Original Paper

EGFR Immunoexpression in Laryngeal Squamous Cell Carcinoma

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ABSTRACT: Epidermal growth factor receptor (EGFR) is a tyrosine kinase molecule associated to the initial stages of neoplastic transformation. High expression of EGFR is connected to aggressive tumor behavior and high risk of metastasis and treatment failure. The aim of our study was to analyze the immunohistochemical expression of EGFR in 38 cases of laryngeal squamous cell carcinomas depending on clinicopathological parameters related to prognosis. The EGFR immunoreactions have statistical significant higher values in high grade carcinomas. Although the EGFR values were superior in advanced stages lesions, the aspect was not significant EGFR may be useful in identifying the aggressive laryngeal squamous carcinomas.

KEYWORDS: *EGFR, laryngeal squamous cell carcinoma*

Introduction

Larynx is an important speaking, breathing and swallowing organ which is affected by cancer in 30% to 40% of the head and neck cancer pathology [1-2]. This pathology is frequently encountered in smoking and drinking alcohol average males [3-4]. Even if early stages diagnosis has higher chances of curability, the majority of patients are diagnosed in stages III and IV, having a high rate of recurrence [5-6]. Most of the cases are diagnosed in advanced stages with a poor prognosis and short life time expectance [7]. Even the initial stages sometimes are associated with an unfavorable evolution because of the treatment failure [8].

One of the molecules involved in the appearance of squamous carcinoma but also the failure of the treatment is EGFR (epidermal growth factor receptor). EGFR is part of the EGFR family with a tyrosine kinase activity that can influence tumor growth and the neoplastic transformation [7]. EGFR activation that is present in head and neck cancer is due to high expression of its ligands, that can induce EGFR dimerization and through this way may activate oncogenic pathways [9]. Metastases is one of the main causes of poor clinical outcome in laryngeal cancer and EGFR expression evaluation may help in identifying the patients with a high-risk to develop metastases [8].

We aimed a study on the immunohistochemical expression of EGFR in the laryngeal squamous cell carcinomas in relation with the clinicopathological parameters.

Material and methods

Our study included a total number of 38 cases of laryngeal squamous cell carcinomas. These cases were selected during a period of three years from the cases admitted and operated in Otolaryngology Clinics of the Emergency County Hospital of Craiova and diagnosed in the Pathology Department. The laryngectomy pieces were fixed in 10% buffered formalin, processed by the usual technique with paraffin embedding and Hematoxylin-Eosin (HE) stain. Histopathological classification and staging have been made in accordance to World Health Organization (WHO) recommendation [5].

We have analyzed in this study age, gender, histological grade, depth of invasion (pT), lymph node metastasis (pN) and tumor stage. In none of the present cases metastases were diagnosed (pM0). The immunoreactions were performed on serial sections using the monoclonal mouse antihuman EGFR antibody (Leica Biosystems), clone EGFR.113, in dilution 1:75 and without antigen retrieval pretreatment.

Immunohistochemical reactions were obtained using LSAB2-HRP (Labeled Streptavidin Biotin-Horseradish Peroxidase) amplification system (DAKO, Redox, Bucharest, code K0675) and for the signal visualization 3,3'-diaminobenzidine tetrahydrochloride (DAB, DAKO, code 3467) as

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chromogen. To validate the obtained reactions, we use external negative (by omitting the primary antibody) and positive (placenta) controls.

A semiquantitative quantification based on a scoring system was used. It was taken into account the reactions intensity and the number of labeled cells. The number of labeled cells was assessed on microscopic field of 200x, and represents an average value of positive cells reported on the total number of cells of the entire specimen. The intensity was scored as mild (score 1), moderate (score 2) and intense (score 3). The number of labeled cells was considered as score 1 for <25% marked cells, score 2 for >26-50% marked cells, and score 3 for 51-74% marked cells and score 4 for >75% marked cells. By the multiplying of the scores for intensity and labeled cells it was obtained final staining score, with values between 1 and 12. For the statistical analysis, the values between 1 and 4 were considered low and the values between 6 and 12 were high.

Statistical analysis was performed using chi-square tests within Statistical Package for the Social Sciences (SPSS) 20 software and p-values <0.05 were considered significant. For the image acquisition were used Nikon Eclipse E600 microscope and Lucia 5 software. The study was approved by the local ethical committee (no.173/11.09.2017), and written informed consent was obtained from all the patients.

