Since 2002

OPEN ACCESS

# The Role of Maspin Expression as Diagnostic Tissue Marker in Pancreaticoduodenal Malignant Tumors and Benign Lesions

Yasmine ElEsawy , Eman Khaled , Badawea Biomy , Samar Elsheikh , Dina El-Yasergy*

*Department of Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt*

## Abstract

**BACKGROUND:** Maspin (a tumor suppressor gene) is down-regulated in breast, prostate, gastric, and melanoma. Although it is not detected in normal pancreatic tissue, it is over-expressed in pancreatic cancer suggesting that maspin may play different activities in different cell types. Pancreatic ductal adenocarcinoma (PC) acquires maspin expression through hypomethylation of its promoter.

**AIM:** Because the discrimination between ampullary and periampullary carcinomas is challenging in advanced cases, this inspired us to search for the use of maspin expression to discriminate between ampullary carcinoma (AC), PC, duodenal adenocarcinoma (DC), and other confusing benign and inflammatory pancreatic lesions.

**METHODS:** Immunostaining for maspin was performed for 80 pancreaticoduodenal lesions. Sixty cases were malignant: 48 cases of pancreatic epithelial tumor (41 PC and 7 solid pseudopapillary neoplasm), 9 AC, and 3 DC. Twenty cases were non-malignant: 12 inflammatory (chronic pancreatitis), 5 benign neoplastic (serous cystadenomas), and 3 normal pancreatic tissue. Cytoplasmic and/or nuclear staining was considered positive as: Focally positive (5–50% of tumor cells), diffusely positive (>50% of tumor cells), or negative (<5% tumor cells).

**RESULTS:** Maspin expression (positive/negative), distribution (focal/diffuse), and nuclear expression are significantly different between PC, solid pseudopapillary neoplasm, AC, and DC. PC shows significantly higher expression with more diffuse positivity and more nuclear expression than other malignant groups. Forty cases of PC (40/41) (97.6%) showed positive expression; 28 of them (28/40) (70%) showed diffuse expression and 82.5% (33 cases) showed nuclear and cytoplasmic expression. Only one case (14.3%) (1/7) of solid pseudopapillary neoplasm showed positive focal cytoplasmic expression. Three AC cases (3/9) (33.3%) showed positive focal cytoplasmic expression. Two cases of DC (2/3) (66.7%) showed positive focal cytoplasmic expression. Maspin expression shows significant positive correlation with poor prognostic variables as tumor grade, lymphovascular invasion, T stage of PC. Minority of chronic pancreatitis and benign lesions are maspin positive with significant difference from the malignant groups.

**CONCLUSION:** Our results suggest that maspin can be of value in differentiating pancreatic adenocarcinoma from ampullary carcinoma, duodenal adenocarcinoma, and other confusing lesions as chronic pancreatitis.

## Introduction

Ampullary carcinoma (AC) is topographically centered in the ampulla of Vater. It mainly shows morphology of intestinal-type or pancreaticobiliary-type adenocarcinoma. Periampullary carcinoma defines as a heterogeneous group of tumors including duodenal adenocarcinoma with secondary involvement of ampulla, distal common bile duct, or pancreatic carcinoma [1].

In advanced cases, it is extremely difficult to distinguish AC from periampullary carcinoma to the extent that the only diagnosis could be rendered is carcinoma of the pancreatobiliary-ampullary region. Although, the clinical presentation and treatment modalities for ampullary and periampullary tumors are the same, their outcome are quite different with that for pancreatic adenocarcinoma being much worse than for the other tumors [2]. This inspired us to search for the use of an immunohistochemical marker expression to discriminate between AC, pancreatic adenocarcinoma, duodenal adenocarcinoma, and other confusing benign and inflammatory pancreatic lesions.

Pancreatic carcinoma (ductal adenocarcinoma) represents 85% of pancreatic malignant tumors [3]. There are three recognized precursors of invasive ductal adenocarcinoma: Pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasm (IPMN), and mucinous cystic neoplasm (MCN) [3]. Risk factors for pancreatic cancer include cigarette smoking, diabetes mellitus, chronic pancreatitis, and family history [3].

Pancreatic ductal adenocarcinoma has the worst 5-year relative survival rate as related to all other solid tumors. It is anticipated that it will become the second leading cause of cancer-related mortality in the United States by 2030 after lung cancer [4]. Cases usually present with metastasis and advanced disease [5]. Prognosis is dependent on stage and size of tumor. The resectable tumors that are less than 4.5 cm in size have favorable prognosis, while late

staged tumors have dismal prognosis. On the other hand, the perineural invasion and vascular invasion are adverse prognostic factors [6]. High histologic grades and the presence of any squamous component also carry unfavorable prognosis [3]. Around 15–20% of patients have resectable disease, but only around 20% of them survive to 5 years [7].

The tumor suppressor gene maspin, a unique member of the serpin superfamily, inhibits cell motility, invasion, and metastasis in some cancers. Maspin is expressed in normal human mammary and prostate epithelial cells. Its expression is down-regulated in breast, prostate, gastric and melanoma cancers. Although maspin expression was not detected in normal pancreatic tissue, it is over-expressed in pancreatic, gallbladder, colorectal, and thyroid cancers suggesting that maspin may play different activities in different cell types [8].

Pancreatic ductal adenocarcinoma acquires maspin expression through hypomethylation of the maspin promoter [8]. Maspin may be helpful in differentiating ductal adenocarcinoma from chronic pancreatitis, once squamous metaplasia is ruled out; as maspin is expressed in squamous cells [9]. The immunoreactivity of maspin was mainly in cytoplasmic staining, with some in nuclear staining [9].

As regard chronic pancreatitis, it is characterized by repeated attacks of pancreatic inflammation with loss of pancreatic parenchyma and replacement by fibrosis [10]. Moving to the cystic lesions of the pancreas, they are being recognized with increasing frequency. Cystic lesions of the pancreas are often either benign or low-grade indolent neoplasia. However, those that are mucinous have well-established malignant potential. Those that are non-mucinous such as serous tumors, congenital cysts, lymphoepithelial cysts, and squamoid cyst of pancreatic ducts have no malignant potential. Only rarely degenerative/necrotic changes in solid neoplasia, such as cystic ductal adenocarcinomas and solid-pseudopapillary neoplasm, can present as complex cystic lesions [10].

