



Subcellular expression of mammary serine proteinase inhibitor in locally advance oral squamous cell carcinoma

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ABSTRACT

Objectives: Mammary serine protease inhibitor (MASPIN) has numerous interactions with tumor pathogenesis and progression. Its relationships with apoptosis and angiogenesis have proven the impact on prognosis. However, its exact role is not known and needs further work in relation to various human cancers. Current study was planned to investigate the subcellular expression of MASPIN in oral squamous cell carcinoma (OSCC) and to observe its relation with tumor grade. **Methods:** It was a descriptive study, conducted at the Department of Morbid Anatomy and Histopathology, University of Health Sciences, Lahore. Histological diagnosis of squamous cell carcinoma was confirmed in 50 cases, and expression of MASPIN was determined by avidin-biotin-peroxidase complex method of immunohistochemical staining. MASPIN expression was scored on the basis of intensity of staining and the percentage of the cells that stained positively. Data was analyzed with SPSS using appropriate statistical procedures. **Results:** Mean age of the patients was 56.84 ± 1.58 years with male to female ratio 1.3:1. All tumors were locally advancing (Stage III). Histologically, 58% tumors were Grade 1, other grades were less common. MASPIN expression was observed in 32 (64%) cases, and it was localized to the cytoplasm of the tumor cells in all cases. Among the positive cases, its expression was focal in 15 (44.1%), diffuse but moderate in 13 (38.2%) and diffuse and intense in 6 (17.7%) cases. MASPIN expression was significantly associated ($P: 0.043$) and negatively correlated ($P: 0.034$) with tumor grade. **Conclusions:** MASPIN expression was observed in the majority of OSCC. However, it was localized to the cytoplasm of tumor cells in all cases. Loss of MASPIN expression was observed more frequently in poorly differentiated cancers.

KEY WORDS: Mammary serine protease inhibitor, oral cancer, squamous cell carcinoma, tumor grade

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INTRODUCTION

Mammary serine protease inhibitor (MASPIN) is a 42 KDA protein that belongs to Serpin superfamily – the family of serine protease inhibitors [1]. Proteases degrade the extracellular matrix so that tumor cells can invade and undergoes the metastasis whereas protease inhibitors restrain this process and thus stop the tumor progression, invasion and metastasis [2]. There are many proposed mechanisms for these tumor inhibitory actions of MASPIN. Some investigators claim that it works by alteration of the integrin profile on the cell surface whereas others advocate that MASPIN has complex interplay with cell adhesion molecules [3,4]. MASPIN is also considered to induce apoptosis of the malignant cells through the activation of caspase pathways [5]. Intracellular MASPIN seems to be responsible for the increased sensitivity of the tumor cells to apoptosis. Tumor cells are affected by apoptosis depending on the N-terminal domain but the exact mechanism is still unknown [6] Proposed mechanism for the MASPIN interaction is through the action of two enzymes glutathione redox system those are glutathione peroxidase and glutathione S-transferase [7].

The clinical importance of MASPIN has been extensively investigated [8]. MASPIN shows varied localization pattern, it can localize to the cytoplasm, nucleus, secretory vesicles and the cell surface [9]. MASPIN expression has diverse implications in oral squamous cell carcinoma (OSCC) and may not only be related in its progression but also in the tumorigenesis and its differentiation [7,10]. Histological grade is the most sensitive indicator of cellular differentiation [11]. The purpose of this study was to determine association between the histological grade of OSCC with MASPIN immunohistochemical expression (nuclear, cytoplasmic or both) in an attempt to find link between MASPIN expression and cellular differentiation in this cancer.

METHODS

It was a cross-sectional descriptive study, conducted at the Department of Morbid Anatomy and Histopathology, University of Health Sciences Lahore. Tumor specimens from 50 patients who were diagnosed as squamous cell carcinoma were included in the study by convenience, non-probability sampling technique.

The tumors specimens were obtained from various teaching hospitals located in Lahore city. The histological diagnosis of OSCC was ascertained with routine hematoxylin and Eosin staining. In difficult cases, immunohistochemical expression of cytokeratin was used to confirm the diagnosis. These tumors were graded into histological Grade 1 to Grade 4 tumors based on the amount of keratinization, intercellular bridges, nuclear pleomorphism, tumor-surrounding interface and leukocytic infiltration.

Subcellular expression of MASPIN was determined by avidin-biotin-peroxidase complex method of immunohistochemical staining with mouse monoclonal antibody for MASPIN (EAW24), provided by Abcam in pre-diluted liquid form. It required phosphate buffer saline based antigen retrieval and 60 min incubation of the primary antibody at 37°C. The entire slide was scanned for immunostaining and MASPIN expression was scored on the basis of intensity of staining and the percentage of the cells that stained positively.

