

Expression and Subcellular Localization of Maspin in Human Ovarian Epithelial Neoplasms: Correlation with Clinicopathologic Features

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ABSTRACT

Background and Purpose: Maspin is an inhibitor of serine proteinases with tumor suppressor activity that is down-regulated in breast and prostate cancer, but over-expressed in pancreatic carcinoma. However, there were very few published data regarding the role of maspin in ovarian carcinoma. The aim of the present study was to evaluate maspin expression in ovarian epithelial neoplasms and correlate its expression with some clinicopathologic parameters.

Material and Methods: Seventy eight paraffin embedded ovarian specimens from patients with ovarian epithelial neoplasms comprised the material of this study. They included 18 benign, 14 low malignant potential (LMP) and 46 malignant epithelial ovarian neoplasms, in addition to seven specimens from normal ovarian tissues as a control.

Results: Immunohistochemical study of maspin expression using streptavidin biotin immunoperoxidase method revealed that, normal ovarian surface epithelium did not express maspin as well as benign serous and mucinous ovarian epithelial neoplasm. However, all benign Brenner ovarian tumors were maspin positive. On the other hand, 57.14% of LMP tumors showed weak maspin expression and 63% of malignant ovarian epithelial tumors showed maspin expression with 39.1% over expression. The two malignant Brenner tumors studied were maspin negative. There was a trend for maspin expression with high grade, high stage, bilateral tumors and tumors with metastasis. Tumors that showed maspin over-expression showed higher mitotic index (MI) ($p=0.02$). Invasive cancers were more likely to have predominantly cytoplasmic staining compared to LMP tumors.

Conclusion: Maspin was expressed in a substantial proportion of ovarian tumors with poor prognostic parameters. These results may offer new insights regarding the role of maspin in ovarian cancer that may also impact diagnosis and treatment strategies. Moreover, variation in maspin expression between Brenner tumor and other epithelial surface ovarian tumors may indicate that the

different histological types probably represent distinct disease entities and involve different molecular pathways.

Key Words: Maspin - Epithelial ovarian neoplasms - Immunohistochemistry.

INTRODUCTION

Although not the most common gynecologic malignancy, ovarian carcinoma is the most lethal. It is the fifth leading cause of cancer related deaths in females and accounts for approximately 50% of all deaths from gynecologic cancers [1]. There has been an association between ovarian cancer with the frequency of ovulation, the repeated proliferation of epithelial cells may increase the chance of a genetic accident that could contribute to the activation of an oncogene or inactivation of a suppressor gene [2]. Epithelial ovarian neoplasms span a histopathologic spectrum ranging from benign adenomas to borderline or low malignant potential tumors (LMP) to high grade invasive carcinomas. Genetic changes in tumors demonstrate different patterns that could be applied to judge their biologic nature, a process not always possible by histopathologic features [3]. Despite attempts at early diagnosis, 60% of patients have extrapelvic spread at the time of diagnosis because the cells have the ability to invade and metastasize [4].

Proteinases and proteinase inhibitors are known to play important roles in tumor invasion and metastasis. Proteinases degrade the extracellular matrix, while their inhibitors antagonize this process. Two classes of proteinases have been extensively studied; serine proteinases and

their inhibitors and metalloproteinases and their inhibitors [5].

Maspin (mammary serine protease inhibitor) is structurally a member of the serpine (serine protease inhibitors) superfamily [6]. Maspin encodes a 375 amino-acid protein (Mr of 42.000) with sequence homology to other inhibitory serpins. The reactive site loop, situated near the COOH terminus is one of the highly variable regions and is the domain that binds to the active site of the serine protease. The maspin gene is part of a serpin locus cluster at chromosome 18q21.3-q23. Several important transcription factor binding sites are present within the 1-Kb promoter region, namely Ets, Ap-1, HRF and R53 [7]. Studies have revealed that maspin is largely an intracellular protein that is soluble in the cytoplasm and is also found associated with secretory vesicles. Maspin is located at the cell membrane interface with the extracellular matrix and does not act as a classical inhibitory serpin with anti protease activity against trypsin-like proteases [8]. Rather small amounts of maspin are secreted but appear to remain associated with distinct structure at the cell surface [9].

Although, at present, the molecular and biological mechanisms of the function (s) of maspin remain largely unknown, there is evidence that maspin interacts with the P53 tumor suppressor pathway and may function as inhibitor to cell motility, invasion, metastasis and angiogenesis in vitro and in vivo [10]. Pemberton et al. [11], have demonstrated the presence of maspin in the epithelium of several normal human organs (such as prostate, thymus, testis, small intestine and colon) and particularly in the myoepithelium of breast tissue, in which it is predominant and probably function both intra and extracellularly. However, maspin was not detected in normal ovary in that study. On the other hand, Sheng and her colleagues [12] reported that maspin appears to have an effect on normal ovarian function, where they found that ovulation efficiency of maspin heterozygous mice is greatly reduced compared with controls. Thus we undertook the present study with the following aims: a) Evaluate the maspin expression in ovarian epithelial neoplasms as well as normal surface epithelium of the ovaries, b) Evaluate whether its expression may be a factor useful in predicting the prognosis of this neoplasm.

