Expression of Maspin, KI-67 and CD105 as Predictors of Postoperative Recurrence in Laryngeal Carcinoma: perioperative planning and proposed reconstructive tools

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Abstract: Background: Maspin, a member of serpin super-family, has multifaceted biological functions and an unique tumor suppressing activity. Several studies showed that maspin suppresses tumor growth, angiogenesis, invasion and metastasis. The present study investigated the relationship between maspin expression, Ki-67 proliferative index (PI), CD105-assessed microvessel density (MVD) and postoperative recurrence in laryngeal squamous cell carcinoma (SCC). **Patients and methods:** Subcellular pattern of maspin expression was immunohistochemically evaluated in 28 cases of laryngeal SCC treated by total laryngectomy with reconstruction but without primary radiotherapy with a follow-up period from 10 to 36 months. The expression and interaction between Ki-67, CD105 and maspin were also studied. **Results:** Two patterns of positive maspin expression; cytoplasmic (n=9) and nuclear-cytoplasmic (n=16) were recognized. Significant inverse correlation between nuclear-cytoplasmic pattern and both Ki-67 PI (P=0.049) and CD105-assessed MVD (P=0.016) were disclosed. Comparing the two groups of patients with (pR+) and without (pR-) evidence of postoperative recurrence, none of the studied clinicopathological parameters (age, sex, pathological grade, tumor stage, and nodal stage) was significantly associated with recurrence (all p>0.05). The nuclear-cytoplasmic maspin expression was significantly higher in pR- patients (p=0.018), while higher Ki-67 PI and CD105-assesed MVD were significantly correlated with pR+ group (p= 0.007 & p = 0.004 respectively). **Conclusion:** The present results suggest that absence of nuclear localization of maspin and high Ki-67 PI and CD105-assessed MVD may predict a higher risk of recurrence in laryngeal SCC patients.

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1. Introduction

Laryngeal cancer is the second most common cancer of the respiratory tract with an estimated incidence rate of 5.1/ 100,000 cases in males worldwide in the year 2008 and the European incidence rate of 10/100,000. The age-standardized mortality (world standard) in laryngeal cancer for males is 2.2/100,000 [1]

Tumor site (glottic, supraglottic and subglottic) and staging determine the treatment modality and influence survival rates. For example, for patients with T1 or T2 laryngeal cancer, the American Society of Clinical Oncology (ASCO) recommends in its clinical practice guidelines an initial treatment that preserves the larynx[2].

On the other hand, a recent article by Lefebvre et al. [3] concluded that it was impossible to give general therapy recommendations because the selected therapy approach should involve a combination of a treatment guideline and a careful patient management process.

Further understanding of the molecular alterations of laryngeal SCC may allow providing more accurate and useful prognostic markers. Molecular and immunohistochemical diagnostics can detect abnormalities in lesions not yet appreciated histologically and thus predict early recurrences [4,5].

Since the discovery of the tumor suppressor gene p53, more than 15 different tumor suppressor genes have been identified [6] .Maspin (mammary serine protease inhibitor), a 42-kDa protein, is known to have a tumor- suppressor function. It belongs to the serpin (serine protease inhibitor) super-family which is categorized to inhibitory and non- inhibitory serpins [7]. Maspin expression has been demonstrated in several normal human tissues including breast, prostate, placenta, skin, oral cavity and lung [8].Previous findings supported the inhibitory effects of maspin as anti –invasive[9], inhibiting angiogenesis by blocking it both in vitro and in vivo models [10,11] and sensitizing apoptosis [12].In spite of being regarded as a tumor suppressor gene, paradoxically, both decreased and increased maspin levels have been described to parallel tumor progression [13,14]. This could be contributed to the fact that maspin demonstrates different subcellular localization that appears to be important in its function. It localizes primarily to the cytoplasm, but may also be located in

the secretory vesicles and the cell surface. Nuclear maspin has only recently been recognized[8,15]. Moreover, it has been noted that maspin nuclear localization is associated with favorable prognosis particularly in lung, gastric, and pancreatic carcinomas[9,14,16] .

The present study investigated the expression of maspin and its value in predicting carcinoma prognosis and recurrence in relation to its subcellular localization in a homogeneous group of laryngeal SCC patients concerning the treatment modality. All cases were treated by total laryngectomy with neck dissection without primary radiotherapy. The relationship between cell proliferation marker; Ki-67, microvessel density-assessing marker; CD-105, and recurrence of laryngeal SCC have also been considered. This might influence decision regarding extent of local excision, lymph node dissection, boundaries of estimated defects, and possible reconstruction tools.

