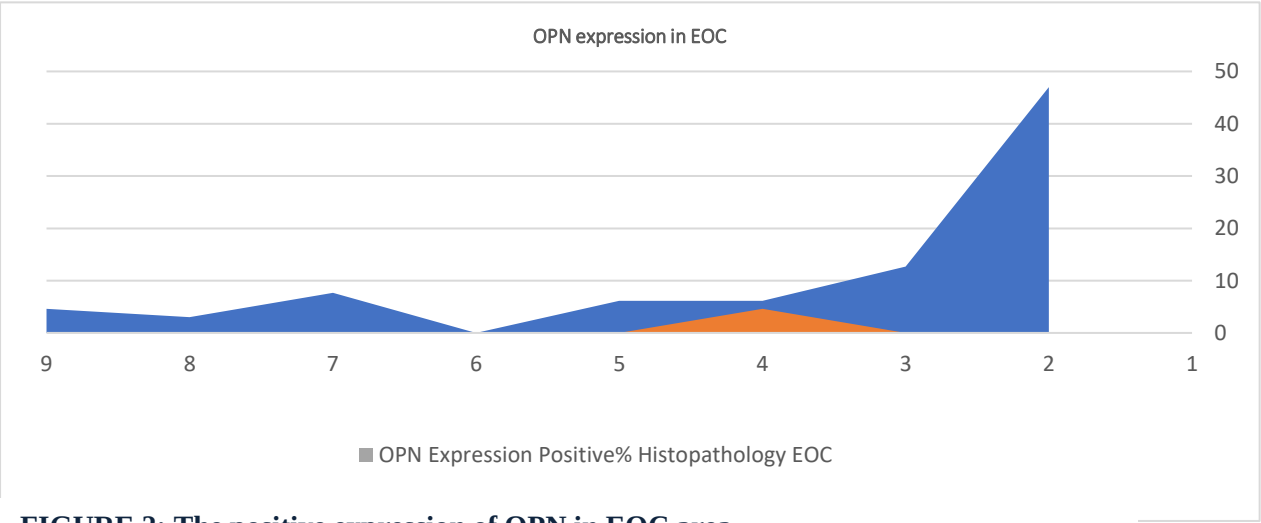


FIGURE 2: The positive expression of OPN in EOC area.