## Materials and Methods

### *Retrieval of cases*

This is a retrospective study in which 80 paraffin blocks of pancreatic, ampullary and duodenal excision biopsies were retrieved. The specimens were obtained from the Department of Pathology, Faculty of Medicine, Cairo University during the period from January 2015 to January 2019. The 80 specimens were already diagnosed as 60 cases of malignant tumors, 17 cases of benign lesions and three cases of normal pancreatic tissue. Some of the pancreatitis and

non-neoplastic normal pancreas were taken from the margins of pancreatic carcinoma cases not enumerated in malignant studied cases. The diagnosis was based on histopathologic evaluation of hematoxylin and eosin-stained sections. Two sections were prepared from each block, 4 μ thick each, one of them was placed on glass slide and stained with hematoxylin and eosin for histopathological evaluation, the other was placed on charged slide for immunohistochemical staining by maspin polyclonal antibody. Clinicopathological data in patient's pathology reports were documented including age, gender and type of operation. For statistical purposes, age was classified into (<52, ≥52). Light microscope Leica was used in histopathological and immune-histochemical slides evaluation.

### *Histopathological evaluation*

Histopathologic classification according to the WHO classification of pancreatic tumors, 2019 was done [11].

The slides were evaluated for the presence of lymphovascular emboli, perineural invasion, and pancreatic margin involvement. Histopathological grading of malignant tumors according to guidelines of American Joint Committee on Cancer (AJCC Cancer Staging Manual 8th ed.) into G1 = Well differentiated, G2 = Moderately differentiated, and G3 = Poorly differentiated [12]. Exact tumor size documentation, extent of tumor invasion, and lymph node involvement of malignant pancreatic epithelial tumors (pancreatic adenocarcinoma and solid pseudopapillary neoplasm) were staged according to guidelines of Eighth Edition of the American Joint Committee on Cancer Staging (AJCC) for pancreatic adenocarcinoma [12]. Extent of tumor invasion and lymph node involvement of ampullary and duodenal adenocarcinoma were staged according to guidelines of AJCC Cancer Staging Manual 8th ed. [12].

### *Maspin immunohistochemical staining and interpretation*

In this study, immunostaining for maspin "mammary serine protease inhibitor" (rabbit polyclonal antibody, code of product YPA1886, Chongqing Biopsies Co, Jiangbei District, Chongqing, China) used at 1:200 dilution - was performed on paraffin sections as recommended by manufactures. Briefly, staining procedure was conducted using automated DAKO immunostainer on 4 mm thick sections of paraffin-embedded tissue sections which were deparaffinized in xylene and rehydrated in descending ethanol series. The antigens were retrieved using citrate buffer. Endogenous peroxidase activity was blocked by immersion for 10 min in 0.3% hydrogen peroxide in methanol solution, followed by single wash in phosphate buffered saline (pH 7.4). The immunostaining was

developed using diaminobenzidine as chromogen. Maspin positivity in normal breast tissue served as a positive control. Negative control was done by omitting the primary maspin antibody.

The whole slide fields were evaluated. Cytoplasmic and/or nuclear staining for maspin was considered positive as: Focally positive (5-50% of tumor cells), diffusely positive (>50% of tumor cells), or negative (<5% tumor cells) [13]. Provided that the staining was limited to the diagnostic cells as well as it did not represent background or artifact, no squamous cellular elements, avoiding the necrotic, and slide marginal areas.

### Statistical analysis

Microsoft Excel 2013 was used for data entry. The Statistical Package for the Social Sciences (SPSS), version 24 (Armonk, New York, United States) was used for data analysis. Numerical values were summarized using means, medians, standard deviations and ranges. Categorical data were grouped as percentages. The maspin immunomarker for pancreatic malignancy sensitivity, specificity as well as overall accuracy with positive and negative predictive values was calculated. Bivariate relationship was displayed in cross tabulations. Comparison of proportions was performed using the Chi-square and Fisher's exact tests where appropriate. The level of significance was set at probability (P) value < 0.05.

## Results

Eighty cases of pancreaticoduodenal lesions were studied. Sixty cases were malignant including: 48 cases of pancreatic epithelial tumor (41 cases of pancreatic adenocarcinoma and seven cases of solid pseudopapillary neoplasm), nine cases of ampullary adenocarcinoma, and three cases of duodenal adenocarcinomas. Twenty cases were non-malignant including: 12 inflammatory (chronic pancreatitis), five benign neoplastic (diagnosed as serous cystadenomas),

and three cases of non-neoplastic normal pancreatic tissue. The age range of the studied cases was from 34 to 70 with mean age 52. Males represented 58.75% of our studied cases while females represented 41.25% with male: female ratio 1.3:1.

Focusing on maspin immunohistochemical expression, our study showed that 51 cases (63.75%) have positive maspin expression (Figure 1), while it was negative in 29 cases (36.25%). All maspin positive studied cases showed cytoplasmic staining. Thirty-seven out of the 51 positive cases (72.55%) showed nuclear expression as well. About 76.7% of malignant cases (46/60) showed positive maspin expression (sole cytoplasmic or combined nuclear/cytoplasmic staining), while 14 cases (23.3%) showed negative maspin expression. In 28 of malignant cases that showed positive maspin expression (60.9%), the expression was diffuse; while in 18 cases (39.1%), the expression was focal.

Regard to maspin expression, there is significant difference between its expression (positive/negative) in pancreatic adenocarcinoma, solid pseudopapillary neoplasm, AC and duodenal adenocarcinoma (p < 0.05). Furthermore, there is significant difference regard to the distribution pattern of the positivity whether focal or diffuse and regard to the nuclear expression pattern among the four groups. Pancreatic carcinoma shows significantly higher maspin expression with more diffuse positivity and more nuclear expression than other tumors (p < 0.05).