2. Patients and Methods:

This study was carried out on 28 cases of primary laryngeal SCC, 19 males and 9 females, ranged from 32-70 years (median 50). Thorough clinical evaluation, routine preoperative, neck ultrasonography, head and neck contrast enhanced computerized tomography or/and magnetic resonance imaging were performed. All cases underwent total laryngectomy without primary radiotherapy at the departments of General Surgery and ENT, Zagazig University Hospital from May 2007 to January 2010. In ten cases the hypopharyngeal tissues left after total laryngectomy were sufficient, so we were not in need of reconstruction technique. In eight of our cases pectoralis major flap based on thoracoacromial vessels used as myocutaneous flap with adding part of the abdominal fascia and overlying skin. We had used this flap as a tubed one[17].

Four cases of our series, was operated by usage of free radial forearm flap. This flap raised on the radial artery, paired venae comitants and cephalic vein[18].

Two cases operated by free jejunal flaps for reconstruction of cervical esophagus. The pedicle of free jejunum is located within the mesentry. While the patients were, supine and two teams were operating simultaneously to decrease time and blood loss[19].

Four cases were reconstructed by the use of gastric pull up technique, and all our cases were followed up for about 10-36 months. All specimens were immediately sent to Pathology Department, processed, diagnosed and evaluated for tumor differentiation according to Geelen et al. [20] as 15 well, 9 moderately ,and 4 poorly differentiated cases.

According to TNM classification of Malignant Tumor of International Union Against Cancer[21] the pathological staging of primary laryngeal SCC (T) was T2 in 16, and T3 in 12 cases. Regional lymph node staging (N) was N1 in 17 and N2 in 11 cases

Immunostaing

It was performed on 4 microns formalin-fixed, paraffin-embedded tissue sections using the avidin – biotin peroxidase complex (ABC) procedure as described by Marioni et al.[22]. Antigen retrieval was performed for each section (microwave 750 w, 10mM citrate buffer, pH 6.0 for 15 minutes).Non specific binding was blocked with 10% normal rabbit serum.

Commercially available antibodies used were as follows: maspin antibody (clone G167-70,BD-Biotechnology, CA, USA, dilution 1:500) , Ki-67 (clone MIB-1,Dako,Glostrup,Denmark, dilution 1:100), and CD105(clone 4G11,Novocastra Lab., Ltd, UK, dilution 1:100). The immuno-staining was developed using diamino-benzidine as chromogen and Meyer's hematoxylin as counter stain.

As negative controls, the primary antibody was replaced by non – immune rabbit serum. Human normal breast tissue sections were used as positive control for maspin while sections of invasive breast cancer as controls for Ki-67 and CD105.

Evaluation criteria

Maspin subcellular pattern of distribution was scored as: negative, cytoplasmic, nuclear, and nuclear-cytoplasmic. The presence of strong cytoplasmic reactivity was investigated at x40 magnification. Weak cytoplasmic staining was considered negative[23].

Proliferative index (PI) was assessed by scoring the percentage of Ki-67 labeled nuclei in at least 5 high power fields (x 400 magnification). A cut-off value of 20% was chosen to separate cases with high and low index[24].

CD105-assessed MVD was measured by counting individual micro-vessel at x200 power field in three areas with the highest density (hot spots). Any brown- stained endothelial cell or cell cluster was considered a single microvessel while vessel lumen was not necessary to define a vessel .The mean count for each specimen was recorded and the median MVD was considered as a cut off value to determine high and low MVD[22].

Statistical analysis was performed using the SPSS program for Windows (SPSS Inc. Chicago, IL, USA), to assess the association between variables, chi-squared or Fisher exact test were used. $P < 0.05$ was considered significant.

3. Results

Normal squamous epithelial cells near SCC showed only weak cytoplasmic maspin expression (Fig. 1). In SCC cases, two positive immunostaining patterns were recognized: cytoplasmic and nuclear-cytoplasmic. Three out of 28 cases (10.8%) showed no or weak cytoplasmic reactivity that was considered negative .Strong cytoplasmic expression was found in 9 cases (32.1%)(Fig.2). Statistical analysis revealed no significant association between cytoplasmic maspin expression and age (p=0.79), sex (p=0.73), pathological grade (p=0.67), Tumor stage (p=0.77), Nodal stage (p=0.39) ,Ki-67 index (p=0.2),and MVD (p=0.73) (Table 1).

