ORIGINAL ARTICLES

UDC: 616.22-006
DOI: https://doi.org/10.2298/vsp191223015R

Clinical significance of matrix metalloproteinase (MMP)-2 and MMP-9 expression in laryngeal squamous cell carcinoma

Klinički značaj ekspresije matriks metaloproteinaza (MMP)-2 i MMP-9 kod skvamocelularnog karcinoma larinksa

Dejan Rašić*, Aleksandar Perić*†, Biserka Vukomanović Djurdjević†‡, Jelena Sotirović*†, Nenad Baletić*†, Milanko Milojević*†, Ljubomir Pavićević*, Aleksandar Dimić*

Military Medical Academy, *Clinic for Otorhinolaryngology, ‡Institute of Pathology, Belgrade, Serbia; †University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Abstract

Background/Aim. The previous researches suggest that matrix metalloproteinases (MMPs) have the ability to degrade extracellular matrix components and play an important role in malignant tumour progression and metastasis. The aim of this study was to investigate MMP-2 and MMP-9 expression in the tissue of laryngeal squamous cell carcinoma (LSCC) and to evaluate their clinical significance. **Methods.** In this prospective study the samples of tumour tissue of seventy patients with LSCC (45 glottic and 25 supraglottic), and samples of laryngeal mucosa of 70 patients with chronic laryngitis were immunohistochemically stained for MMP-2 and MMP-9. We studied the relationships between MMPs tissue expression and clinical and histological characteristics of the patients with LSCC in comparison to the patients with chronic laryngitis. **Results.** MMP-2 and MMP-9 expression was significantly higher in the tissue of both glottic and supraglottic LSCC than in chronically inflamed laryngeal mucosa ($p < 0.001$ and $p < 0.001$, respectively). There were positive correlations between epithelial MMP-2 expression and the presence of perineural invasion ($r = 0.515$, $p < 0.008$), lymphovascular invasion ($r = 0.559$, $p < 0.004$) and disease recurrence ($r = 0.415$, $p < 0.039$) in supraglottic LSCC, as well as between MMP-2 expression and the presence of the exophytic type of tumour growth ($r = 0.347$, $p < 0.020$) in glottic LSCC. Epithelial MMP-9 expression was associated with lymphovascular invasion ($r = 0.331$, $p < 0.026$) and the presence of the exophytic tumour growth ($r = 0.474$, $p < 0.001$) in glottic LSCC. **Conclusion.** MMP-2 and MMP-9 can be used as potential biomarkers for the assessment of LSCC progression.

Key words:
biomarkers; glottis; immunohistochemistry; larynx, neoplasms; laryngitis; matrix metalloproteinases.

Apstrakt

Uvod/Cilj. Prethodna istraživanja sugerišu da matriks-metaloproteinaze (MMP) imaju sposobnost da razgrađuju komponente vanćelijskog matriksa i imaju veliku ulogu u progresiji malignih tumora i nastanku metastaza. Cilj ove studije je bio da se ispita ekspresija MMP-2 i MMP-9 u tkivu skvamocelularnog karcinoma larinksa (SCKL) i da se proceni njihov klinički značaj. **Metode.** U prospektivnoj studiji, uzorci tumorskog tkiva 70 bolesnika sa SCKL (45 sa karcinomom glotisa i 25 sa karcinomom supraglotisa) i uzoraka tkiva sluznice larinksa 70 bolesnika sa hroničnim laringitisom bojeni su imunohistohemijski na MMP-2 i MMP-9. Ispitivana je povezanost između nivoa imunohistohemijske ekspresije MMP i kliničkih i histoloških karakteristika bolesnika sa SCKL u odnosu na bolesnike sa hroničnim laringitisom. **Rezultati.** Ekspresija MMP-2 i MMP-9 bila je značajno viša u tkivu glotičnog i supraglotičnog SCKL u odnosu na ekspresiju ispitivanih MMP u tkivu hronično inflamirane sluznice larinksa ($p < 0,001$ i $p < 0,001$, redom). Nađena je pozitivna korelacija između epitelne ekspresije MMP-2 i perineuralne invazije ($r = 0,515$; $p < 0,008$), limfovaskularne invazije ($r = 0,559$; $p < 0,004$) i pojave recidiva bolesti ($r = 0,415$; $p = 0,039$) kod supraglotičnog SCKL, kao i između ekspresije MMP-2 i pojave egzofitičnog tipa rasta tumora ($r = 0,347$; $p = 0,020$) kod glotičnog SCKL. Ekspresija MMP-9 u epitelu bila je povezana sa limfovaskularnom invazijom ($r = 0,331$; $p = 0,026$) i pojavom egzofitičnog tumorskog rasta ($r = 0,474$; $p < 0,001$) kod glotičnog SCKL. **Zaključak.** Endopeptidaze MMP-2 i MMP-9 mogu biti korišćene kao potencijalni biomarkeri za procenu progresije SCKL.