Forty cases of pancreatic adenocarcinoma (40/41) (97.6%) showed positive expression; 28 of them (28/40) (70%) showed diffuse expression and 82.5% (33 cases) showed nuclear and cytoplasmic expression (Figure 1). Only one case (14.3%) (1/7) of solid pseudopapillary neoplasm showed positive focal cytoplasmic maspin expression (Figure 2). Three ampullary adenocarcinoma cases (3/9) (33.3%) showed positive focal cytoplasmic maspin expression (Figure 3). Two cases of duodenal adenocarcinoma (66.7%) showed positive focal cytoplasmic maspin expression (Figure 4). About 76% (35/46) of the positive malignant cases show nuclear and cytoplasmic expression, while only 24% (11/46) show cytoplasmic expression only (Table 1).
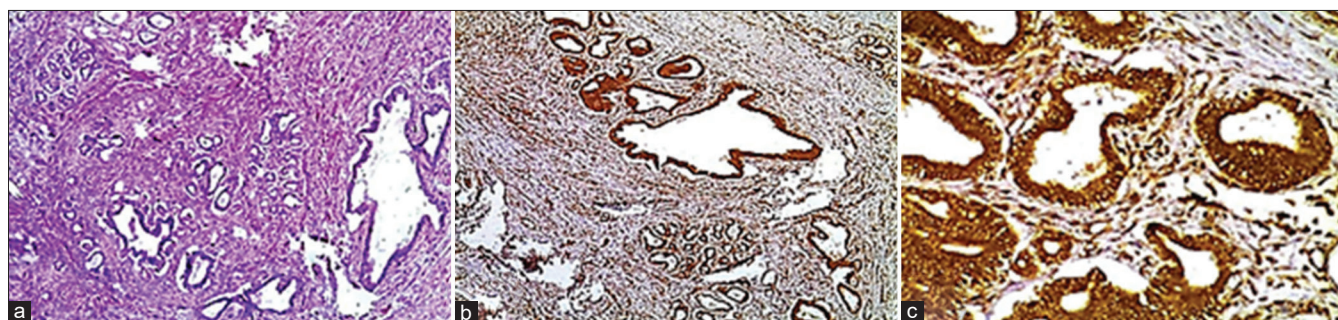


Figure 1: A case of pancreatic adenocarcinoma showing malignant acini separated by desmoplastic stroma (a) (H and E, ×40) (original magnification). The same case with combined nuclear and cytoplasmic diffuse maspin expression (b and c) – (Maspin, ×40, ×200) (original magnification)
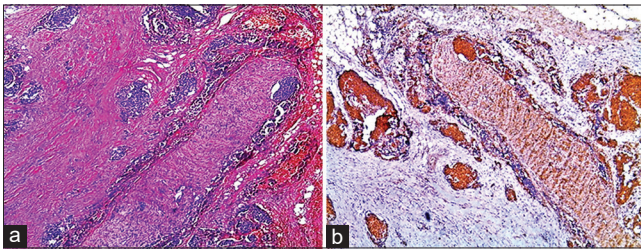
*Figure 2: A case of solid pseudopapillary neoplasm showing nests of cells with eosinophilic cytoplasm surrounding blood vessels forming pseudopapillary pattern with positive maspin expression (a) H and E, (b) maspin, ×100 original magnification*

In this study, significant relationship was found between the presence of tumor vascular emboli on one side with maspin expression (positive/negative), distribution (focal/diffuse), and nuclear expression on the other side among the four groups of malignant tumors. About 74% of malignant positive cases (34/41) show lymphovascular emboli, mainly diffuse, and nuclear expression (p < 0.05). Furthermore, there was significant relationship between grade of malignant tumors and maspin positivity and expression distribution (p < 0.05). No significant relationship between maspin expression and margin involvement or perineural invasion by the malignant tumors (Table 2 and Figures 5 and 6).

A significant relationship was found between maspin expression and T stage (tumor size) of pancreatic malignant epithelial tumors (p = 0.05). No significant relation was found between maspin expression distribution and nuclear expression of the positive malignant pancreatic epithelial tumors with T stage (p value > 0.05). No significant relation between N stage of malignant pancreatic epithelial tumors and maspin positivity, or distribution pattern or nuclear expression pattern (p > 0.05) (Table 3).
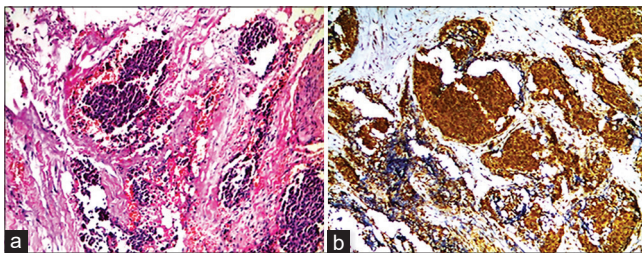


*Figure 3: A case of periampullary adenocarcinoma showing malignant acini separated by desmoplastic stroma, with positive maspin expression (a) H and E, 40×, (b) maspin, ×40 and ×200, original magnification*

In our study, there was insignificant relationship between maspin positivity, T and N stages of ampullary adenocarcinoma or duodenal adenocarcinoma (Tables 4 and 5).

Regarding the sensitivity and specificity of maspin in pancreatic carcinoma, counting on the maspin nuclear staining, it was found that sensitivity = 82.5%, specificity = 63.6%, positive predictive value (PPV) = 89.2%, negative predictive value (NPV) = 50.0%, and accuracy = 78.4%. On the other side, by counting on sole maspin cytoplasmic staining, it was found that Sensitivity = 100%, Specificity = 0%, PPV = 78.4%, and NPV = 0%.
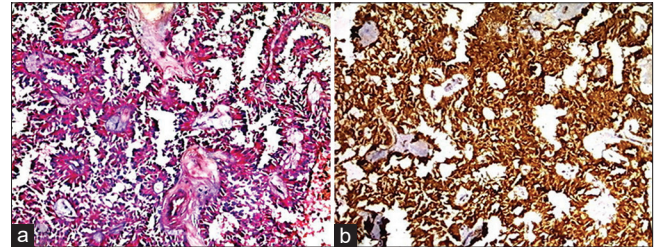


*Figure 4: A case of duodenal adenocarcinoma showing malignant acini with positive maspin expression (a) H and E, (b) maspin, ×100 original magnification*

In this study, we noted that minority of non-malignant cases (only 5/20 cases) showed positive maspin expression as follows: Three pancreatitis cases, and two benign serous cystadenomas. All positive pancreatitis cases showed a sole cytoplasmic focal maspin expression distribution. The two positive cases of benign serous cystadenoma showed combined nuclear and cytoplasmic expression (one with focal and the other with diffuse expression). All the three cases of normal pancreas showed negative maspin expression (Figures 7–9).