Nuclear-cytoplasmic pattern appear-ed in 16/28 cases (57.1%) (Fig.3 A&B). This pattern was not statistically correlated with the studied clinicopathological parameters {age (p=0.66), sex (p=0.77), pathological grade (p=0.93), T stage (p=0.50), and nodal stage (p=0.7)}. However, a significant inverse correlation was apparent between nuclear-cytoplasmic maspin expression and both Ki-67 index (p=0.049) and CD105 –assessed MVD (p=0.016) as shown in Table 1.

Only 11/28 (39.3%) cases developed loco-regional recurrence after a mean period of 25 +/-13 months. Table 2 showed comparison between the two patients' groups with (pR+) and without (pR-) postoperative recurrence. No significant difference was found with patient's age, sex, pathological grade, tumor stage, nodal stage and cytoplasmic maspin expression (all P>0.05).

However, nuclear-cytoplasmic expression was significantly higher in the pR- group (P= 0.018). On the other hand, both high Ki-67 index (Fig. 4) and CD105-assessed MVD (Fig. 5) were significantly associated with the pR+ group (P= 0.007 and 0.004 respectively)

Table(1) Relationship between clinicopathological, immunohistochemical results and subcellular maspin expression in laryngeal squamous cell carcinoma.

Variables	Score	+ ve maspin expression (n=25)					
		Cytoplasmic (n=9)		P value	Nuclear – cytoplasmic (n=16)		P value
		No.	%		No.	%	
Age	**≤ 50**	5	55.6	0.79	8	50	0.66
	> 50	4	44.4		8	50	
Sex	**Male**	7	77.8	0.73	11	68.8	0.77
	Female	2	22.2		5	31.2	
Pathological	**Well**	4	44.5		9	56.3	
grade	**Moderate**	3	33.3	0.67	5	31.3	0.93
	Poor	2	22.2		2	12.5	
Tumor stage	**T_2**	3	33.3	0.77	6	37.5	0.50
	T_3	6	66.7		10	62.5	
Nodal stage	**N1**	7	77.8	0.39	9	56.2	0.7
	N_2	2	22.2		7	43.8	
Ki-67 index	**≥ 20%**	5	55.6	0.2	3	18.8	0.049*
	< 20%	4	44.4		13	81.2	
Microvessel	**High**	3	33.3	0.73	2	12.5	0.016*
density	**Low**	6	66.7		14	87.5	

Table (2) Evaluation of clinicopathological and immunohistochemical parameters in laryngeal squamous cell carcinoma patients according to presence (pR+) or absence (pR-)of recurrence.

Variable	Score	PR + (n=11)		PR - (n=17)		P value
		No	%	No	%	
Age	**≤ 50**	6	54.5	10	58.8	0.86
	> 50	5	45.5	7	41.2	
Sex	**Male**	7	63.6	12	70.6	0.57
	Female	4	36.4	5	29.4	
Pathological grade	**Well**	5	45.5	10	58.8	0.48
	Moderate	4	36.3	5	29.4	0.97
	Poor	2	18.2	2	1.8	0.93
Tumor stage	**T_2**	6	54.5	10	58.8	0.86
	T_3	5	45.5	7	41.1	
Nodal stage	**N1**	7	63.6	10	58.8	0.59
	N_2	4	36.4	7	41.2	
Maspin expression	**Negative**	2	18.2	1	5.9	0.68
	Cytoplasmic	6	54.5	3	11.8	0.09
	Nuclear.-cytoplasmic	3	27.3	13	82.3	0.018*
Ki-67 index	**≥ 20**	9	81.8	2	11.8	0.007**
Microvessel density	**High**	7	63.6	1	5.9	0.004**

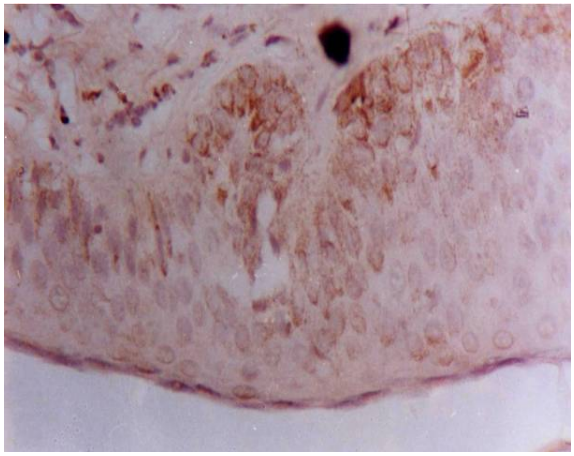


Fig (1): Normal laryngeal epithelial cells near SCC showed weak cytoplasmic maspin staining (ABC, Meyer's hematoxylin counter- stain, original magnification X400).