Ključne reči:
biomarkeri; glotis; imunohistohemija; larinks, neoplazme; laringitis; matriks metaloproteinaze.

Correspondence to: Dejan Rašić, Military Medical Academy, Clinic for Otorhinolaryngology, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: dejan_rasic@ymail.com.

Introduction

Laryngeal malignant tumours make about 1%–2% of all malignant tumours in the human population [1]. Laryngeal squamous cell carcinoma (LSCC) is more common in male than in female population, occurring usually between the fifth and seventh decade of life. The most important etiological factors for LSCC occurrence are tobacco smoking, consumption of alcoholic drinks, gastroesophageal reflux, human papillomavirus (HPV) infections, air pollution, a diet low in fruits and vegetables. Alcohol in combination with tobacco significantly increases the risk of laryngeal cancer [1].

The prognosis of LSCC depends on the region involved in malignant disease formation, actual stage of disease, which can be determined by Tumour Node Metastasis (TNM) classification. Survival is significantly reduced due to the cancer development and spread of the disease (local or regional recurrence and distant metastasis occurrence) [2]. In recent years, molecular investigations have discovered many mechanisms of oncogenesis in malignant tumours. The expression of the specific tumour markers is associated with development and biological behaviour of laryngeal carcinoma. Prognostic value identification of some tumour markers would be of great help in selecting treatment modalities, which is of crucial significance for the prognosis of the disease. Matrix metalloproteinases (MMPs) are the family of 23 endopeptidases, or gelatinases, structurally very similar proteolytic enzymes which have the ability to degrade extracellular matrix components [3]. Thus, MMPs play important role in tumour progression and metastasis. The elevated levels of MMPs can be detected in the tumour tissue and serum of patients with advanced cancer. MMPs may be activated by various agents including other protease. Depending on the type of substrate which degrades or structural domains, MMPs can be divided into 6 subgroups. MMP-2 and MMP-9 have the ability to destroy the type IV collagen, which is a very important component of basement membrane [4]. This is a key moment, which is necessary for tumour growth and distant metastasis onset. Some published papers have shown that MMP-2 and MMP-9 may play important roles in the progression of the head and neck squamous cell carcinoma [4, 5]. However, the results regarding the correlation of MMP-2 and MMP-9 expression levels with clinical and pathological characteristics, as well as with prognosis of LSCC, are not consistent. The identification of patients with a high risk of tumour development, the presence of subsequent locoregional tumour recurrence, as well as the existence of regional metastases, are very important for the prognosis of the disease, as well as for the choice of the best treatment modality.

The aim of the study was to evaluate the clinical significance of MMP-2 and MMP-9 expression in the tissue samples of patients with LSCC, comparing their expression in glottic and supraglottic region of the larynx. To our knowledge, this is the first study completely investigating the relationships between the MMPs expression, clinical characteristics of LSCC, 5-year survival rates, and the presence of lymphovascular and perineural invasion of the malignant cells in patients with LSCC.

Methods

Study population

This prospective, observational, cross-sectional study included a total of 140 patients, 70 with histologically verified LSCC and 70 with chronic laryngitis. The patients with the malignant tumour were divided into groups with respect to: the localization of the tumour in the larynx (supraglottic, glottic); TNM stages of the disease; a degree of tumour differentiation (good, moderate, poor); the disease stages (I, II, II, IV); the types of tumour growth (infiltrative, exophytic, mixed); the development of locoregional recurrence (with or without disease recurrence); disease-free interval (with or without that), and 5-year survival (with or without 5-year survival). The patients diagnosed, treated and followed-up for LSCC for a period of minimlly 5 years (between 2010 and 2017) in our Clinic for Otorhinolaryngology were included in this prospective, observational study. The control group consisted of patients diagnosed and treated for chronic laryngitis in the same period. Pathological and immunohistochemical analyses of tissue specimens were performed at the Institute of Pathology by the same experienced pathologist. The research was approved by the Ethics Committee of our Institution. A written informed consent was obtained from all participants to use their medical data.