## Discussion

Pancreatic cancer is an aggressive malignancy and is the seventh leading cause of global cancer deaths in industrialized countries and the third most common in the USA. Based on GLOBOCAN 2018 estimates, pancreatic cancer was ranked the 11th most common cancer in the world [14].

Maspin is a member of the serine protease

**Table 1: Relationship between maspin expression (positive/negative), distribution (focal/diffuse), and expression pattern (nuclear/cytoplasmic) in malignant cases (60 cases)**

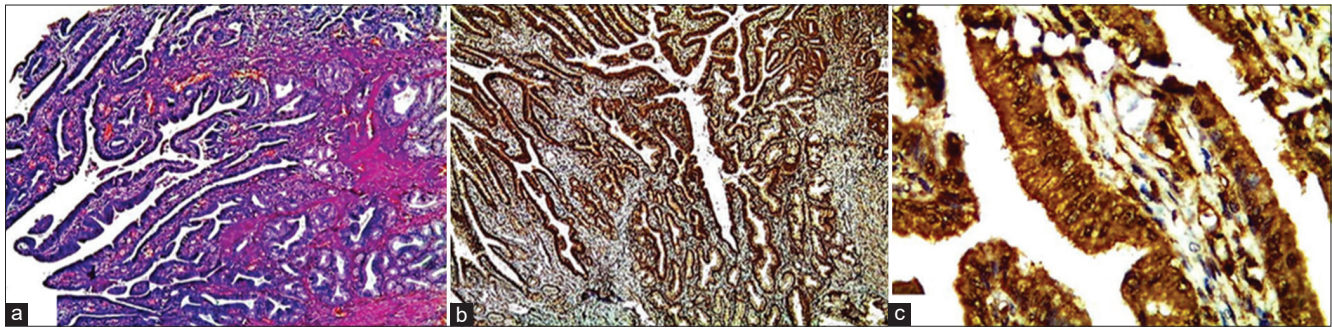| Malignant cases | Expression | | | Distribution | | | Nuclear Expression Pattern | | |
|---|---|---|---|---|---|---|---|---|---|
| | -ve | +ve | Total | Focal | Diffuse | Total | Present | Absent | Total |
| Pancreatic carcinoma | 1 | 40 | 41 | 12 | 28 | 40 | 33 | 7 | 40 |
| | 2.4% | 97.6% | 100% | 30% | 70% | 100% | 82.5% | 17.5% | 100% |
| Solid pseudopapillary tumor | 6 | 1 | 7 | 1 | 0 | 1 | 0 | 1 | 1 |
| | 85.7% | 14.3% | 100% | 100% | 0% | 100% | 0% | 100% | 100% |
| Ampullary adenocarcinoma | 6 | 3 | 9 | 3 | 0 | 3 | 2 | 1 | 3 |
| | 66.7% | 33.3% | 100% | 100% | 0% | 100% | 66.7% | 33.3% | 100% |
| Duodenal adenocarcinoma | 1 | 2 | 3 | 2 | 0 | 2 | 0 | 2 | 2 |
| | 33.3% | 66.7% | 100% | 100% | 0% | 100% | 0% | 100% | 100% |
| Total | 14 | 46 | 60 | 18 | 28 | 46 | 35 | 11 | 46 |
| | 23.3% | 76.7% | 100% | 39.1% | 60.9% | 100% | 76% | 24% | 100% |
| p value | p < 0.0001 | | | p = 0.013 | | | p = 0.014 | | |

*Figure 5: A case of pancreatic adenocarcinoma showing tumor perineural invasion with positive maspin expression (a) H and E, (b and c) maspin, ×40 original magnification*

inhibitor/non-inhibitor superfamily whose expression is regulated at the level of transcription in a cell type-specific manner [15]. Most epithelial cells display abundant expression of maspin, whereas mesenchymal cells do not express maspin, with the exception of corneal stromal cells [16]. Its expression is down-regulated in breast, prostate, gastric cancers, and melanoma; but over-expressed in pancreatic, gallbladder, colorectal, and thyroid cancers suggesting that maspin may play different activities in different cell types [8].
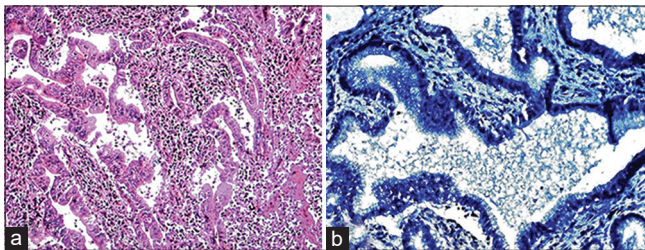


*Figure 6: A case of pancreatic adenocarcinoma showing tumor lymphovascular emboli with positive maspin expression (a) H and E, (b) maspin, ×40 original magnification*

In this study, we examined maspin immunoreactivity on 80 specimens that were already diagnosed as 60 cases of malignant tumors, 17 cases of non-malignant lesions, and three cases of normal pancreatic tissue. Cytoplasmic and/or nuclear staining for maspin was considered positive, either focal (5–50% tumor cells) or diffuse positive (>50% tumor cells) or negative (<5% tumor cells) [13].
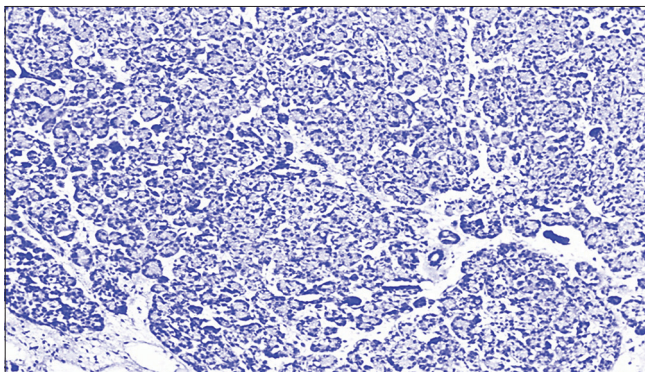


*Figure 7: Normal pancreatic acini and ducts showed negative maspin immunostaining (×100 original magnification)*