Results

We included in the present study a total of 38 laryngeal squamous cell carcinomas and we observed a predominance in males and an average age of 59.8±8.0 years old (Table 2). The majority of the tumors were moderate differentiated (22 cases), without lymph node metastases (26 cases) and in advanced stages (19 cases for the stage III and 10 cases for the stage IV) (Table 1).

Table 1. Cases distribution according to the investigated clinicopathological parameters

Parameter	Variable
Age	<50 years old=2 >50 years old=36
Gender	Females=3; Males=35
Differentiation degree	WD*=7; MD*=22; PD*=9
Depth of invasion (pT)	T1=3; T2=9; T3=22; T4=4
Lymph node metastasis (pN)	N0=26; N1=4; N2=8
Tumor stage	I=3; II=6; III=19; IV=10

***WD: well differentiated; MD: moderate differentiated; PD: poorly differentiated**

In this study, the analysis of EGFR expression was present in 34 cases, the reactions being observed in the membrane and cytoplasmic level, with variable distribution and intensity.

Analyzing the marker expression for well differentiated cases we found the average marked cells of 26,04±8,4, variable intensity and a mean score of 2.8 (Fig.1, Table 2). For the 18 moderate differentiated cases, the mean value was 53,3±13,9, moderate intensity and a mean score of 6,5 (Fig.2, Table 2). The higher values were present in poorly differentiated cases where we obtained an average percentage of marked cells of 79,9±12,2, variable intensities and mean score of 6.7 (Fig.3, Table 2).

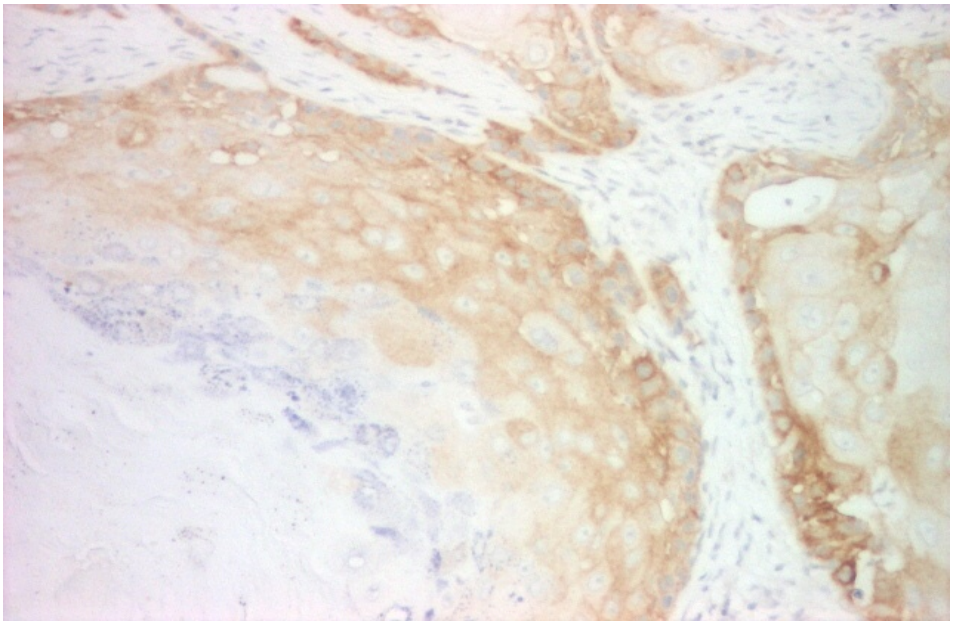

Fig.1. Well differentiated laryngeal squamous cell carcinoma, EGFR immunostaining, x100