The criteria for inclusion in the study were histologically verified LSCC and histologically verified chronic inflammatory changes in laryngeal mucosa, including the patients with keratosis (hyperkeratosis, parakeratosis, etc). All patients enrolled in the study underwent a primary surgical treatment. They were interviewed about their profession, history of malignant diseases in the family, their habits and health status. The criteria for exclusion from the study were other malignant diseases, previous radiotherapy or chemotherapy, previously performed surgical treatment of laryngeal tumour, systemic diseases affecting the larynx, laryngeal benign pseudotumours (vocal fold oedema, polyps, granulomas, etc).

Histopathological examination

Tissue specimens, that were obtained during the laryngomicroscopy or laryngectomy, were fixed for 24 h in 4% buffered formaldehyde solution. Then, they were washed by water and dehydrated by concentrated ethanol (70% up to absolute), then lipofilled in xylene and embedded in paraffin. Paraffin blocks were sectioned at the thickness of 3–5 μm. The sections were stained with hematoxylin-eosin (HE). The type and dimensions of tumour, histological and nuclear grade, perineural and lymphovascular invasion, keratinisation of tumour, spread beyond the larynx or in the regional lymph nodes were determined by the preparations. Determination of TNM status was done according to the TNM classification of American Joint Committee on Cancer (AJCC) [6].

Immunohistochemical staining

Immunohistochemical staining includes a series of technological procedures: deparaffining after cutting the sections of 3–4 μm form paraffin mold and drying phase following the sinking in xylene, alcohol and distilled water then proteolytic digestion. Deparaffined sections were cooked twice in a microwave oven in a cuvette with 250 mL of citrate buffer solution (10 mmol/L) at a maximum temperature, for five min. After that, sections were cooled in a citrate buffer at the room temperature for 30 min and washed with distilled water two times for thirty sec. The next phase involved the blocking of endogenous peroxidase: tissue sections were placed in 3% hydrogen peroxide for five min; then washed with distilled water, overlaid with a phosphate-buffered saline three times for two min. Immunohistochemical staining was performed with human anti-MMP-2 and anti-MMP-9 antibodies (R&D Systems, Inc, Minneapolis, USA). The analysis of MMP-2 and MMP-9 expression levels was performed semi-quantitatively on the basis the intensity of staining cytoplasm of epithelial and stromal cells by light microscopy. The staining was negative when no cells stained for MMP-2 or MMP-9 were found. Immunoreactivity was graded from 1 to 3 : 1 = weak (0%–10% stained cells), 2 = moderate (10%–50% stained cells) and 3 = strong (more than 50% stained cells).

Study power and the number of participants

According to the results of the previous study performed by Peschos et al. [7], we expected the difference of 20% in the expression of MMP-9 between LSCC tissue and chronic laryngitis tissue. The type I error (α-level) was set to 0.05. Using z-test (differences in proportions between independent groups), we calculated the minimum of 70 participants would be required in each group to raise the study power of 80%. For the calculation of the number of participants in each group, the G*Power 3.1.9 programme (Heinrich Heine Universität, Düsseldorf, Germany) was used.

Statistical analysis

Statistical analysis of the data was done with the statistical software package, SPSS Statistics 18 (SPSS INC, Chicago, Illinois, USA). As the majority of variables were expressed as categorical data (frequencies), we used the χ^2 test to compare the values of different frequencies. In case of continuous data, the variables were presented as mean value ± standard deviation (SD), median, min and max values. Kolmogorov-Smirnov test was used for the evaluation of the normality of the data distribution. The levels of statistical significances between the two groups were assessed by the *t*-test and Mann-Whitney test. A one-way analysis of variance (ANOVA) was used to calculate the differences among three groups of participants. To assess the levels of the statistical differences, we performed a *post-hoc* Dunn-Bonferroni correction. For the comparison of the disease free interval and 5-year survival between glottic and supraglottic LSCC, we used Kaplan-Meier analysis. The Pearson's and Spearman's correlation analyses were used to establish the value of relation between parameters. All the differences were estimated at $p < 0.05$ to be statistically significant.

Results

The mean age of patients with LSCC was 59.4 (± 7.1) years, range from 40–79 years. There were 60 (85.7%) male and 10 (14.3%) female patients. Among them, 45 (64.3%) of patients had glottic LSCC and 25 (35.7%) of patients had supraglottic LSCC, mean age of 59.7 (± 6.8) years and 58.8 (± 7.7), respectively. The majority of patients with glottic LSCC were in T1/T2 stage, whereas the majority of supraglottic LSCC patients were in T3/T4 stage. Table 1 shows the distribution of patients according to T and N stages. The most common stage of glottic LSCC was I (53.3%), and the most common stage in supraglottic LSCC was IV (40.0%). It was found that more frequent lymph node metastases (N+ status) were present in supraglottic than in glottic LSCC (40% *vs* 4.4%) (Table 1). The 5-year survival rates for glottic LSCC and supraglottic LSCC were 77.8% and 76.0%, respectively. The disease-free interval was present in 71.1% of the glottic LSCC patients and 68.0% of the supraglottic LSCC. We found no significant difference regarding the disease-free interval and 5-year survival between glottic and supraglottic LSCC ($p = 0.835$ and $p = 0.776$, respectively).