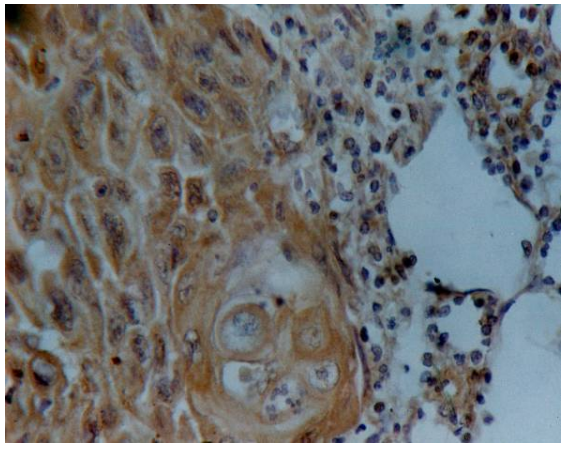
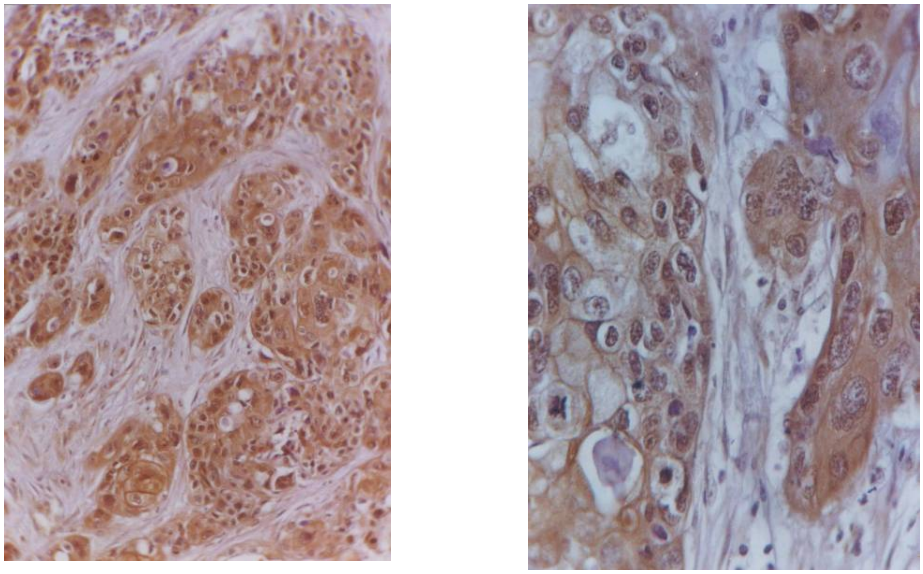


Fig. (2): Strong maspin cytoplasmic pattern of expression in a well differentiated laryngeal SCC (ABC, Meyer's hematoxylin counter-stain, original magnification X400).



A B

Fig. (3): Maspin nuclear-cytoplasmic pattern of expression in a moderately differentiated (A) and a poorly differentiated (B) SCC (ABC, Meyer's hematoxylin counter-stain, original magnification X200&X400 respectively).

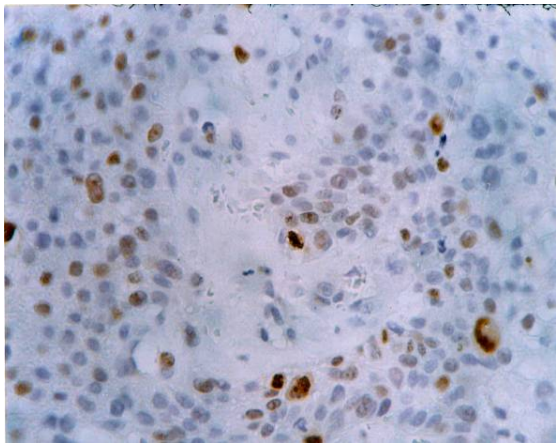


Fig. (4): Ki-67 immunoreactivity in a poorly differentiated SCC showing diffusely scattered positive nuclei with high labeling index (ABC, Meyer's hematoxylin counter-stain, original magnification X400).