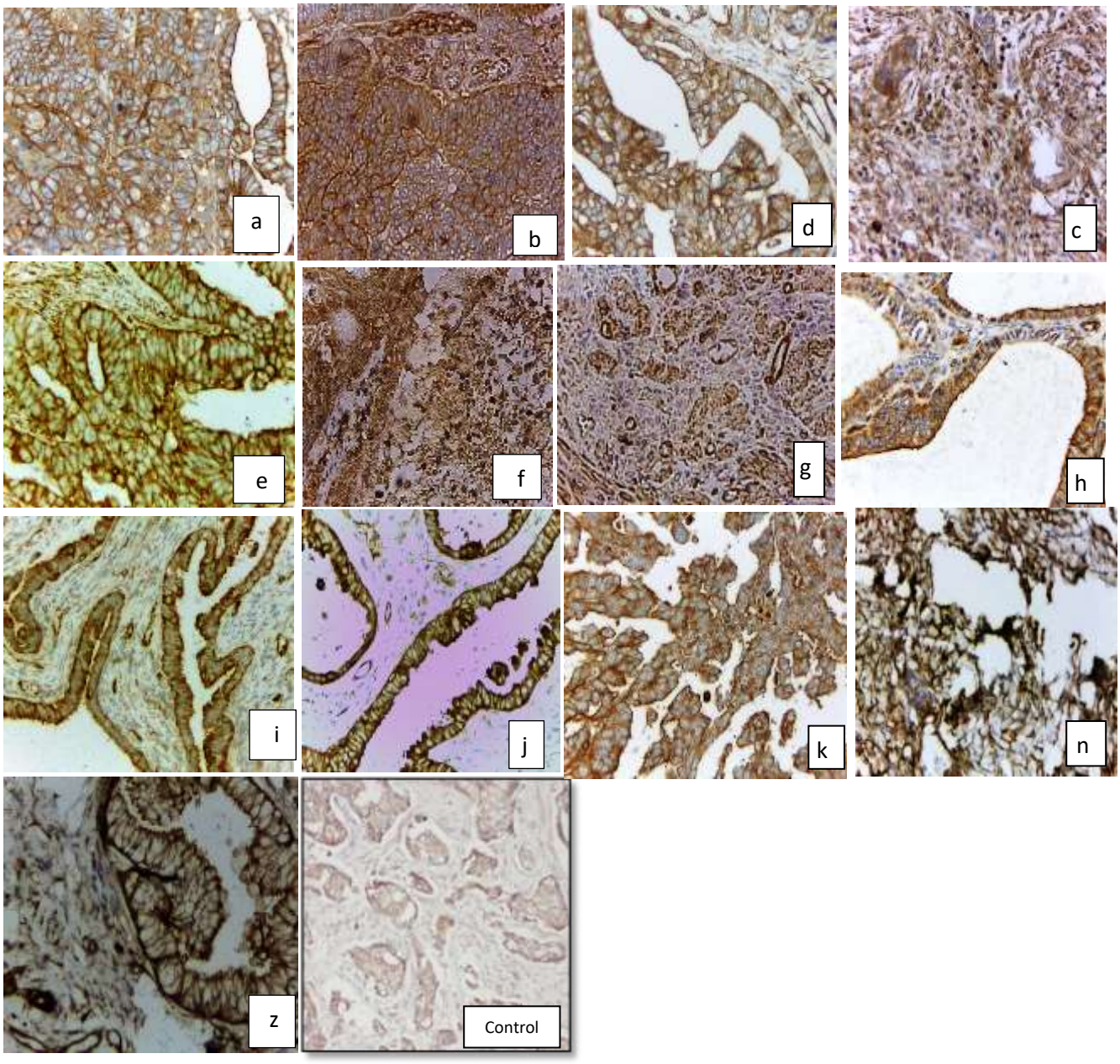


Figure 2: Immunohistochemical staining of OPN: (a. b .c) high expression of OPN in HGSOC +3 strong cytoplasmic expression or complete membrane expression. (d) +2 moderate cytoplasmic expression or incomplete membrane expression pf OPN in HGSOC. (e) +3 strong cytoplasmic expression or complete membrane expression OF OPN in ECO. (f) +2 moderate cytoplasmic expression or incomplete membrane expression in ECO. (g) +2 moderate cytoplasmic expression or incomplete membrane expression in CCC**. (**h) +3 strong cytoplasmic expression or complete membrane expression OF OPN in MOC. (i ,j) +3 strong cytoplasmic expression or complete membrane expression OF OPN in SBT .(k) strong cytoplasmic expression or complete membrane expression OF OPN in synchronous.(n ,z) benign tumours.(×40).

Osteopontin, a soluble protein found in all bodily fluids, participates in signaling pathways that regulate interactions between extracellular matrix and adhesion, which in turn affects a variety of cellular processes, including angiogenesis, inflammation, and tumor metastasis (34). Immunohistochemistry studies have demonstrated increased OPN protein expression in ovarian cancer tissues compared to normal ovarian epithelium (35). Additionally, higher OPN levels correlate with advanced tumor stage, metastasis, and poorer patient outcomes. Analysis of OPN expression across different ovarian cancer histological subtypes has revealed significant heterogeneity (36). HGSOC consistently display the highest OPN expression levels, which drive their aggressive phenotype (37). Clear cell and endometrioid ovarian cancers exhibit moderate OPN expression. All other subtypes of ovarian carcinoma were detected for positive expression of OPN including benign ovarian tumor. Only mucinous cancers have comparatively negative results for the expression of OPN (38). The molecular mechanisms inducing OPN overexpression specifically in the HGSOC subtype include copy number alterations of the OPN gene *SPP1,* and dysregulation of transcriptional networks that control OPN. Targeting OPN signaling pathways may provide therapeutic benefit, especially for the high-risk serous subtype (39).

Numerous studies have analyzed OPN expression levels in clinical ovarian cancer samples and consistently shown that HGSOC exhibits the highest levels of OPN positivity compared to other subtypes (40). Immunohistochemistry data indicates 60-90% of HGSOC samples display strong positive staining for OPN protein, whereas other subtypes like clear cell and endometrioid carcinomas exhibit only 10-30% OPN positivity (41). The high frequency of OPN overexpression in HGSOC positively correlates with advanced FIGO stage, tumor metastasis, platinum resistance, and reduced progression-free/overall survival (42). Mechanistically, OPN promotes HGSOC proliferation, invasion, and angiogenesis through binding integrin receptors and CD44 (43). These findings suggest OPN may serve as an important biomarker and therapeutic target in HGSOC. Targeting the oncogenic OPN pathway represents a promising approach for improving outcomes in this high-risk ovarian cancer subtype (44).

This study demonstrates significantly higher expression of MMP14 and OPN proteins in ovarian carcinoma. These data suggest MMP14 and OPN are potential biomarkers and

therapeutic targets that could improve prognosis and treatment of aggressive types of ovarian cancer like HGSOC.

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