The positive MMP-2 expression in epithelial cells was detected in 65 (92.9%) of patients with LSCC (Figure 1a). In stromal cells, positive immunostaining was found in 66 (94.3%) of cases. In patients with the diagnosis of chronic laryngitis, the MMP-2 expression was negative in 72.9% of specimens (Figure 1b).

The positive MMP-9 epithelial immunostaining was detected in all LSCC specimens (Figure 2a). Positive stromal expression of MMP-9 was detected in 69 (98.6%) of LSCC

Table 1

Distribution of patients according to T and N stages

Region of larynx	T	n (%)	N	n (%)
Glottis	T1/T2	36 (80)	N0	43 (95.6)
	T3/T4	9 (20)	N+	2 (4.4)
Glottis total		45 (100)		45 (100)
Supraglottis	T1/T2	7 (28)	N0	15 (60)
	T3/T4	18 (72)	N+	10 (40)
Supraglottis total		25 (100)		70 (100)

T1-T4 – tumour stage; N – lymph node; N0 – without lymph node metastasis; N+ – with lymph node metastasis.

specimens. In patients with chronic laryngitis, moderate MMP-9 expression was found in 47.1% of tissue specimens (Figure 2b). Our results revealed significantly higher MMP-2 and MMP-9 expression in both epithelium and stromal tissue specimens of patients with glottic and supraglottic LSCC in comparison to patients with chronic laryngitis ($p < 0.001$ and $p < 0.001$, respectively). These results are presented in Figure 3.

In patients with glottic LSCC, we found a positive correlation between the epithelial and stromal MMP-2 expression ($r = 0.315$, $p < 0.05$). We also found a positive relationship between the epithelial MMP-2 and epithelial MMP-9

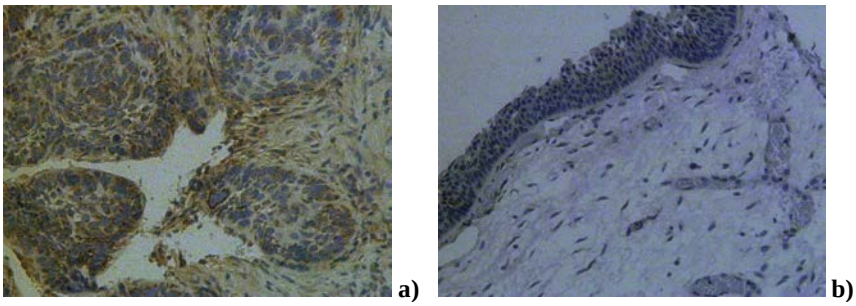

Fig. 1 – Immunoreactivity of matrix metalloproteinase (MMP)-2 in (hematoxylin-eosin, x40): a) laryngeal squamous cell carcinoma (LSCC); b) chronic laryngitis.

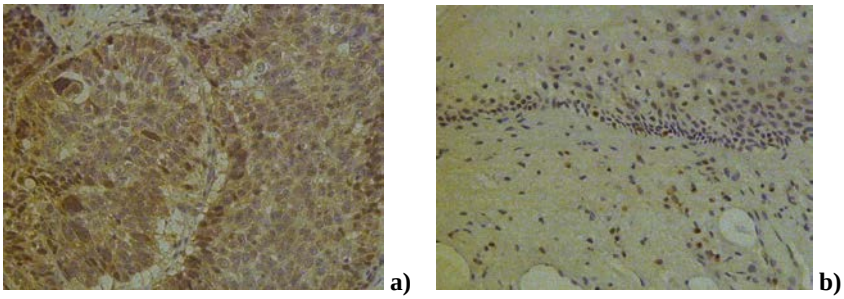

Fig. 2 – Immunoreactivity of matrix metalloproteinase (MMP)-9 in (hematoxylin-eosin, x40): a) laryngeal squamous cell carcinoma (LSCC); b) chronic laryngitis.