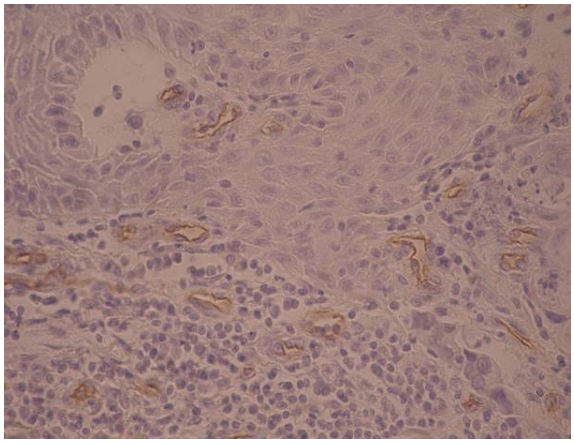


Fig. (5): CD105 immunoreactivity in a well differentiated SCC showing high microvessel density (ABC, Meyer's hematoxylin counter-stain, original magnification X400).

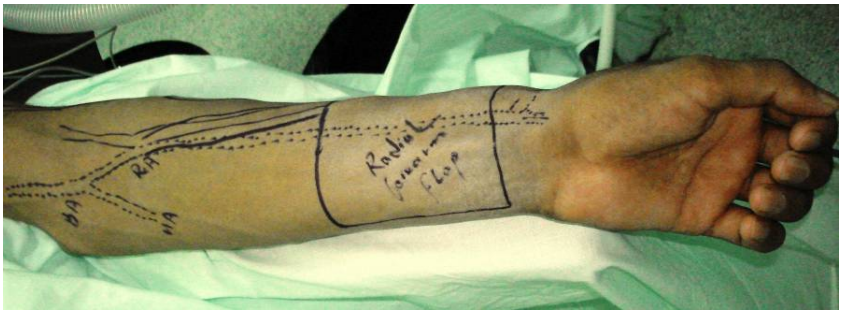


Fig. (6): Drawing of radialforearm flap

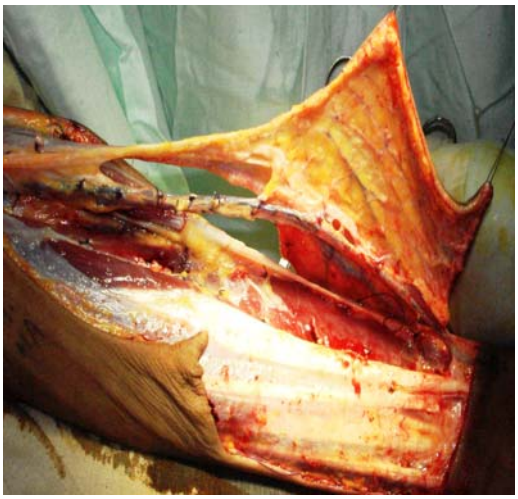


Fig. (7): Raising of radialforearm flap.

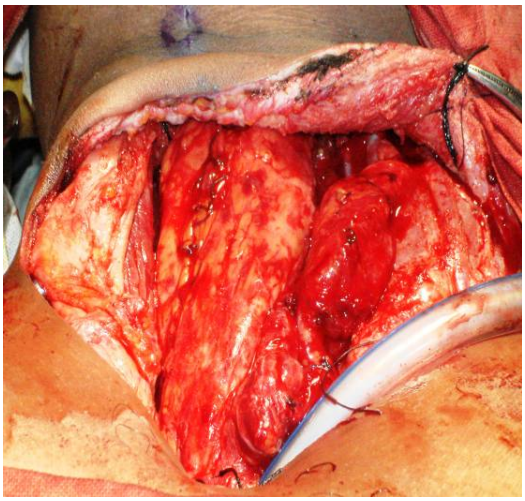


Fig. (8): Insetting of the flap after microsurgery anastomosis.

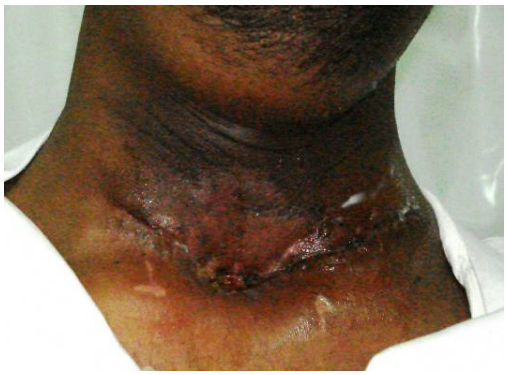


Fig. (9): postoperative.