PATIENTS AND METHODS

I- Patient Selection:

Seventy eight surgical ovarian specimens were taken from patients who attended the Obstetric and Gynecology Department, Faculty of Medicine, Menoufiya University during the period between January 2000 and December 2003. The cases were randomly selected. They were 18 benign, 14 LMP and 46 malignant epithelial ovarian neoplasms. In addition, seven specimens of normal ovarian tissue from patients who underwent total abdominal hysterectomy with salpingo-oophorectomy for other causes than ovarian tumors were obtained.

Clinical data such as; age, parity family history, presence of ascites, bilaterality, presence of metastasis, type of operation and adjuvant therapy as well as the patients' follow up data were obtained from the patients' medical records.

CA125 and carcinoembryonic antigen (CEA) were performed for the malignant cases. For CA125, values above 35u/ml were considered abnormal and for CEA serum level above 5.1mg/ml were considered abnormal.

II- Histopathological Examination:

Histopathological examination of surgical specimens was performed at the Pathology Department, Faculty of Medicine, Menoufiya University. The gross appearance of tumors and presence of associated endometrial lesions were assessed. Paraffin sections of all cases were routinely stained with hematoxylin & eosin (H&E) to diagnose the cases and for malignant tumors to determine the histologic tumor type and grades according to the criteria of WHO. All the patients were staged according to the International Federation of Gynecology & Obstetric (FIGO), surgical staging system. Mitotic and apoptotic indices (MI & AI) were also calculated [13,14].

Immunohistochemistry (IHC):

Formalin-fixed, paraffin-embedded tissues were sectioned at 4µm to confirm the previous diagnosis and then stained with IHC. The steps of IHC are summarized as follows:

All the slides were deparaffinized using xylene, 100% then 95% ethanol followed by a thorough deionized water wash. A water bath

antigen-recovery technique using citrate buffer (PH=6.0) was performed on all slides. The IHC staining for maspin was performed by mouse antihuman maspin clone EAW24, IgG2a, Kappa (Dako, Copenhagen). The antigens used for immunostains is prokaryotic recombinant protein corresponding to the N-terminal region of the human maspin molecule (dilution 1:50). After deparaffinization and antigen recovery, slides were washed in trisbuffered saline and Tween 20 (TBST). Three blocking steps were applied: 0.03% hydrogen peroxide (Dako, Copenhagen) for 15 mins, followed by TBST wash, avidin and biotin blocks were applied for 15 mins followed by a TBST wash and finally the protein block serum-free was applied for 15 mins. The primary antibody was applied to the slides and incubated for 60 mins, slides were rinsed in TBST. This procedure was followed by incubation with an avidin-biotin peroxidase complex (ABC) reagent for 20 mins. Colour was produced by using Diamynobenzidine (DAB) (brown) substrate (Dako, Copenhagen) for 3 mins. Slides were counterstained with Mayer's hematoxylin for 5-10 mins.

Evaluation of IHC:

Maspin expression was determined by assessing semiquantitatively the percentage of stained cells, the staining intensity as well as the subcellular localization [10].

- The percentage of positive cells was rated as follows: 0 point; no +ve cells. 1 point; 0-5%. 2 points; 6-50%. 3 points >50% positive cells.
- The staining intensity was rated as follows: 1 point; weak intensity. 2 points; moderate intensity. 3 points; strong intensity.
- Points for intensity and percentage of positive cells were added and an overall maspin score (OMS) (0-3) was assigned. Lesions were categorized into four groups [10]: negative (OMS=0): <5% stained cells regardless of intensity, weak expression (OMS=1): 3 points, moderate expression (OMS=2): 4-5 points, strong expression (OMS=3): 6 points.

OMS 2 & 3 were considered as over-expression.

- The subcellular localization of staining was assessed; whether the maspin expression was localized to cytoplasm, nucleus or both.

III- Statistical Analysis:

Statistical analysis was performed using an Epi Info computer package for statistics. Chi square test (X^2), fisher's exact test and non parametric methods were used. Also, survival analysis was done to study survival distribution with maspin expression using Kaplan Meier survival curve for overall survival. p value ≤ 0.05 was considered statistically significant [15].

RESULTS

This study was carried out on 78 patients with ovarian epithelial neoplasms; 18 were benign, 14 were LMP and 46 were malignant epithelial ovarian neoplasms. In addition 7 normal ovarian tissues as a control group were included.

All the patients underwent surgical treatment. For the malignant cases studied cytoreduction was done as the initial treatment then the treating gynecologic oncologist determined the adjuvant therapy.

The age of patients with benign epithelial ovarian tumors ranged from 18 years (ys) to 60ys with a mean \pm S.D. of 48.43 ± 12.46 ys and a median of 50.0ys. The age of LMP studied cases ranged from 30 to 52ys with a mean of 39.07 ± 6.49 and median of 39.50ys. The age of the malignant group ranged from 26 to 85ys with a mean of 52.76 ± 12.64 and a median of 51.5ys. However, there was no statistically significant difference between the three groups regarding the mean age of the studied cases. The clinicopathologic data of the studied cases demonstrated in tables (1,2,3).