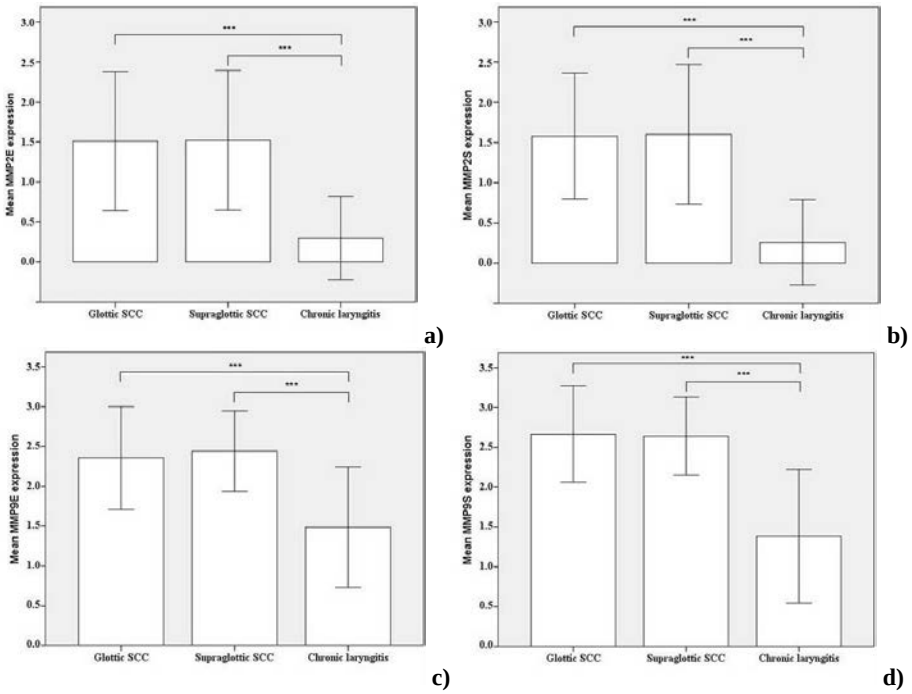

Fig. 3 – Comparison of matrix metalloproteinase (MMP) expression in tissue specimens of patients with glottic squamous cell carcinoma (SCC), supraglottic SCC, and chronic laryngitis: a) expression of MMP-2 in epithelium (MMP-2E); b) expression of MMP-2 in stroma (MMP-2S); c) expression of MMP-9 in epithelium (MMP-9E); d) expression of MMP-9 in stroma (MMP-9S). ***$p < 0.001$ *vs* chronic laryngitis.

immunostaining (r = 0.313, p = 0.05). Also, there was a positive correlation between the epithelial and stromal MMP-9 expression (r = 0.435, p = 0.01). In patients with supraglottic LSCC, there was a statistically significant relationship between the epithelial and stromal MMP-2 expression (r = 0.397, p = 0.05), also between the epithelial MMP-2 and epithelial MMP-9 (r = 0.517, p = 0.01) and stromal MMP-9 expression (r = 0.491, p = 0.05). Also, we found a positive correlation between the epithelial and stromal MMP-9 expression (r = 0.497, p = 0.05) in supraglottic LSCC. All results regarding the correlations between tested marker expressions are presented in Table 2.

The correlations between stromal MMP-2 and MMP-9 expressions and clinicopathological characteristics of both glottic and supraglottic LSCC were not presented due to the statistical insignificances. The statistically significant correlations between the expression of MMP-2 in epithelial cells and clinicopathological features of LSCC are shown in Table 3.

Our study showed that epithelial MMP-2 expression is not in correlation with T or N stages, nuclear grade and clinical stages, disease-free interval and 5-year survival.

In supraglottic LSCC, the MMP-2 epithelial expression was in correlation with the presence of perineural (r = 0.515, p = 0.008) and lymphovascular (r = 0.559, p = 0.004) invasion. A correlation between the epithelial MMP-2 expression and local recurrences of the primary supraglottic cancer (r = 0.415, p = 0.039) was noticed as well. Also, a significant negative correlation was recorded between the MMP-2 epithelial expression and histological stage (r = -0.521, p = 0.008) in supraglottic LSCC. The MMP-2 tumour cell expression was in correlation with the presence of exophytic type of tumour growth in glottic LSCC (r = 0.347, p = 0.020). In supraglottic LSCC, we found no association between the type of tumour growth and the level of MMP-2 expression.

The most important correlations between the MMP-9 epithelial expression and clinicopathological features of LSCC are presented in Table 3.