4. Discussions

The cure rates of laryngeal SCC have improved little over the last few decades. Despite the higher number of therapeutic and histopathologic studies performed, there are no specific parameters available to predict the outcome in these patients [25]. Maspin expression is down-regulated or absent in breast and esophageal cancers [13,26].Conversely, it is up-regulated in pancreatic, ovarian and gastric cancers [9,14,27]. Concerning maspin expression in head and neck SCC, Yoshizawa et al.[28] reported that higher maspin expression in oral SCC was correlated with the absence of lymph node metastasis and better prognosis.

In this current study, both cytoplasmic (32.1%) and nuclear- cytoplasmic (57.1%) patterns of maspin expression were demonstrated in laryngeal SCC. Cytoplasmic maspin expression was not significantly associated with the studied clinicopathological parameters including the pathological grade (P>0.05). This finding was not completely in accord with other head and neck SCC whereas low maspin expression was found to be associated with high tumor grade [29]. The precise mechanism of significant maspin expression in the cytoplasm of laryngeal SCC cells remains unclear. A possible explanation may be that during laryngeal carcinogenesis, some trigger factors stimulate the regulation mechanism of maspin. It is known that maspin expression results in tumor suppression, however, the high cytoplasmic concentration of maspin may result in auto-inhibition of its activity by polymerization[30].

Maspin function in the nucleus is less investigated and probably more complex. In the absence of a nuclear localization signal, maspin must either be chaperoned to the nucleus or cross the nuclear membrane by passive diffusion[31].

Nuclear-cytoplasmic expression of maspin in the present study showed no significant association with the clinico-pathological parameters, while a significant inverse correlation was found with both Ki-67 index (P=0.049) and CD105-assessed MVD(P=0.016).In agree-ment with these results, Marioni et al [23] concluded that nuclear localization of maspin was associated with less proliferative laryngeal SCC. MVD was also found to be significantly lower in laryngeal SCC with nuclear maspin than in carcinomas with cytoplasmic pattern[11]. These results confirmed the crucial role of nuclear maspin in reducing the proliferative activity and angiogenesis of laryngeal SCC.

Comparing the two groups of patients with and without postoperative carcinoma recurrence (pR+ versus pR-), a statistically significant difference in maspin nuclear-cytoplasmic expression was found in the present study (p=0.018), whereas this pattern was higher in the pR- group. This finding is consistent with the results achieved by Marioni et al. [12] who observed a highly significant lower recurrence rate in the group of patients with nuclear maspin localization (P=0.0086). Their study supported the hypothesis of an apoptosis-sensitizing effect of nuclear maspin in laryngeal carcinoma with the potential perspective of a clinical use of the tumor suppressive pro-apoptotic function of maspin.

Similar results were reported in other carcinomas. Lee et al. [14] evaluated that gastric carcinoma cases with nuclear-cytoplasmic maspin expression survived longer than those with only cytoplasmic expression. Also, Frey et al. [32] concluded that nuclear maspin in stage I lung carcinoma is an important predictor of improved survival supporting the hypothesis that maspin tumor inhibitor properties may be linked to its nuclear localization.

Regarding the Ki-67 PI, pR+ group in the current study had a statistically significant correlation with higher proliferating laryngeal SCC (P=0.007). Similarly, Calgaro et al. [24] and Cordes et al.[33] observed that the patients' group with low proliferating laryngeal SCC had a statistically longer absolute and recurrence –free 5-year survival time than patients with a highly proliferating cancers.

Angiogenesis is one of the critical mechanisms to postoperative recurrence and metastasis in carcinomas. CD105 (endoglin) was not expressed in the vascular endothelial cells of normal tissue in contrast to CD34 that reacts with normal vessels trapped within the tumor. So, the use of antibodies to CD105 had a higher specificity than pan-endothelial markers in the identification of new microvessels and could reduce the false positive staining spots[34].

The results of Marioni et al [22] and Zvrko et al. [35] revealed a strong association between high CD105 –assessed MVD and recurrence in laryngeal SCC (P=0.009 and 0.012 respectively). The present study results were consistent with the previous studies (p= 0.004) reflecting the role of this marker in identification of high risk patients for recurrence. Interestingly, CD105-assessed MVD was correlated with prognosis and recurrence of other cancers including hepatocellular and breast carcinomas[36,37].