Follow up data were obtained for the studied malignant cases and ranged from 1 to 30 months (ms) with a mean of 13.064 ± 4.68 ms. Five cases died of the disease at 3,4,7,9 & 21ms.

Regarding the normal ovarian tissue studied, the age of patients ranged from 38 to 60ys with a mean of 49.29 ± 6.97 ys. All the patients were multiparous and complaining of causes other than ovarian neoplasms that indicated total abdominal hysterectomy with salpingo-oophorectomy; 6 were complaining of vaginal bleeding (adenomatous endometrial hyperplasia) and one was complaining of abdominal mass (multiple fibroids).

Immunohistochemistry:

Normal ovarian surface epithelial cells did not express maspin (7 cases). The same result was exhibited by benign serous (7 cases) and mucinous (5 cases) epithelial tumors of the ovary whereas the 6 Brenner tumor cases studied showed moderate to strong maspin expression. On the other hand, 8/14 (57.14%) of LMP tumors showed weak positive expression of maspin (5/8 of serous tumors and 3/6 of mucinous tumors). Among the malignant ovarian epithelial tumors; 29/46 (63.0%) were ranked positive for maspin and 18/46 (39.1%) over-expressed maspin (OMS 2 & 3). The difference in maspin expression between the three groups studied with the exclusion of Brenner tumors (benign, LMP and malignant) were highly significant as well as regarding the maspin intensity between LMP and malignant group ($p=0.001$) (Table 4). The two malignant Brenner tumor cases studied showed negative maspin expression (Table 5).

Subcellular Localization:

- All benign Brenner tumors showed cytoplasmic expression of maspin (6 cases).
- In LMP tumors, 3/8 (37.5%) of positive cases showed cytoplasmic expression; whereas 5/8

(62.5%) showed both cytoplasmic and nuclear expression.

- In malignant cases studied, 20/29 (68.96%) showed only cytoplasmic expression; whereas 8/29 (27.58%) showed both cytoplasmic and nuclear expression and only 1/29 (3.44%) showed nuclear maspin expression.

Correlation between Maspin Expression and Clinicopathologic Data in the Studied Group:

In borderline cases, only bilaterality of the tumor showed trend for positive maspin expression in serous and mucinous epithelial ovarian tumors, where all the cases that showed bilateral tumors were positive for maspin expression (Table 6). As regards malignant cases, 70.8% of positive cases were more than 50ys of age and the mean age was higher in positive cases than that with negative maspin expression (54.9ys versus 49.0ys) but the difference was statistically insignificant ($p=0.25$). 56.3% of bilateral tumors, 62.1% of cases with positive metastasis, 56.8% of high stage tumors and 62.5% of high grade tumors were positive for maspin expression but the differences did not reach significant values (Table 7).

Table (1): Clinicopathologic data of the studied benign ovarian epithelial tumors.

Variables	Serous (7)	Mucinous (5)	Brenner (6)	<i>p</i> value
<i>Age No. (%)</i> :				
≤50	4 (33.3)	4 (33.3)	4 (33.3)	0.830
>50	3 (50)	1 (16.7)	2 (33.3)	
<i>Parity No. (%)</i> :				
Nullipara	1 (100)	–	–	0.999
Multipara	6 (35.3)	5 (29.4)	6 (35.3)	
<i>Associated endometrial lesion No. (%)</i> :				
–ve	2 (22.2)	3 (33.3)	4 (44.4)	0.496
+ve	5 (55.6)	2 (22.2)	2 (22.2)	
<i>Bilaterality No. (%)</i> :				
–ve	5 (33.3)	4 (26.7)	6 (40)	0.588
+ve	2 (66.7)	1 (33.3)	–	
<i>Symptoms No. (%)</i> :				
Abdominal mass	2 (25)	3 (37.5)	3 (37.5)	0.456
Bleeding	5 (62.5)	2 (25)	1 (12.5)	
Pain	–	–	2 (100)	

Test of significance: Fisher test exact.
p values ≤0.05 are considered significant.

Table (2): Clinicopathologic data of the studied borderline ovarian epithelial neoplasms.

Variables	Serous (8)	Mucinous (6)	<i>p</i> value
<i>Age No. (%)</i> :			
≤50	7 (58.3)	5 (41.7)	0.857
>50	1 (50)	1 (50)	
<i>Parity No. (%)</i> :			
Nullipara	–	–	
Multipara	8 (57.1)	6 (42.9)	
<i>Associated endometrial lesions No. (%)</i> :			
–ve	8 (57.1%)	6 (42.9)	
+ve	–	–	
<i>Bilaterality No. (%)</i> :			
–ve	6 (54.5)	5 (45.5)	0.769
+ve	2 (66.7)	1 (33.3)	
<i>Symptoms No. (%)</i> :			
Abdominal mass	8 (57.1)	6 (42.9)	
Bleeding	–	–	
Pain	–	–	

Test of significance: Fisher Free-man-Halton exact.
p values ≤0.05 are considered significant.