In our study, the mean age of the studied cases was 52, ranging from 34 to 70 and the median
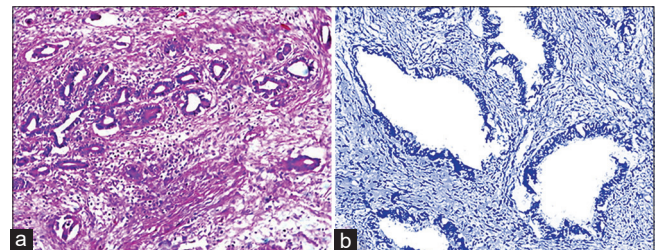


*Figure 8: A case of chronic pancreatitis showing periductal fibrosis and stromal chronic inflammatory cellular infiltrate with negative maspin expression (a) H and E ×40 and (b) maspin ×400 original magnification*

age was 55. Those figures were near to what was reported in a study performed by Cao *et al.*, [13] in which the mean age was 66 years, ranging from 32–89 years and similar to study conducted by Lim *et al.*, [17] in which the median age of the patients was 59.8 years. The wider age spectrum in our study can be interpreted by having non-malignant cases together with malignant ones, unlike those studies that were concerned by the malignant cases only. Males represented 58.75% of our studied cases with male: female ratio 1.3:1. This was similar to the study of Xin *et al.*, [18] in which they studied primary and metastatic pancreatic cancers revealing male: female ratio of 1.3:1.
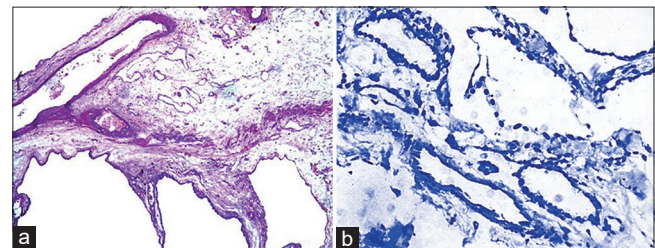


*Figure 9: A case of pancreatic serous cystadenoma showing cystic spaces lined by small cuboidal cells, with negative maspin expression (a) H and E, (b) maspin ×100 original magnification*

Regard to maspin immunohistochemical expression in all studied lesions, our study showed that 51 cases (63.75%) showed positive maspin expression. Twenty-nine cases (56.86%) showed diffuse pattern of expression. Thirty-seven positive maspin cases (72.55%) showed nuclear pattern of staining. Forty-six malignant cases (76.7%) showed positive maspin expression, while fourteen cases (23.3%) showed negative maspin

expression. Combined nuclear/cytoplasmic maspin expression was found in thirty-five cases (76.1% of positive malignant cases), all maspin positive cases showed cytoplasmic staining. In twenty-eight of cases that showed positive maspin expression (60.9%), the expression was diffuse. Most of the malignant cases were pancreatic adenocarcinoma. Forty cases of pancreatic adenocarcinoma (40/41) (97.6%) showed positive cytoplasmic maspin expression; twenty-eight of them (28/40) (70%) showed diffuse maspin expression.

The results were similar to that conducted by Oh et al. [19] and *Nash et al.* [9] who reported that cases of ductal adenocarcinoma showed diffuse staining in most cases. Our results are in concordance with Liu et al. [20] and Cao et al., [13] who demonstrated that more than 90% of cases of ductal adenocarcinoma were positive for maspin.

In our study, only one case (14.3%) (1/7) of solid pseudopapillary neoplasm showed positive focal

cytoplasmic maspin expression, that is different from what documented by Oh et al., [19] who examined sections from 107 pancreatic benign and malignant neoplasms that were immunostained with anti-maspin antibody using an EnVision + System. Maspin was expressed in all ductal adenocarcinomas. In contrast, solid-pseudopapillary tumors demonstrated no Maspin expression. The difference may be due to different number of included cases in both studies or due to different immunostaining methods.

Three periampullary adenocarcinoma cases (3/9) (33.3%) showed positive focal cytoplasmic maspin expression. This is similar to that documented by Blandamura et al., [21].

We noted significant relationship between maspin positivity and presence of vascular emboli (p < 0.05) that is different from Cao et al., [13] who reported that maspin expression was not associated with vascular invasion. This difference may be due to that Cao et al. study investigated maspin protein expression in a large series of 223 surgically resected pancreatic ductal adenocarcinomas only using tissue microarrays. We also encountered significant relationship between maspin positivity, distribution and grade of malignant tumors (p < 0.05). Maspin positivity is inversely proportional to tumor grade, being more among poorly differentiated cases. These results were similar to that conducted on Maspin expression in breast carcinoma by Helal and El-Guindy, Lee et al., Umekita and Yoshida [22], [23], [24], [25] who reported a strong association between Maspin expression and higher histologic grade. On the other hand, Jason et al., [9] did not find a correlation between maspin staining and histologic grade of the pancreatic tumors. Furthermore, Ohike et al. [26] noted greater staining in well to moderately differentiated tumors than in poorly differentiated pancreatic tumors. In this study and similar to Ohike et al. [26], 35 cases out of the 41 positive malignant cases (76.1%) are Grade II, while nine cases (19.6%) are Grade III, this may be due to the relatively small number of poorly differentiated tumors in these studies.

**Table 2: Relationship between maspin expression (positive/negative), distribution (focal/diffuse) and expression pattern (nuclear/cytoplasmic) in malignant cases and various histopathological characteristics of malignant cases (margin involvement, vascular emboli, perineural invasion and grade) (60 cases)**