5. Conclusion:

Nuclear expression of maspin may be useful to identify patients at lower risk of malignancy recurrence and less aggressive laryngeal SCC. On the other hand, high Ki-67 PI and CD105-assessed MVD can be valuable parameters for selecting patients who should be treated with more aggressive therapies.

So, we recommend that using preoperative biopsies of laryngeal SCC immunostained for maspin, Ki-67 and CD105 may be useful to predict patients at risk for developing regional recurrence. This might influence decision regarding therapeutic management.

References

1. Dietz A, Boehm A, Horn IS, Kruber P, Bechmann I, Golusinski W: Assay-based response evaluation in head and neck oncology: requirements for better decision making. Eur Arch Otorhinolaryngol, 267:483–494, 2010.
2. Pfister DG, Laurie SA, Weinstein GS, Mendenhall WM, Adelstein DJ: American society of clinical oncology clinical practice guideline for the use of larynx-preservation strategies in the treatment of laryngeal cancer. J Clin Oncol 24:3693–3704, 2006.
3. Lefebvre JL, Ang KK, Panel LPC: Larynx preservation clinical trial design: key issues and recommendations—a consensus panel summary. Int J Radiat Oncol Biol Phys 73:1293–1303, 2009.
4. Loyo M. and Pai S.I.: The molecular genetics of laryngeal cancer. Otolaryngol. Clin .North Am., 41:657-672, 2008.
5. Syed M.I., Syed S., Minty F., et al.: Gama tubulin : a promising indicator of recurrence in squamous cell carcinoma of the larynx . Oto-laryngol .Head Neck Surg., 140:498-504, 2009.
6. Beltran A., Parikh S., and Liu Y., et al.: reactivation of a dormant tumor suppressor gene maspin by designed transcription factors.Oncogene, 26:2791-2798, 2007.
7. Marioni G., Staffieri C., Staffieri A.,et al.: Maspin tumor-suppressing activity in head and neck squamous cell carcinoma: emerging