Table (3): Clinicopathologic data of the studied malignant epithelial ovarian neoplasms.

Variables	Serous (21)	Mucinous (13)	Endometrioid (5)	Malig. Brenner (2)	Undifferentiated ca (5)	<i>p</i> value
<i>Age No. (%)</i> :						
≤50	10 (45.5)	6 (27.3)	1 (4.5)	1 (4.5)	4 (18.2)	0.513
>50	11 (45.8)	7 (29.2)	4 (16.7)	1 (4.5)	1 (4.2)	
<i>Parity No. (%)</i> :						
Nullipara	–	1 (50)	–	–	1 (50)	0.252
Multipara	21 (47.7)	12 (27.3)	5 (11.4)	2 (4.5)	4 (9.1)	
<i>Family history No. (%)</i> :						
–ve	21 (45.7)	13 (28.3)	5 (10.9)	2 (4.3)	5 (10.9)	
+ve	–	–	–	–	–	
<i>Symptoms No. (%)</i> :						
Abdominal mass	18 (43.9)	11 (26.8)	5 (12.2)	2 (4.9)	5 (12.2)	0.999
Bleeding	2 (66.7)	1 (33.3)	–	–	–	
Pain	1 (50)	1 (50)	–	–	–	
<i>CEA No. (%)</i> :						
Normal	11 (32.4)	12 (35.3)	5 (14.7)	2 (5.9)	4 (11.8)	0.049
Elevated	10 (83.3)	1 (8.3)	–	–	1 (8.3)	
<i>CA125 No. (%)</i> :						
Normal	9 (42.9)	10 (47.6)	2 (9.5)	–	–	0.018
Elevated	12 (48.0)	3 (12.0)	3 (12.0)	2 (8.0)	5 (20.0)	
<i>Assoicated endometrial lesions No. (%)</i> :						
–ve	18 (58.1)	6 (19.4)	1 (3.2)	2 (3.2)	5 (16.1)	0.004
+ve	3 (21.4)	7 (50)	4 (28.6)	–	–	
<i>Bilaterlity No. (%)</i> :						
–ve	8 (57.1)	4 (28.6)	2 (14.3)	–	–	0.525
+ve	13 (40.6)	9 (28.1)	3 (9.4)	2 (6.5)	5 (15.6)	
<i>Ascites No. (%)</i> :						
–ve	8 (50)	6 (37.5)	2 (12.5)	–	–	0.403
+ve	13 (43.3)	7 (23.3)	3 (10)	2 (6.7)	5 (16.7)	
<i>Presence of metastasis No. (%)</i> :						
–ve	5 (50)	3 (30)	–	1 (10)	1 (10)	0.716
+ve	16 (44.4)	10 (27.8)	5 (13.9)	1 (2.8)	4 (11.1)	
<i>LN No. (%)</i> :						
–ve	15 (45.5)	12 (36.4)	3 (9.1)	1 (3)	2 (6.1)	0.114
+ve	6 (46.2)	1 (7.7)	2 (15.4)	1 (7.7)	3 (23.1)	
<i>Stage No. (%)</i> :						
I & II	5 (55.6)	4 (44.4)	–	–	–	0.508
III & IV	16 (43.2)	9 (24.3)	5 (13.5)	2 (5.4)	5 (13.5)	
<i>Grade No. (%)</i> :						
I	4 (36.4)	7 (63.6)	–	–	–	0.024
II	5 (45.5)	4 (36.4)	2 (18.2)	–	–	
III	12 (50)	2 (8.3)	3 (12.5)	2 (8.3)	5 (20.8)	
<i>MI:</i>						
Mean ± S.D.	*,‡2.095± 868	*,‡1.384± 1.325	*,‡2.400± 1.1402	6.000± 1.414	5.600± 1.1402	**16.96 <i>p</i> =0.002
Minimum	0.00	0.00	1.00	5.00	4.00	
Maximum	5.00	4.00	4.00	7.00	7.00	
<i>AI:</i>						
Mean ± S.D.	#2.809± 2.015	1.5385± 1.3914	#4.200± 0.836	1.500± 0.7071	#3.600± 2.302	**10.40 <i>p</i> =0.034
Minimum	0.00	0.00	3.00	1.00	0.00	
Maximum	6.00	4.00	5.00	2.00	6.00	

MI : Mitotic index.

AI : Apoptotic index.

L.N. : Lymph node.

CEA : Carcinoembryonic antigen.

*: Significant than malignant Brenner.

‡: Significant than malignant Brenner.

#: Significant than undifferentiated carcinoma.

**: X² (for Kruskal Wallis test).*p* values ≤0.05 are considered significant.

Table (4): Maspin expression in the studied cases with the exclusion of Brenner tumors.