| Pathological parameters | Expression | | Distribution | | Nuclear Pattern | |
|---|---|---|---|---|---|---|
| | -ve | +ve | Focal | Diffuse | Present | Absent |
| Margin involvement | | | | | | |
| -ve | 14 | 41 | 17 | 24 | 30 | 11 |
| | 100% | 89.1% | 94.4% | 85.7% | 85.7% | 100% |
| +ve | 0 | 5 | 1 | 4 | 5 | 0 |
| | 0% | 10.9% | 5.6% | 14.3% | 14.3% | 0% |
| p value | 0.19 | | 0.35 | | 0.184 | |
| Vascular emboli | | | | | | |
| -ve | 12 | 12 | 10 | 2 | 6 | 6 |
| | 85.7% | 26% | 55.6% | 7.1% | 17.1% | 54.5% |
| +ve | 2 | 34 | 8 | 26 | 29 | 5 |
| | 14.3% | 74% | 44.4% | 92.9% | 82.9% | 45.5% |
| p value | 0.0001 | | 0.0002 | | 0.013 | |
| Perineural Invasion | | | | | | |
| -ve | 13 | 38 | 16 | 22 | 27 | 10 |
| | 92.9% | 82.6% | 88.9% | 78.6% | 77.1% | |
| +ve | 1 | 8 | 2 | 6 | 8 | 1 |
| | 7.1% | 17.4% | 11.1% | 21.4% | 22.9% | |
| p value | 0.347 | | 0.37 | | 0.315 | |
| Grade | | | | | | |
| G I | 6 | 2 | 2 | 0 | 1 | 1 |
| | 42.9% | 4.3% | 11.1% | 0.0% | 2.9% | 9.1% |
| G II | 7 | 35 | 15 | 20 | 25 | 10 |
| | 50% | 76.1% | 83.3% | 71.4% | 71.4% | 90.9% |
| G III | 1 | 9 | 1 | 8 | 9 | 0 |
| | 7.1% | 19.6% | 5.6% | 28.6% | 25.7% | 0% |
| p value | 0.001 | | 0.043 | | 0.136 | |
| Total | 14 | 46 | 18 | 28 | 35 | 11 |
| | 100% | 100% | 100% | 100% | 100% | 100% |

**Table 3: Relationship between maspin expression and T, N stage of malignant pancreatic epithelial tumors**

| Malignant epithelial T | Expression (48 cases) | | | | p value | Distribution (41 cases) | | | | p value | Pattern (nuclear) (41 cases) | | | | p value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Negative | | Positive | | | Focal | | Diffuse | | | Cytoplasmic only | | Nuclear/cytop | | |
| | Count | % | Count | % | | Count | % | Count | % | | Count | % | Count | % | |
| T stage | | | | | | | | | | | | | | | |
| T1 | 6 | 85.7% | 8 | 19.5% | 0.005 | 3 | 23.1% | 5 | 17.9% | 0.706 | 4 | 50% | 4 | 12.1% | 0.115 |
| T2 | 1 | 14.3% | 15 | 36.6% | | 6 | 46.2% | 9 | 32.1% | | 2 | 25% | 13 | 39.4% | |
| T3 | 0 | 0.0% | 10 | 24.4% | | 2 | 15.4% | 8 | 28.6% | | 1 | 12.5% | 9 | 27.3% | |
| T4 | 0 | 0.0% | 8 | 19.5% | | 2 | 15.4% | 6 | 21.4% | | 1 | 12.5% | 7 | 21.2% | |
| Malignant epithelial T | Expression (48 cases) | | | | p value | Distribution (41 cases) | | | | p value | Pattern (nuclear) (41 cases) | | | | p value |
| | Negative | | Positive | | | Focal | | Diffuse | | | No | | Yes | | |
| | Count | % | Count | % | | Count | % | Count | % | | Count | % | Count | % | |
| N stage | | | | | | | | | | | | | | | |
| N0 | 6 | 85.7% | 25 | 61.0% | 0.209 | 6 | 46.2% | 19 | 67.9% | 0.028 | 7 | 87.5% | 18 | 54.5% | 0.219 |
| N1 | 0 | 0.0% | 13 | 31.7% | | 4 | 30.8% | 9 | 32.1% | | 1 | 12.5% | 12 | 36.4% | |
| N2 | 1 | 14.3% | 3 | 7.3% | | 3 | 23.1% | 0 | 0.0% | | 0 | 0% | 3 | 9.1% | |
| Total | 7 | 100% | 41 | 100% | | 13 | 100% | 28 | 100% | | 8 | 100% | 33 | 100% | |

**Table 4: Relationship between maspin expression and T, N stage of ampullary adenocarcinoma (nine cases)**

| Malignant ampullary T | Expression (9 cases) | | | | p value | Pattern (3 cases) | | | | p value |
|---|---|---|---|---|---|---|---|---|---|---|
| | Negative (6) | | Positive (3) | | | Cytoplasmic only | | Nuclear/cytoplasmic | | |
| | Count | % | Count | % | | Count | % | Count | % | |
| T stage | | | | | | | | | | |
| T1a | 0 | 0% | 1 | 33.3% | 0.199 | 1 | 100% | 0 | 0% | 0.223 |
| T1b | 0 | 0% | 1 | 33.3% | | 0 | 0% | 1 | 50% | |
| T2 | 3 | 50% | 0 | 0% | | 0 | 0% | 0 | 0% | |
| T3a | 2 | 33.3% | 1 | 33.3% | | 0 | 0% | 1 | 50% | |
| T3b | 1 | 16.7% | 0 | 0% | | 0 | 0% | 0 | 0% | |
| Malignant ampullary T | Expression (9 cases) | | | | p value | Pattern (nuclear) (3 cases) | | | | p value |
| | Negative | | Positive | | | No | | Yes | | |
| | Count | % | Count | % | | Count | % | Count | % | |
| N stage | | | | | | | | | | |
| N0 | 5 | 83.3% | 2 | 66.7% | 0.276 | 0 | 0% | 2 | 100% | 0.083 |
| N1 | 1 | 16.7% | 0 | 0% | | 0 | 0% | 0 | 0% | |
| N2 | 0 | 0% | 1 | 33.3% | | 1 | 100% | 0 | 0% | |
| Total | 6 | 100% | 3 | 100% | | 1 | 100% | 2 | 100% | |

**Table 5: Relationship between maspin expression and T and N stage of duodenal adenocarcinoma (3 cases)**

| Malignant duodenal | Expression (3 cases) | | | | p value |
|---|---|---|---|---|---|
| | Negative | | Positive | | |
| | Count | % | Count | % | |
| T stage | | | | | |
| T1 | 0 | 0% | 2 | 100% | 0.083 |
| T3b | 1 | 100% | 0 | 0% | |
| Malignant duodenal | Expression (3 cases) | | | | p value |
| | Negative | | Positive | | |
| | Count | % | Count | % | |
| N stage | | | | | |
| N0 | 1 | 100% | 2 | 100% | 0.083 |
| Total | 1 | 100% | 2 | 100% | |

A non-significant relationship was found between maspin distribution and N stage of malignant pancreatic epithelial tumor (p >0.05). Contrary to our results, Helal and El-Guindy, Terashima et al., and Oh et al. [19], [22], [27] found significant relation between immunoreactivity for maspin and nodal metastasis. They noted that maspin expression was higher in lymph node-positive carcinomas than in lymph node-negative carcinomas. In our study, there was a significant relationship between maspin positivity and T stage (tumor size) of malignant epithelial tumors (p < 0.05).