Statistical Analysis

Quantitative variables were presented in terms of means \pm standard error of the mean. Independent samples *T*-test and Pearson correlation were used to determine association and correlation respectively. Qualitative variables were projected as ratios and percentages, which were analyzed for statistical association with Chi-square and Fisher's exact test.

RESULTS

Demographic Profile of Patients

Mean age of the patients of OSCC was 56.84 ± 1.58 years with male to female ratio 1.3:1. Smoking was the most commonly reported risk factor as 25 (50%) patients were cigarette smokers. Pan-chewing (12%), poor orodental hygiene (6%) and acid reflux disease (4%) were other identifiable risk factors.

Clinical Parameters of OSCC

The commonest site of the tumor was tongue (32%) followed by lower lip (22%) and buccal mucosa (20%). Most common presentation was exophytic lesion (68%), many of which also had an ulcer (61.8%). The tumor size was >2.0 cm in majority (66%) of cases. All cases were advance stage OSCC.

Pathological Assessment of Tumors

Histologically, 58% tumors were Grade 1 as shown in Figure 1, other grades were less common. MASPIN expression was observed in 32 (64%) cases, and the intensity of MASPIN expression in OSCC is shown in Table 1. MASPIN was localized to the cytoplasm of the tumor cells in all cases as shown in Figure 2. Among the positive cases, its expression was focal in 15 (44.1%), diffuse but moderate in 13 (38.2%) and diffuse and intense in 6 (17.7%) cases. MASPIN expression was significantly

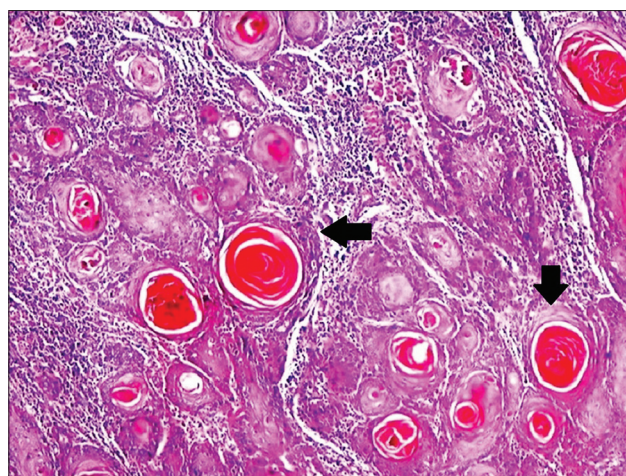


Figure 1: A well-differentiated oral cavity squamous cell carcinoma (H and E, $\times 200$)

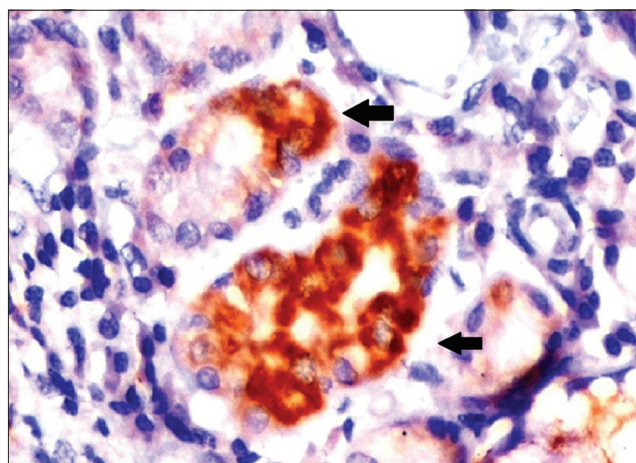


Figure 2: Mammary serine protease inhibitor expression in a well-differentiated oral cavity squamous cell carcinoma (immunohistochemistry, $\times 400$)

Table 1: Intensity of MASPIN expression in OSCC

Intensity of MASPIN	Frequency	Percentage
Weak	15	44.1
Moderate	13	38.2
Strong	6	17.7

MASPIN: Mammary serine protease inhibitor; OSCC: Oral squamous cell carcinoma

associated ($P: 0.043$) with histological grades of the OSCC as shown in Table 2. MASPIN expression was found to be negatively correlated ($P: 0.034$) with tumor grade of OSCC as shown in Table 3.