Variables	Benign (12)	LMP (14)	Malignant (44)	<i>p</i> -value
<i>Expression No. (%):</i>				
-ve	12 (36.4)	6 (18.2)	15 (45.5)	<0.001
+ve	–	8 (21.8)	29 (78.4)	
<i>Staining intensity of positive cases No. (%):</i>				
Weak	–	8 (72.7)	3 (27.3)	<0.001
Moderate	–	–	13 (100)	
Strong	–	–	13 (100)	
<i>OMS No. (%):</i>				
0	12 (36.4)	6 (18.2)	15 (45.5)	<0.001
1	–	8 (42.1)	11 (57.5)	
2	–	–	7 (100)	
3	–	–	11 (100)	
<i>Subcellular localization No. (%):</i>				
Cytoplasmic	–	3 (13)	18 (87)	0.401
Nuclear	–	–	1 (100)	
Both	–	5 (38.5)	10 (61.5)	

OMS: Overall maspin score.

p values ≤0.05 are considered significant.

Table (5): Maspin expression in malignant epithelial ovarian neoplasms.

Variables	Serous (21)	Mucinous (13)	Endometrioid (5)	Malig. Brenner (2)	Undifferentiated ca (5)	<i>p</i> -value
<i>Expression No. (%):</i>						
-ve	9 (52.9)	4 (23.5)	–	2 (11.8)	2 (11.8)	0.153
+ve	12 (41.4)	9 (31)	5 (17.2)	–	3 (10.3)	
<i>Staining intensity of positive cases No. (%):</i>						
Weak	3 (100)	–	–	–	–	0.324
Moderate	5 (38.5)	5 (38.5)	3 (23.1)	–	–	
Strong	4 (30.8)	4 (30.8)	2 (15.4)	–	3 (23.1)	
<i>OMS No. (%):</i>						
0	9 (60.0)	4 (23.5)	–	2 (11.8)	2 (11.8)	0.219
1	5 (45.45)	3 (27.27)	3 (27.27)	–	–	
2	3 (42.85)	2 (28.57)	2 (28.57)	–	–	
3	4 (36.4)	4 (36.4)	–	–	3 (27.3)	
<i>Subcellular localization No. (%):</i>						
Cytoplasmic	7 (38.9)	6 (33.3)	5 (27.8)	–	–	0.041
Nuclear	–	1 (100)	–	–	–	
Both	5 (50)	2 (20)	–	–	3 (30)	

OMS: Overall maspin score.

p values ≤0.05 are considered significant.

Table (6): Comparison between maspin expression and clinicopathologic parameters in borderline tumors.

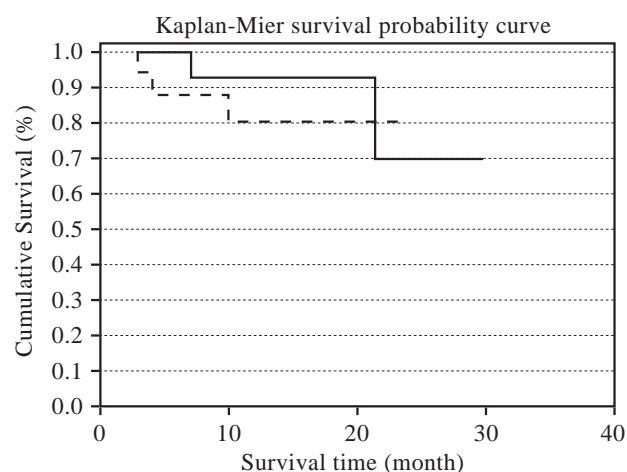
Variables	Maspin expression		<i>p</i> -value
	-ve	+ve	
<i>Age No. (%):</i>			
≤50	5 (41.7)	7 (58.3)	0.999
>50	1 (50)	1 (50)	
<i>Bilaterality No. (%):</i>			
-ve	6 (54.5)	5 (45.5)	0.208
+ve	–	3 (100)	
<i>Type No. (%):</i>			
Serous	4 (50)	4 (50)	0.627
Mucinous	2 (33.3)	4 (66.7)	

p values ≤0.05 are considered significant.

Table (7): Comparison between maspin expression and clinicopathologic prognostic parameters in malignant epithelial ovarian tumors studied.

Variables	Maspin expression		Test of significance	p-value
	-ve (17)	+ve (29)		
Age No. (%):				
≤50	10 (45.5)	12 (54.5)	Fisher exact test	0.274
>50	7 (29.2)	17 (70.8)		
CEA No. (%):				
Normal	11 (32.4)	23 (67.6)	Fisher exact test	0.314
Elevated	6 (50)	6 (50)		
CA 125 No. (%):				
Normal	11 (52.4)	10 (47.6)	Fisher exact test	0.067
Elevated	6 (24)	19 (76)		
Bilaterality No. (%):				
-ve	3 (21.4)	11 (78.6)	Fisher exact test	0.194
+ve	14 (43.8)	18 (56.3)		
Ascites No. (%):				
-ve	2 (12.5)	14 (87.5)	Fisher exact test	0.023
+ve	15 (50)	15 (50)		
Presence of metastasis No. (%):				
-ve	3 (30.0)	7 (70)	Fisher exact test	0.722
+ve	14 (38.9)	22 (62.1)		
LN No. (%):				
-ve	10 (30.3)	23 (69.7)	Fisher exact test	0.181
+ve	7 (53.8)	6 (46.2)		
Stage No. (%):				
I & II	1 (11.1)	8 (88.9)	Fisher exact test	0.124
III & IV	16 (43.2)	21 (56.8)		
Grade No. (%):				
I	5 (45.5)	6 (54.5)	(X ²) 0.787	0.668
II	3 (27.3)	8 (72.7)		
III	9 (37.5)	15 (62.5)		
MI:				
Mean ± S.D.	2.588±2.399	2.413±1.918	*0.12	0.991
AI:				
Mean ± S.D.	2.294±1.723	2.827±2.01	*0.877	0.381