Those findings were different from that reported by Maass et al., [28] who found a lower frequency of lymph node metastasis in maspin positive cases. This is also different from Cao et al. and Lim et al., [13], [17] who reported that there was no statistical significance between the maspin expression and clinicopathologic parameters including lymph node metastasis and tumor size. The difference may be due to the difference in number of cases.

In our study, the sensitivity and specificity of maspin in pancreatic carcinoma were evaluated. Counting on the maspin nuclear staining, it was found that sensitivity = 82.5%, specificity = 63.6%, PPV = 89.2%, NPV = 50.0%, and accuracy = 78.4%. On the other side, by counting on sole maspin cytoplasmic staining, it was found that sensitivity = 100%, specificity = 0%, PPV = 78.4%, and NPV = 0%.

This is similar to Aksoy-Altinboga et al., [29] who studied maspin expression in pancreatic cell blocks and found that the diagnostic sensitivity for malignancy in maspin was 87.5%. When maspin, IMP3, and S100P expression were used together as triple test, sensitivity was 62.5% and specificity 100%. However, when any two of each three markers were evaluated (triple test/dual response), sensitivity reached 93.8% and specificity 100%. This is in concordance with Mamdouh et al., [30] who combined the use of maspin, CK17 and Ki-67 together as a triple test (at least one of them is positive) achieved the highest sensitivity of 98.8%, specificity of 100%, PPV of 100%, NPV of 96.2%, and accuracy of 99% in the differentiation between PDAC and benign pancreatic tissue.

Regarding maspin expression in non-malignant cases, we found that minority of non-malignant cases (only five cases) showed positive maspin expression (three pancreatitis cases (25%) and two benign serous cystadenomas (40%). All the three cases of normal pancreas showed negative maspin expression. Our results, as regards chronic pancreatitis and normal pancreas, were similar to that done by Maass et al., Jason et al., Lim et al., Liu et al., and Oh et al., [9], [17], [19], [21], [28], who noted only rare expression of maspin in normal pancreatic ducts and chronic pancreatitis adjacent to tumors. As for serous cystadenoma; our results were different from that reported by Oh et al. [19] who examined the expression of maspin in various pancreatic neoplasms that demonstrated no maspin expression in serous cystadenoma. The difference may be due to that they used monoclonal anti-maspin antibody and we used polyclonal one.

Moreover, the expression of maspin might be useful as a prognostic and possibly predictive factor for patients with particular types of cancer. Its expression in circulating tumor cells could be also useful in clinical practice along with other factors to select the best therapy to be carried out [8]. Regarding maspin expression in pancreatic tissue, maspin expression seems to increase with increasing malignancy from normal pancreas tissue via precancerous lesions to invasive carcinomas. These findings indicate that maspin expression is of biological relevance in vivo for the development of pancreatic cancers [31].

## Conclusion

On the basis of our results and published data, maspin immunoreactivity is useful in differentiating malignant epithelial pancreatic tumors from duodenal adenocarcinoma, ampullary adenocarcinoma and non-malignant pancreatic lesions. However, to yield more precise results, it should be combined with other markers.

## References

1. Sunnapwar AA, Nagar A, Katre R, Khanna L, Sayana HP. Imaging of ampullary and periampullary conditions. J Gastrointest Abdom Radiol. 2021;4(03):214-28. https://doi.org/10.1055/s-0041-1726663

2. Komine R, Kojima M, Ishi G, Kudo M, Sugimoto M, Kobayashi S, et al. Recognition and pathological features of periampullary region adenocarcinoma with an indeterminable origin. Cancer Med. 2021;10(11):3499-510. https://doi.org/10.1002/cam4.3809
   PMid:34008914

3. Bosman FT, Carneiro F, Hruban RH. WHO Classification of Tumours of the Digestive System. Vol. 3. Lyon, France: IARC Press, World Health Organization Classification of Tumours; 2010.

4. Chugh S, Barkeer S, Rachagani S, Nimmakayala RK, Perumal N, Pothuraju R, et al. Disruption of C1galt1 gene promotes development and metastasis of pancreatic adenocarcinomas in mice. Gastroenterology. 2018;155(5):1608-24. https://doi.org/10.1053/j.gastro.2018.08.007
   PMid:30086262

5. Mizrahi JD, Surana R, Valle JW, Shroff RT. Pancreatic cancer. Lancet. 2020;395:2008-20. https://doi.org/10.1016/s0140-6736(20)30974-0

6. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics. CA Cancer J Clin. 2021;71(1):7-33.
   PMid:33433946

7. McGuigan A, Kelly P, Turkington RC, Jones C, Coleman HG, McCain RS. Pancreatic cancer: A review of clinical diagnosis, epidemiology, treatment and outcomes. World J Gastroenterol. 2018;24(43):4846-61. https://doi.org/10.3748/wjg.v24.i43.4846
   PMid:30487695

8. Berardi R, Morgese F, Onofri A, Mazzanti P, Pistelli M, Ballatore Z, et al. Role of maspin in cancer. Clin Transl Med. 2013;2(1):8. https://doi.org/10.1186/2001-1326-2-8
   PMid:23497644

9. Nash JW, Bhardwaj A, Wen P, Frankel WL. Maspin is useful in the distinction of pancreatic adenocarcinoma from chronic pancreatitis: A tissue microarray based study. Appl Immunohistochem Mol Morphol. 2007;15(1):59-63. https://doi.org/10.1097/01.pai.0000203037.25791.21
   PMid:17536309

10. Basturk O, Coban I, Adsay NV. Pancreatic cysts: Pathologic classification, differential diagnosis, and clinical implications. Arch Pathol Lab Med. 2009;133(3):423-38. https://doi.org/10.5858/133.3.423
    PMid:19260748

11. Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, et al. The 2019 WHO classification of tumours of the digestive system. Histopathology. 2020;76(2):182-8. https://doi.org/10.1111/his.13975
    PMid:31433515