DISCUSSION

In current study, MASPIN expression was checked in clinical tissue specimens of human OSCC using avidin-biotin-peroxidase complex method of immunohistochemical staining with mouse monoclonal antibody for MASPIN protein. As all the tumors were in Stage III due to little awareness about the

Table 2: Association of MASPIN and grade of OSCC

MASPIN expression	Tumor grade			Total
	Grade 1	Grade 2	Grade 3	
Negative	7	2	6	15
Positive	22	10	3	35
Total	29	12	9	50

MASPIN: Mammary serine protease inhibitor, OSCC: Oral squamous cell carcinoma

Table 3: Correlation between MASPIN and grade of OSCC

Spearman's rho	Histological grade	MASPIN expression
Histological grade		
Correlation coefficient	1.000	-0.300*
Sig. (2-tailed)	0	0.034
N	50	50
MASPIN expression		
Correlation coefficient	-0.300*	1.000
Sig. (2-tailed)	0.034	0
N	50	50

MASPIN: Mammary serine protease inhibitor, OSCC: Oral squamous cell carcinoma

disease among the local population, delay in seeking proper healthcare and fear of costs. The histological tumor grade of OSCC was found to significant association with MASPIN expression and its correlation was negative which means that with the increase in tumor grade increases (or decrease in cellular differentiation) of OSCC, MASPIN expression decreased. Thus, MASPIN expression may be regarded as a reliable indicator of cellular differentiation and moreover, it also provides an indirect clue that MASPIN plays an important role in the carcinogenesis of OSCC. In a recent study, MASPIN expression in OSCC has been found to be associated with the histological tumor differentiation grade and observed as a predictor of better prognosis in OSCC and that MASPIN probably plays a role in tumor progression [12]. However, Zhang and coworkers failed to establish significant relation between the expression of MASPIN and histological grade or tumor size in OSCC. They concluded that MASPIN gene may down-regulate the expression of VEGF gene and thus play an important role in the tumor progression through modulation of angiogenesis [13].

In the current study, MASPIN localization was cytoplasmic in all (100%) cases. In a recent study, MASPIN expression has been reported to be a significant marker in predicting prognosis of OSCC [14]. In another study, the higher nuclear expression of MASPIN was found to be related to significantly reduced recurrence rate and thus associated with longer disease-free survival [15]. It may be bad news of the patients of the current study as all had cytoplasmic expression of MASPIN and thus may have more tendencies of distant metastasis and regional lymph node involvement. However, the survival data were not collected for the patients of current study due to logistic feasibility, which is an important limitation of this study so the authors could not determine the role of MASPIN expression and its localization on patients' survival. In a recent study, significantly longer disease-free interval and overall survival periods for tumors

immunohistochemically positive for MASPIN than for tumors negative for MASPIN was observed and the absence of MASPIN expression was found more frequently in cases of subsequent cervical lymph node metastasis than in cases without metastasis. Thus decreased MASPIN expression may be a significant factor associated with the metastatic potential of stage I and II oral SCCs [16]. An important study reported different localization patterns of MASPIN in oral SCC came to the view in which association between MASPIN cytoplasmic expression and pN stage was found in OSCC. This study was an effort to explain the paradoxical expression of MASPIN in the progression of tumor that is due to regulation of different functions at the subcellular level [8].

Historically, Xia *et al.* (in year 2000) examined the expression levels of MASPIN in 44 biopsies of OSCC for the first time and found a positive relation between the higher MASPIN expressions with the absence of lymph node metastases. Higher MASPIN expression was significantly associated with longer disease free interval and thus good prognosis. They thought MASPIN to be a favorable prognostic marker [17]. MASPIN's role was also investigated in pharyngeal carcinoma and loss of MASPIN expression has been related to the tumor grade but no association with cancer location, lymph node involvement or stage of the tumor established [18]. Very recently, it has been reported that loss of heterozygosity in MASPIN gene (18q21) was more frequently identified in the OSCC as compared to the normal mucosal tissues and the expression of MASPIN was also higher in tumor tissues which means that MASPIN has important role in the carcinogenesis of OSCC [10]. In oral lesion, intense immunohistochemical expression of MASPIN in the middle third of the epithelium could be regarded as a diagnostic sign of epithelial dysplasia and an indication of carcinoma in the upper third. The correlations between MASPIN and controlling factors (e.g. p63 and p53) may be events with key roles in the development of OSCC [19]. In 2008, MASPIN's role in OSCC was proved by noticing the expression of MASPIN in OSCC itself, paratumor tissue and normal tissue by immunohistochemistry and the results showed that its expression decreases from normal to the progression of OSCC indicating its significance [20]. MASPIN expression has been determined in actinic cheilitis and squamous cell carcinoma and loss of MASPIN expression has been linked to an increase in epithelial dysplasia pointing towards an increase in cancer progression and severity [21]. MASPIN expression has also been observed in CIN 3, microinvasive SCC and invasive SCC of uterine cervix and the results showed that the MASPIN expression decreased as the cancer progresses towards invasiveness [22].

CONCLUSION

In conclusion, we conclude that the MASPIN expression is negatively correlated with histological grades of OSCC and its subcellular localization is cytoplasmic in all cases included in the current study. However, due to lack of survival data current study gives no idea about the role of MASPIN expression with patient outcome.

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ETHICAL APPROVAL

The project was approved by Ethics Committee and Board of Advance Studies and Research, University of Health Sciences Lahore.

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