MI : Mitotic index. p values ≤0.05 are considered significant.
 AI : Apoptotic index. *: Mann Whitney (U test).
 L.N.: Lymph.



Curve (1): Kaplan Meier survival probability curve by maspin expression in malignant epithelial ovarian tumors.

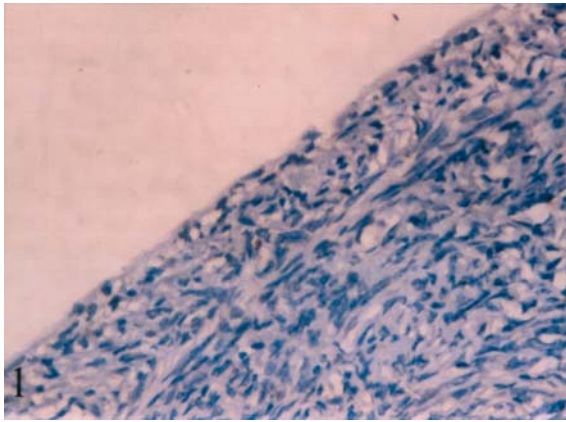


Fig. (1): Normal surface epithelium of the ovary showing negativity for maspin expression. Immunoperoxidase (DAB) X 200.

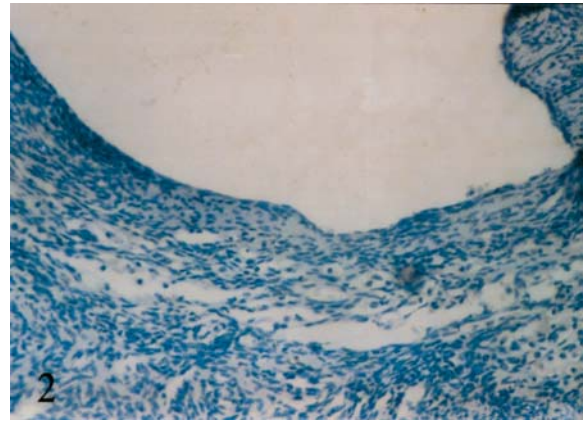


Fig. (2): Wall of benign serous cystadenoma showing negativity for maspin immunohistochemistry. Immunoperoxidase (DAB) X 100.

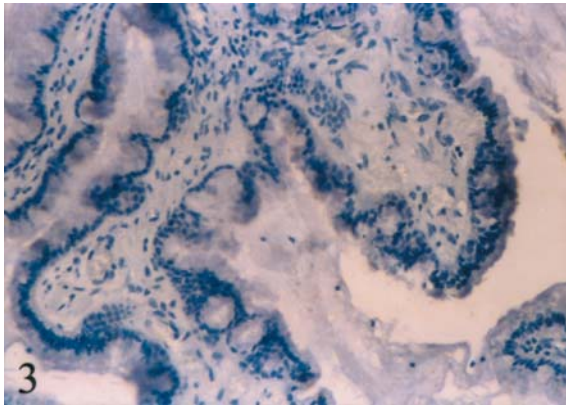


Fig. (3): Benign mucinous cystadenoma showing negativity for maspin expression. Immunoperoxidase (DAB) X 200.

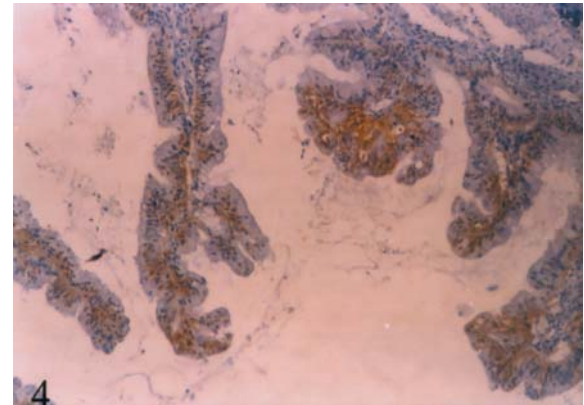


Fig. (4): Mucinous tumor of low malignant potential, showing positivity for maspin expression. Immunoperoxidase (DAB) X 100.