The present study revealed neither the correlation be-

Table 2

Correlations between MMPs expressions in glottic and supraglottic region of the larynx

Region of larynx			MMP-2E	MMP-2S	MMP-9E	MMP-9S
Glottis	MMP-2E	r	–	0.315	0.313	0.180
		p		0.035	0.037	0.236
	MMP-2S	r			0.259	0.229
		p			0.086	0.131
	MMP-9E	r			–	0.435
		p				0.003
Supraglottis	MMP-2E	r	–	0.397	0.517	0.491
		p		0.049	0.008	0.013
	MMP-2S	r		–	0.110	0.347
		p			0.601	0.089
	MMP-9E	r			–	0.497
		p				0.012

***r* – Spearman's correlation coefficient; *p* – probability; MMP – matrix metalloproteinase; E– expression in epithelium; S – expression in stroma.**

Table 3

Correlations between expressions of MMP-2 and MPP-9 in epithelial cells and clinicopathological features of LSCC

Region of larynx	Clinicopathological features	**MMP-2** (n = 70)		**MMP-9** (n = 70)	
		r	*p*	*r*	*p*
Glottis	Histologic grade	-0.188	0.215	-0.257	0.284
	LV invasion	0.083	0.588	0.031	0.026
	PN invasion	0.074	0.542	0.100	0.409
	Local recurrence	-0.211	0.164	0.178	0.332
	Exophytic growth	0.347	0.020	0.474	0.001
Supraglottis	Histologic grade	-0.521	0.008	-0.358	0.187
	LV invasion	0.559	0.004	0.345	0.092
	PN invasion	0.515	0.008	0.161	0.442
	Local recurrence	0.415	0.039	0.183	0.401
	Exophytic growth	0.265	0.201	-0.228	0.273

***r* – Pearson's correlation coefficient; *p* – probability; MMP – matrix metalloproteinase; LV – lymphovascular; PN – perineural; LSCC – laryngeal squamous cell carcinoma.**

tween the epithelial MMP-9 expression and some clinicopathological characteristics (T stage, N stage, clinical stage, nuclear stage, histological stage), nor the correlation with disease-free interval and 5-year survival.

Epithelial MMP-9 expression significantly correlated with the presence of lymphovascular invasion (r = 0.334 p = 0.026) and with the presence of exophytic type of the tumour growth (r = 0.474, p = 0.001) in glottic LSCC. In patients with supraglottic localization of SCC, there were no similar relationships. We found no statisticaly significant relationship between the expression of MMP-9 in epithelial cells and perineural invasion in the LSCC.

Discussion

Our research, as well as the previous studies [7–18], demonstrated that MMP-2 and MMP-9 are highly expressed in tissue of LSCC in comparison with the mucosa of patients with chronic laryngitis, benign laryngeal pseudotumours (vocal fold polyps, oedemas, granulomas, etc), and normal laryngeal mucosa. MMPs are produced by various types of cells such as epithelial, inflammatory cells, fibroblasts, macrophages in both epithelium and stroma [3–5, 7–11]. As well as in the study of Uloza et al. [8], we demonstrated that MMP-2 and MMP-9 are produced by both epithelial and stromal cells in LSCC. However, Uloza et al. [8] found a significantly higher expression of these markers in stromal than in epithelial cells. We found no statistical differences between stromal and epithelial expression of the examined enzymes. Also, we found a positive relationship between the epithelial and stromal MMP-2 and MMP-9 expression in both glottic and supraglottic region of the larynx.

Earlier studies presented the contradictory results related to expression of the MMPs and their relationships with clinical and pathological features of LSCC. Our study demonstrated no statistically significant relationship between MMP-2 and MMP-9 expression and the clinical stage of LSCC, which is in accordance with the studies presented by Liu et al. [10] and Wael and Manal [11].

The presence of locoregional lymph node metastases is an important prognostic factor. We found no correlation between MMP-2 and MMP-9 expression and the presence of lymph node metastases, which is in accordance with the study performed by Akdeniz et al. [12]. On the other hand, in the studies presented by Sarioglu et al. [13] and by Yuce et al. [14], overexpression of MMP-2 and MMP-9 was found to be significantly higher in cases of lymph node involvement in patients with glottic [13] and supraglottic [14] LSCC.