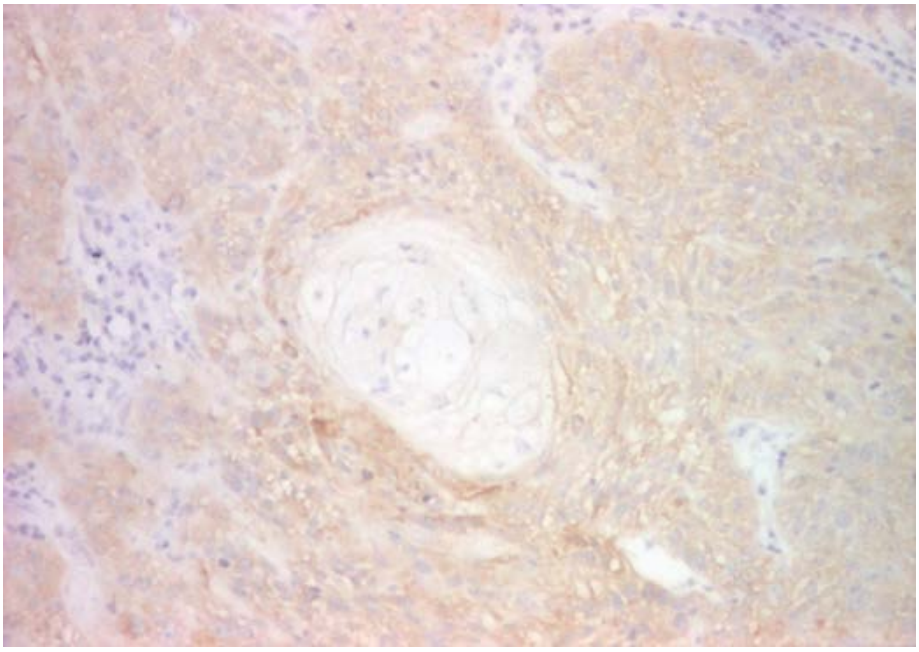

Fig.2. Moderate differentiated laryngeal squamous cell carcinoma, EGFR immunostaining, x100

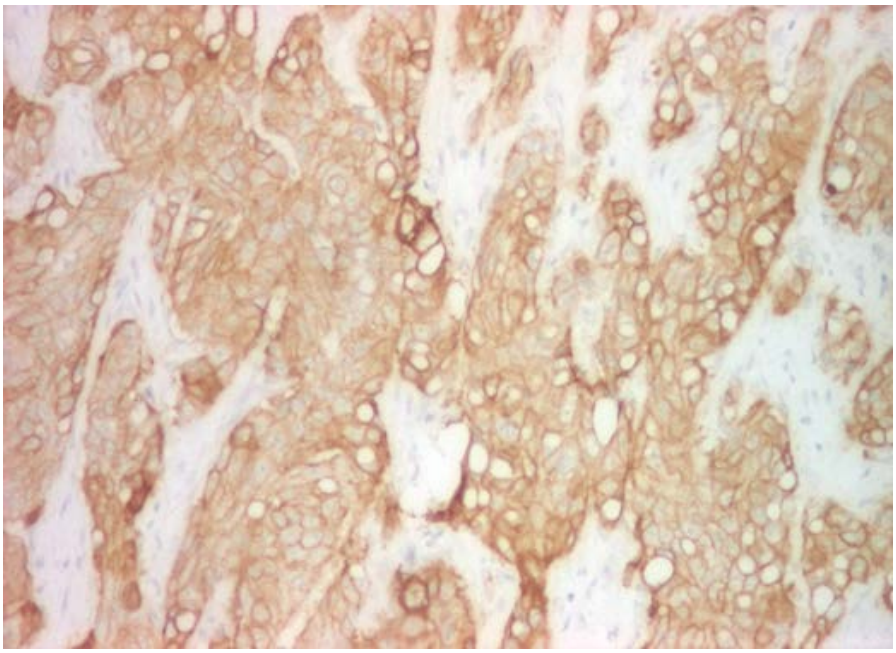

Fig.3. Poorly differentiated laryngeal squamous cell carcinoma, EGFR immunostaining, x100

Referring to the tumor stages we observed in the stage I a mean value of 41,8±22,7 labelled cells, moderate intensity and an average score of 3, while in stage II the values were 52,7±27 and 6.6.

By comparison in stages III and IV the values were 59,1±20,5 and 52,2±23,9 for the marked cells, the reaction intensities were moderate, and the scores were 6.7 and 4.6 (Table 2)

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Table 2. Immunostaining scores in relation with clinicopathological parameters

Parameters		No. cases	EGFR		p values
			% cells ± SD*	Mean score	
Age	<50	2	56,2±6,2	4,5	0,794
	>50	32	54,6±23,03	5,9	
Gender	F	3	74,9±18,4	10	0,129
	M	31	52,8±21,9	5,4	
Differentiation degree	BD	7	26,04±8,4	2,8	0,015
	MD	18	53,3±13,9	6,5	
	SD	9	79,9±12,2	6,7	
Depth of invasion (pT)	T1	3	41,8±22,7	3	0,122
	T2	9	61,2±27,4	6,8	
	T3	18	56,1±19,5	6	
	T4	4	43,5±22,7	5	
Lymph node metastasis (pN)	N0	23	51,5±19,7	6,1	0,534
	N1	4	70,1±32,7	6,2	
	N2	7	56,5±24,1	4,5	
pTNM stage	I	3	41,8±22,7	3	0,066
	II	6	52,7±27	6,6	
	III	16	59,1±20,5	6,7	
	IV	9	52,5±23,9	4,6	