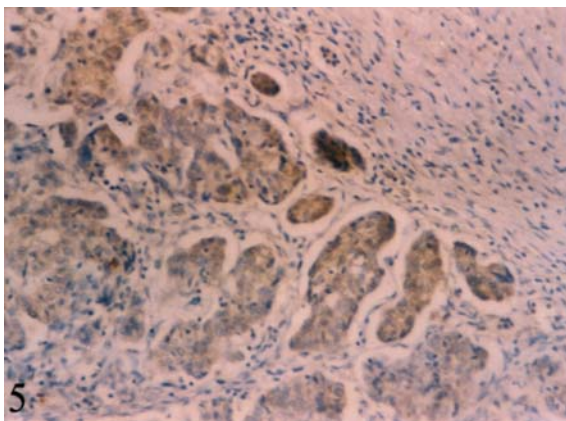


Fig. (5): Papillary serous cystadenocarcinoma showing positivity for maspin staining. Immunoperoxidase (DAB) X 100.

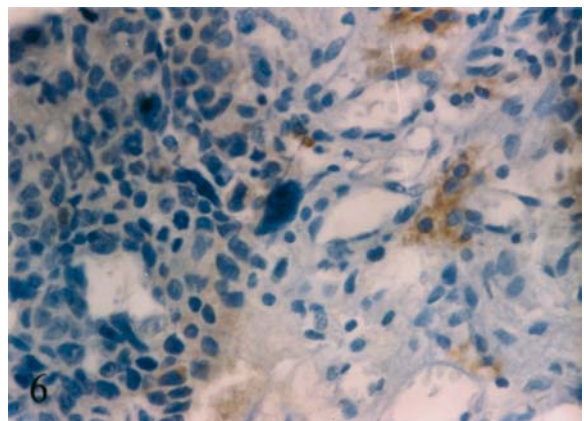


Fig. (6): Mucinous cystadenocarcinoma showing cytoplasmic maspin expression. Immunoperoxidase (DAB) X 400.

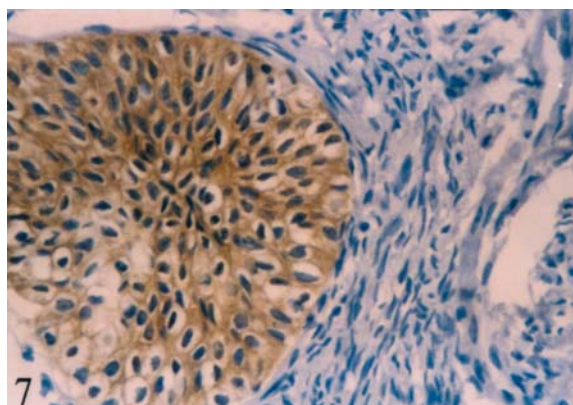


Fig. (7): Benign Brenner tumor showing strong maspin expression. Immunoperoxidase (DAB) X 400.

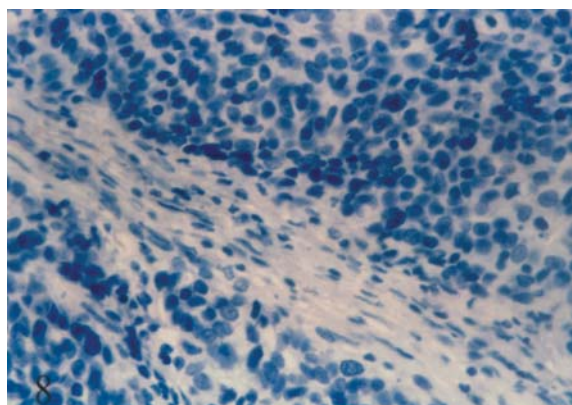


Fig. (8): Malignant Brenner tumor showing negativity for maspin immunohistochemistry. Immunoperoxidase (DAB) X 400.

Regarding the survival analysis, no significant difference was observed between patients with negative maspin expression and those with positive expression (curve 1).

61.1% of bilateral tumors, 77.8% of cases with metastasis, 77.8% of high stage tumors, 61.1% of high grade tumors, 55.6% of patients with ascites showed over-expression of maspin (OMS 2 & 3). In addition, tumors that exhibited maspin over-expression showed higher MI (2.66 ± 2.19 versus 1.21 ± 1.3 & $p=0.02$), higher AI (3.05 ± 2.18 versus 1.57 ± 1.74 & $p=0.02$) and shorter follow up period than that of other positive cases (10.029 ± 7.89 versus 13.50 ± 11.50 & $p=0.304$). Of the 5 patients who died of the disease, 2 cases were negative for maspin and 3 were positive (2 were OMS 1 and 1 was OMS 3).

DISCUSSION

In 1997, Pemberton and his colleagues [11], demonstrated the presence of maspin in the epithelium of several normal human organs; however, maspin was not detected in normal ovary in that study. This goes with our results, where maspin was not detected in normal ovarian epithelium (7 cases). Also, Maass et al. [16], reported that maspin expression was not detected in normal pancreatic tissues. On the other hand, Sood et al. [10], reported weak expression of maspin in normal ovarian epithelium.