12. Chun YS, Pawlik TM, Vauthey JN. 8th Edition of the AJCC cancer staging manual: Pancreas and hepatobiliary cancers. Ann Surg Oncol 2018;25(4):845-7. https://doi.org/10.1245/s10434-017-6025-x
    PMid:28752469

13. Cao D, Zhang Q, Wu L, Salaria SN, Winter JW, Hruban RH, et al. Prognostic significance of maspin in pancreatic ductal adenocarcinoma: Tissue microarray analysis of 23 surgically resected cases. Modern Pathol. 2007;20(5):570-8. https://doi.org/10.1038/modpathol.3800772
    PMid:17396143

14. Rawla P, Sunkara T, Gaduputi V. Epidemiology of pancreatic cancer: Global trends, etiology and risk factors. World J Oncol. 2019;10(1):10-27. https://doi.org/10.14740/wjon1166
    PMid:30834048

15. Futscher BW, Oshiro MM, Wozniak RJ, Holtan N, Hanigan CL, Duan H, et al. Role for DNA methylation in the control of cell type specific maspin expression. Nat Genet. 2002;31(2):175-9. https://doi.org/10.1038/ng886
    PMid:12021783

16. Ngamkitidechakul C, Burke JM, O'Brien WJ, Twining SS. Maspin: Synthesis by human cornea and regulation of in vitro stromal cell adhesion to extracellular matrix. Invest Ophthalmol Vis Sci 2001;42(13):3135-41.
    PMid:11726614

17. Lim YJ, Lee JK, Jang WY, Song SY, Lee KT, Paik SW, et al. Prognostic significance of maspin in pancreatic ductal adenocarcinoma. Korean J Intern Med 2004;19(1):15-8. https://doi.org/10.3904/kjim.2004.19.1.15
    PMid:15053038

18. Xin W, Yun KJ, Ricci F, Zahurak M, Qiu W, Su GH, et al. MAP2K4/MKK4 expression in pancreatic cancer: Genetic validation of immunohistochemistry and relationship to disease course. Clin Cancer Res. 2004;10(24):8516-20. https://doi.org/10.1158/1078-0432.ccr-04-0885
    PMid:15623633

19. Oh YL, Song SY, Ahn G. Expression of maspin in pancreatic neoplasms: Application of maspin immunohistochemistry to the differential diagnosis. Appl Immunohistochem Mol Morphol. 2002;10(1):62-6. https://doi.org/10.1097/00129039-200203000-00011
    PMid:11893038

20. Liu H, Shi J, Anandan V, Wang HL, Diehl D, Blansfield J, et al. Reevaluation and identification of the best immunohistochemical panel (pVHL, Maspin, S100P, IMP-3) for ductal adenocarcinoma of the pancreas. Arch Pathol Lab Med. 2012;136(6):601-9. https://doi.org/10.5858/arpa.2011-0326-oa
    PMid:22646265

21. Blandamura S, D'alessandro E, Guzzardo V, Giacomelli L, Moschino P, Parenti A, et al. Maspin expression in adenocarcinoma of the ampulla of vater: Relation with clinicopathological parameters and apoptosis. Anticancer Res. 2007;27(2):1059-65.
    PMid:17465244

22. Helal DS, El-Guindy DM. Maspin expression and subcellular localization in invasive ductal carcinoma of the breast: Prognostic significance and relation to microvessel density. J Egypt Natl Canc Inst. 2017;29(4):177-83. https://doi.org/10.1016/j.jnci.2017.09.002
    PMid:29126758

23. Tsoli E, Tsantoulis PK, Papalambros A, Perunovic B, England D, Rawlands DA, et al. Simultaneous evaluation of maspin and CXCR4 in patients with breast cancer. J Clin Pathol.

2007;60(3):261-6. https://doi.org/10.1136/jcp.2006.037887

PMid:16751302

24. Lee MJ, Suh CH, Li ZH. Clinicopathological significance of maspin expression in breast cancer. J Korean Med Sci. 2006;21(2):309-14. https://doi.org/10.3346/jkms.2006.21.2.309

PMid:16614520

25. Umekita Y, Yoshida H. Expression of maspin is up-regulated during the progression of mammary ductal carcinoma. Histopathology. 2003;42(6):541-5. https://doi.org/10.1046/j.1365-2559.2003.01620.x

PMid:12786889

26. Ohike N, Maass N, Mundhenke C, *et al*. Clinicopathological significance and molecular regulation of maspin expression in ductal adenocarcinoma of the pancreas. Cancer Lett. 2003;199(2):193-200. https://doi.org/10.1016/s0304-3835(03)00390-2

PMid:12969792

27. Terashima M, Maesawa C, Oyama K, Ohtani S, Akiyama Y, Ogasawara S, *et al*. Gene expression profiles in human gastric cancer: Expression of maspin correlates with lymph node metastasis. Br J Cancer. 2005;92(6):1130-6. https://doi.org/10.1038/sj.bjc.6602429

PMid:15770218

28. Maass N, Hojo T, Ueding M, Lüttges J, Klöppel G, Jonat W, *et al*. Expression of the tumor suppressor gene Maspin in human pancreatic cancers. Clin Cancer Res. 2001;7(4):812-7.

PMid:11309327

29. Aksoy-Altinboga A, Baglan T, Umudum H, Ceyhan K. Diagnostic value of S100p, IMP3, Maspin, and pVHL in the differantial diagnosis of pancreatic ductal adenocarcinoma and normal/chronic pancreatitis in fine needle aspiration biopsy. J Cytol. 2018;35(4):247-51. https://doi.org/10.4103/joc.joc_18_17

PMid:30498299

30. Mamdouh MM, Okasha H, Shaaban HA, Hafez NH, El-Gemeie EH. Role of maspin, CK17 and Ki-67 immunophenotyping in diagnosing of pancreatic ductal adenocarcinoma in endoscopic ultrasound-guided fine needle aspiration cytology. Asian Pac J Cancer Prev. 2021;22:3299-307. https://doi.org/10.31557/apjcp.2021.22.10.3299

PMid:34711007

31. Tarafa G, Villanueva A, Farré L, Rodríguez J, Musulén E, Reyes G, *et al*. DCC and SMAD4 alterations in human colorectal and pancreatic tumor dissemination. Oncogene. 2000;19:546-55. https://doi.org/10.1038/sj.onc.1203353

PMid:10698524