SD=Standard deviation, F:Female, M: Male, WD=Well differentiated, MD=Moderate differentiated, PD=Poorly differentiated

The examination of different parameters in our study indicated significant increased EGFR values in poorly differentiated carcinomas compared with moderate and well differentiated ones (p=0.0015, chi square test) (Fig.4). We did not find any other statistical relation of EGFR expression and the investigated parameters.

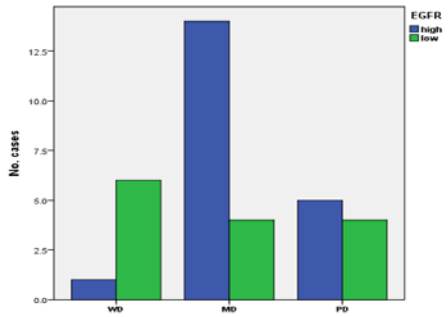

Fig.4. Cases distribution according to the scores of EGFR and the differentiation degree

Discussions

EGFR is one of the molecules involved in the appearance of the malignant phenomena. Interferes with the control over the apoptotic process, it sustains through different mechanisms the cellular proliferation, angiogenesis and the metastatic process [8]. This molecule it is described as being involved not only in the head and neck carcinomas initiation but also in mammary and pulmonary carcinomas [10-12]. More than 80% of the head and neck carcinomas are associated with an elevated expression of EGFR. This higher expression of EGFR has been noticed in the cancer genesis, beginning from the initial stages and it was related to the lesions severity [12]. Overexpression of EGFR was also described in laryngeal squamous cell carcinomas, it's presence being related to a less favorable prognosis with a shorter life expectancy [8,13-14].

In this study, the EGFR immunoexpression analysis was positive in 34 cases, from the 38 laryngeal squamous cell carcinomas analyzed. The membranous and cytoplasmic expression localization was present in 54.7% of the cases, the higher values being noticed in the moderate and poorly differentiated cases by comparison to the well differentiated ones. Searching through the literature we found different values for the marker, like the study conducted by Şimşek H et al. on 92 patients where the EGFR was positive in 54% of the cases [15]. According to other studies, higher values up to 87.5% were obtained [16]. It has been also investigated the relation between the EGFR marker and the prognosis. The higher intensity expression of the marker has been reported as an indicator of poor prognosis, with a short disease-free survival and also overall survival [17].

Hypoxia is a phenomenon present in head and neck carcinomas. It is associated with an unfavorable prognosis, because it is involved in the reduced survival time and also interferes with the treatment. Normally the hypoxia-inducible factors HIF are degraded but in cancer these genes are stabilized and mediate the activation of EGFR, leading to more aggressive tumors with a higher metastatic potential [18].

Cancer recurrence has been linked to the nodal metastases [19]. EGFR next to TNF-α may be helpful in identifying, from the beginning, the patients with a higher risk of recurrence and also guide the treatment [9,19]. EGFR expression is linked to the treatment. The literature associates overexpression of EGFR to the radio and chemotherapy failure. These cases, with an increased resistance to the oncological treatment

can be identified at the time of the diagnosis and may benefit from more aggressive treatment with improved results [8].

The purpose of the chemotherapy is to block the EGFR function. In order to obtain the blockade are used monoclonal antibodies and tyrosine kinase inhibitors. These monoclonal antibodies main targets are to block the ligand binding, induce some types of receptors degradation and to activate antitumoral immune response [7]. Regarding the tyrosine kinase inhibitors their main function is to inhibit the EGFR phosphorylation.

Currently, better results for the advanced stages of head and neck carcinomas were obtained using the combined therapy, radiotherapy with Cetuximab, a monoclonal antibody [7].

Conclusions

In this study, the laryngeal squamous carcinomas with high grade and advanced stages indicated overexpression of EGFR. We obtained significant increased EGFR values in poorly differentiated carcinomas compared with moderate and well differentiated ones. The EGFR immunoexpression can be helpful in identifying the high risk squamous cell carcinoma cases that may develop metastases and resistance to treatment.

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