evidence and therapeutic perspectives. Acta. Otolaryngol., 129:476-480,2009
8. Bialey CM., Khalkhali-Ellis Z., Sefter EA., and Hendrix MJ: Biological functions of maspin. J Cell Phsiol. 209:617-24,2006.
9. Hong S., Lee J., Choe W., et al.: The effect of aberrant maspin expression on the invasive ability of pancreatic ductal adenocarcinoma cells. Oncol.Rep. 21:425-430, 2009.
10. Solomon L.A., Munkarah A., Schimp V.L., et al.: Maspin expression and localization: impact on angiogenesis and prognosis in ovarian cancer. Gynecol. Oncol., 101: 385-389, 2006.
11. Marioni G.,D'Allessandro E., Giacomelli L., et al.,: Maspin nuclear localization is related to reduced density of tumor-associated microvessels in laryngeal carcinoma. Anticancer Res ., 26:4927-4932,2006..
12. Marioni G., Giacomelli L., D'Alessandro E., et al.: Nuclear localization of maspin :is its impact on the prognosis in laryngeal carcinoma due to a pro-apoptotic effect? Am J Otolarngol.,29: 156-162,2008.
13. Cai Z., Zhou Y., Lei T., Chiu J.,and He Q.: Mammary serine protease inhibitor inhibits epithelial growth factor-induced epithelial-mesenchymal transition of esophageal carcinoma cells.Cancer,115:36-48, 2009.
14. Lee D.Y., Park C.S., Kim H.S. et al.: Maspin and p53 protein expression in gastric adenocarcinoma and its clinical applications. Appl. Immunohistochem. Mol. Morphol.,16:13-18,2008.
15. Zheng H.C., Saito H., Masuda S., et al : Cytoplasmic and nuclear maspin expression in lung carcinomas :an immunohistochemical study using tissue microarrays. Appl. Immunohistochem. Mol Morphol.,16:459-465,2008.
16. Takanami I., Abiko T.,and Koizumi S.: Expression of maspin in non- small cell lung cancer: correlation with clinical features. Clin.. Lung Cancer,9:361-336,2008.
17. Russell R, Fellet A, Elliott LF,et al : the extended pectoralis major myocutaneous flap: uses and indications. Plast Reconst Sur., 90: 676-680, 1991.
18. Inhinose A, Tahara S, Yokoo S,et al: Fail-safe drainage procedure in free radial forearm flap transfer. J Reconstr Microsurg;19:371-375, 2003.
19. Carlson GW, Temple JR, and Condor MA: reconstruction os the pharynx and overlying soft tissue by partitioned free jejunal flap. Plast Reconstr Surg 1996; 97:460.
20. Geelen G., Hordijck G., Ravasz L., and Terhard C.: Degree of tumor differentiation as a prognostic factor in advanced laryngeal cancer. Acta. Otorhinolaryngol. Belg. 49:1-4, 1995.
21. International Union against Cancer. TNM classification of malignant tumors, 5th ed. New York.Wiley-Liss,1997
22. Marioni G., Giacomelli L., D'Alessandro E., et al.: Laryngeal carcinoma recurrence rate and disease-free interval are related to CD105 expression but not to vascular endothelial growth factor2 expression. Anticancer Res., 28:551-557, 2008.
23. Marioni G., Blandamura S., Giacomelli L., et al.: Nuclear expression of maspin is associated with a lower recurrence rate and a longer disease-free interval after surgery for squamous cell carcinoma of the larynx. Hitopathology, 46: 576-582, 2005.
24. Calgaro N., Marioni G., Blandamura S., et al.: P27 and MIB-1 expression is related to malignancy recurrence in laryngeal carcinoma treated with partial laryngectomy: Preliminary results. J Otolaryngol., 36:98-105,2007.
25. Saussez S., Decaestecker C., Lorfevre F., et al. : High level of galectin-1 expression is a negative prognostic predictor of recurrence in laryngeal squamous cell carcinomas. Int. J Oncol., 30:1109-1117,2007.
26. Kim D.H., Yoon D.S., Dooley W.C. et al.: Association of maspin expression with the high histological grade and lymphocyte -rich stroma in early-stage breast cancer. Histopathology,42:37-42,2003.
27. Bolat F., Gumurdulu D., Erkanli S., et al.: Maspin over-expression correlates with increased expression of vascular endothelial growth factors A,C and D in human ovarian carcinoma. Pathol. Res. Pract. , 204:379-387 ,2008.
28. Yoshizawa K. Nozaki S., Okamune A., et al.: Loss of maspin is a negative prognostic for invasion and metastasis in oral squamous cell carcinoma. J Oral Pathol., 38:535-539,2009.
29. Mhawech-Fauceglia P., Dulguerov P., Bech A., et al.: Value of maspin and nm23-H1 protein expression in predicting outcome of patients with head and neck squamous cell carcinoma treated with radical radiotherapy. J.Clin.Pathol., 60:185-189,2007.
30. Umekita Y. and Yoshida H.: Expression of maspin is up-regulated during the progression of mammary ductal carcinoma. Histopathology, 42:541-545, 2003.
31. Blandamura S., Giacomelli L., Leo G., et al: Nuclear maspin detection in renal cell tumors:

possible diagnostic role and correlation with p53 status. Histopathology, 49:274-282, 2006.
32. Frey A., Soubani A., Adam A., et al.: Nuclear compared with nuclear-cytoplasmic expression of maspin is linked in lung adenocarcinoma to reduced VEGF-A level and in stage I, improved survival. Histopathology, 54:590-597, 2009.
33. Cordes C., Munzel A., Rudolph P. et al.: Immunohistochemical staining of Ki-67 using the monoclonal antibody Ki-S11 is a prognostic indicator for laryngeal squamous cell carcinoma. Anticancer Res., 29:1459-1465, 2009.
34. Marioni G.,D'Alessandro E.,Giacomelli L.,and Staffieri A.: CD105 is a marker of tumor vasculature and a potential target for the treatment of head and neck squamous cell carcinoma. J Oral Pathol.Med.39:361-367,2010
35. Zvrko E., Mikic A., and Vuckovic L. :CD105 expression as a measure of micro vessel density in supraglottic laryngeal squamous cell carcinoma. Eur. Arch. Otorhinolaryngol .266:1961-1976,2009
36. Yang L., Lu W.,Huang G., and Wang W.,: Correlation between CD105 expression and postoperative recurrence and metastasis of hepatocellular carcinoma. BMC cancer,6:110-118,2006.
37. Dales J., Garcia S., Bonnier P., et al .: CD105 expression is a marker of high metastatic risk and poor outcome in breast carcinoma. Am J Clin Pathol., 199:374-380,2003.

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