In the present study, maspin was not detected in benign serous and mucinous epithelial ovarian

tumors but it was expressed in 57.1% of LMP serous and mucinous tumors where they showed weak expression. On the other hand, malignant cases studied showed maspin expression in 63% and over-expression in 39.1%. As over expression was only detected in malignant cases studied, this may serve as a useful marker for detection of ovarian cancer and provide an aid as a diagnostic tool in ovarian epithelial tumors. This confirms the data of other groups [10] that maspin is, in contrast to other tumor entities, over expressed in ovarian cancer.

Ovarian cancer continues to result in a high mortality rate among gynecological malignancies. This is reflective of the advanced stage at which most patients present [1]. In the present study, there was a trend for maspin expression in tumors with high stage, high grade, bilateral tumors, tumors with metastasis and older patients but with no significant values. Also, tumors that showed maspin over-expression had higher MI ($p=0.02$) and shorter follow up period. Moreover, Sood et al. [10] found a positive correlation of maspin expression with advanced tumors. Therefore, maspin expression may have a role in ovarian cancer progression.

In the present study, there was a positive correlation between maspin over-expression and AI ($p=0.02$). A previous study had demonstrated that maspin sensitize breast carcinoma cells to induce apoptosis as judged by increased fragmentation of DNA increased proteolytic inactivation of poly-(ADP-ribose) polymerase (PARP) as well as increased activation of caspase-3. Taken together, these provide new

insights into the complex molecular mechanisms of maspin [17].

As regards the survival analysis, no significant difference was observed between patients with negative and those with positive maspin expression. This could be due to the fact that most of our patients were lost for follow up after a short period of time. Therefore, it was not considered as a reliable predictor of prognosis.

The surface epithelium of the ovary is mesodermal in origin, the same origin of the Mullerian duct. This explains the Mullerian like differentiation observed in surface epithelial ovarian tumors (e.g. tubal, endometrioid and endocervical mucinous differentiation). Several studies indicated that the different histologic types of ovarian neoplasms probably represent distinct disease entities and involve different molecular pathways [1]. Brenner tumors are characterized by dense fibrous stroma and nests of transitional cells resembling urinary transitional or rarely columnar epithelium [18]. The most interesting result in this study was that benign Brenner tumors (6 cases) showed strong maspin expression in contrast to the other benign surface epithelial tumors studied (serous and mucinous). To our knowledge to date, there are no published reports evaluating maspin expression in various surface epithelial tumors. However, previous studies reported positivity of Brenner tumors with other markers like uroplakin (urothelial specific proteins) and thrombomodulin, where these markers were not expressed in other benign and malignant lesions or normal tissues of the female genital tract [19,20,21]. These results support the hypothesis that the Brenner tumor represents true urothelial (transitional cell) differentiation. Therefore, this could explain the controversy in maspin expression in Brenner tumor cases studied and other surface epithelial ovarian tumors. So, understanding the molecular basis of each morphological type of surface epithelial ovarian tumor and its biological behavior is very important and will eventually lead to the development of more specific and effective treatments for ovarian neoplasms [22].

In 2004, Friedrich and his colleagues [23] reported that normal transitional epithelium expressed maspin, whereas maspin was lost with progression to transitional cell carcinoma.

This goes with our findings in Brenner tumors where maspin was expressed in benign Brenner and lost in malignant Brenner cases studied. Therefore, our results may indicate that maspin expression in Brenner tumor could act as a tumor suppressor gene as it was reported in transitional carcinoma of the bladder [23]. As the result on Brenner tumor of the ovary was an unexpected finding, so another study on a large number of cases will be carried out trying to understand the biological characteristics of these tumors.

It is possible that the subcellular localization of maspin may play a critical role in its biological function [10]. In this study, 57.1% of the LMP tumors of the ovary expressed maspin; 62.5% of them showed both cytoplasmic and nuclear expression and 37.5% showed only cytoplasmic expression. However, the majority of invasive ovarian cancers expressed cytoplasmic maspin expression (68.96%). The cytoplasmic localization of maspin in invasive tumors was reported by Sood et al. [10], and they speculated that maspin expression in these tumors may be inactive and supported their finding by the data with SKOV3 cell line where they noticed that invasion was not affected by an inhibitory antibody. However, definite proof for this hypothesis will require a functional assay of maspin activity, which is presently unavailable.

Although at present, the molecular and biological mechanisms of maspin function are unknown, several authors adhere to the hypothesis that maspin functions at the level of invasion and metastasis by blocking tumor cell migration and proliferation [24,25,26]. The finding of Maass et al. [16], showing the up regulation of maspin in pancreatic cancer and the results of Sood et al. [10], demonstrating the up regulation of maspin in ovarian cancer as well as our findings, provide new information about factors that regulate tumor cell development and progression.

In conclusion, we have demonstrated that maspin may play a role in the development and/or progression of ovarian cancer. Its relationship to carcinoma of ovarian epithelium opens a new field of discussion on its function in cancer. Moreover, variation in maspin expression between Brenner tumors and other surface epithelial ovarian tumors may indicate that the

different histological types of ovarian epithelial tumors probably represent distinct disease entities and involve different molecular pathways. Therefore, understanding the molecular basis of each morphologic type and its biological behavior is very important.

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