The histological grade of tumour may be a predictor of cancer behaviour and contribute to the finding of the best modality of the treatment. In order to determine the significance of MMP-2 and MMP-9 expression, many researchers have evaluated the relationship between the MMP tumour expression and the level of tumour histological differentiation. However, the results are not consistent. Sarioglu et al. [13] indicated that MMP-2 expression is not associated with the level of differentiation of LSCC. Our study generally demonstrated no such relation, but in patients with supraglottic carcinoma localisation, a negative correlation was found between the expression of MMP-2 and the histological grade of the tumour. So, our results suggest that lower level of epithelial MMP-2 expression could be associated with the higher level of differentiation of supraglottic LSCC.

The previous studies demonstrated that the higher expression of MMP-2 and MMP-9 is associated with poor outcome of oncological treatment [4, 5, 7]. Many researchers pointed out high MMP-2 and MMP-9 expression as a potential marker of worse prognosis in patients with laryngeal cancer. Although we found no such relationship, Mallis et al. [15] showed a statistically significant difference ($p < 0.05$) for the 5-year overall survival rate between the groups with positive and negative MMP-2 expression in patients with glottic LSCC.

Numerous studies assessed the MMP-2 and MMP-9 immunostaining in the laryngeal cancer. In only few studies, the laryngeal subregions were investigated separately [7, 10, 15]. Our study compared the immunoreactivity of MMP-2 and MMP-9 in patients with supraglottic and glottic LSCC. We found that the level of MMP-2 expression is significantly related to the presence of perineural and lymphovascular invasion, and the presence of local disease recurrence in the supraglottic region. So, according to our results, MMP-2 could be considered as a potential predictive factor for the estimation of spreading the disease in patients with supraglottic LSCC.

Our results demonstrated a positive correlation between epithelial MMP-9 expression and the presence of lymphovascular invasion in patients with glottic LSCC. Wittekindt et al.[17] found a positive correlation between MMP-9 expression and blood vessel density, suggesting that MMP-9 may be a potential target to disrupt tumour neovascularisation during the oncological therapy of LSCC. A recent study by Colovic et al. [18] demonstrated the overexpression of MMP-9 in patients with tumour relapses. MMP-9 could be considered as a potential predictive factor for the assessment of spreading and recurrence of primary disease in glottic region of the larynx. Finally, our results showed a highly significant positive correlation between the level of MMP-2 and MMP-9 immunostaining and the presence of exophytic tumour growth only in glottic LSCC, suggesting the importance of MMP-2 and, especially, MMP-9 expression as potential predictors for the feature of vegetant type of tumour growth in glottic LSCC.

However, our study had some limitations. Due to the financial reasons, we did not perform the measurement of MMP-2 and MMP-9 levels in the serum of patients with LSCC. Also, we did not evaluate the quantitative real-time polymerase chain reaction reactivity for the messenger RNA (mRNA) production for MMP-2 and MMP-9. The previous investigations demonstrated important relationships between the serum level/mRNA tissue immunostaining for MMP-2/MMP-9 and clinical stage, nodal status and survival rates in patients with LSCC [19–21].

Conclusion

This study demonstrated a higher expression of MMP-2 and MMP-9 in both epithelium and stroma of patients with LSCC in comparison to patients with chronic laryngitis. Our results suggest that MMP-2 expression can serve as a potential parameter for the detection of patients with supraglottic LSCC who are at a high risk of perineural and lymphovascular invasion and developing disease recurrence. MMP-9 expression can be a strong predictor of the presence of exophytic type of tumour growth, as well as of the presence of lymphovascular invasion in glottic LSCC.

R E F E R E N C E S

1. *Smee R, Williams JR, Kotevski DP.* Management of locally advanced T3-4 glottic laryngeal carcinomas. J Laryngol Otol 2018; 132(7): 642−50.
2. *Tang D, Tao L, Zhou L, Zhang M, Wu X, Li X,* et al. Retrospective analysis of 659 laryngeal squamous cell carcinoma patients treated with open laryngeal function-preserving operations. Acta Otolaryngol 2018; 138(11): 1043−50.
3. *Whittaker M, Ayscough A.* Matrix metalloproteinases and their inhibitors: current status and future challenges. Celltransmissions 2001; 17(1): 3−14.
4. *Gou X, Chen H, Jin F, Wu W, Li Y, Long J,* et al. Expressions of CD147, MMP-2 and MMP-9 in laryngeal carcinoma and its correlation with poor prognosis. Pathol Oncol Res 2014; 20(2): 475−81.
5. *Rydlova M, Holubec L Jr, Ludvikova M Jr, Kalfert D, Franekova J, Povysil C,* et al. Biological activity and clinical implications of the matrix metalloproteinases. Anticancer Res 2008; 28(2B): 1389−97.
6. *Deschler DG, Moore MG, Smith RV.* Quick Reference Guide to TNM Staging of Head and Neck Cancer and Neck Dissection Classification, 5th ed. Alexandria, VA: American Academy of Otolaryngology – Head and Neck Surgery Foundation; 2014.
7. *Peschos D, Damala C, Stefanou D, Tsanou E, Assimakopoulos D, Vougiouklakis T,* et al. Expression of matrix metalloproteinase-9 (gelatinase B) in benign, premalignant and malignant laryngeal lesions. Histol Histopathol 2006; 21(6): 603−8.
8. *Uloza V, Liutkevicius V, Pangonyte D, Lesauskaite V.* Characteristics of expression of matrix metalloproteinases (MMP-2 and MMP-9) in glottic squamous cell carcinoma and benign vocal fold lesions. Clin Exp Otorhinolaryngol 2015; 8(1): 57−64.
9. *Lotfi A, Mohammadi G, Saniee L, Mousaviagdas M, Chavoshi H, Tavassoli A.* Serum level of matrix metalloproteinase-2 and -9 in patients with laryngeal squamous cell carcinoma and clinical significance. Asian Pac J Cancer Prev 2015; 16(4): 1327−30.
10. *Liu WW, Zeng ZY, Wu QL, Hou JH, Chen YY.* Overexpression of MMP-2 in laryngeal squamous cell carcinoma: a potential indicator for poor prognosis. Otolaryngol Head Neck Surg 2005; 132(3): 395−400.
11. *Wael S, Manal S.* Expression of nm23H1 and MMP2 in laryngeal carcinoma and its role in aggressiveness of the tumour and node metastasis. Egypt J Otolaryngol 2013; 29(2): 86−92.
12. *Akdeniz O, Akduman D, Haksever M, Ozkarakas H, Muezzinoglu B.* Relationships between clinical behavior of laryngeal squamous cell carcinomas and expression of VEGF, MMP-9 and E-cadherin. Asian Pac J Cancer Prev 2013; 14(9): 5301−10.
13. *Sarioglu S, Ozer E, Kirimca F, Sis B, Pabuccuoglu U.* Matrix metalloproteinase-2 expression in laryngeal preneoplastic and neoplastic lesions. Pathol Res Pract 2001; 197(7): 483−6.
14. *Yuce I, Bayram A, Cagli S, Canoz O, Bayram S, Guney E.* The role of CD44 and matrix metalloproteinase-9 expression in predicting neck metastasis of supraglottic laryngeal carcinoma. Am J Otolaryngol 2011; 32(2): 141−6.
15. *Mallis A, Teymoortash A, Mastronikolis NS.* MMP-2 expression in 102 patients with glottic laryngeal cancer. Eur Arch Otorhinolaryngol 2012; 269(2): 639−42.
16. *Cao XL, Xu RJ, Zheng YY.* Expression of type IV collagen, metalloproteinase-2, metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in laryngeal squamous cell carcinomas. Asian Pac J Cancer Prev 2011; 12(12): 3245−9.
17. *Wittekindt C, Jovanovic N, Guntinas-Lichius O.* Expression of matrix metalloproteinase-9 (MMP-9) and blood vessel density in laryngeal squamous cell carcinomas. Acta Otolaryngol 2011; 131(1): 101−6.
18. *Colovic Z, Pesutić-Pisac V, Poljak NK, Racić G, Cikojević D, Kontić M.* Expression of matrix metalloproteinase-9 in patients with squamous cell carcinoma of the larynx. Coll Antropol 2013; 37(1): 151−5.
19. *Kalfert D, Ludvikova M, Topolcan O, Windrickova J, Malirova E, Pesta M,* et al. Analysis of preoperatice serum levels of MMP-1, -2, -9 in patients with site-specific head and neck squamous cell cancer. Anticancer Res 2014; 34(12): 7431−41.
20. *Li JZ, Gao W, Lei WB, Zhao J, Chan JY, Wei WI,* et al. MicroRNA 744-3p promotes MMP-9 mediated metastasis by simultaneously suppressing PDCD4 and PTEN in laryngeal squamous cell carcinoma. Oncotarget 2016; 7(36): 58218−23.
21. *Popov TM, Stancheva G, Goranova TE, Rangachev J, Konov D, Todorov S,* et al. Strong correlation between mRNA expression levels of HIF-2α, VEGFR1, VEGFR2 and MMP2 in laryngeal carcinoma. Pathol Oncol Res 2016; 22(4): 741−6.

Received on December 23, 2019
Revised on February 3, 2020
Accepted on February 4, 2020
Online First February, 2020