Assessment of epidermal growth factor receptor expression in N0 laryngeal squamous cell carcinoma

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Abstract

Objectives: to determine the Epidermal growth factor receptor (EGFR) over-expression in laryngeal carcinoma primary tumor and in first echelon node in N0 neck. Design: A Cross sectional retrospective study. Setting: Otolaryngology and Pathology Departments, Suez Canal University Hospital (Ismailia, Egypt). Participants: Twenty five patients. Main outcome measures: EGFR expression is linked to early neoplastic transformation, cellular proliferation, and the metastatic process in laryngeal cancer. Twenty paraffin preserved primary laryngeal squamous cell carcinoma (LSCC) and their cervical lymph nodes stained with hematoxylin & eosin and EGFR using immunohistochemical technique with scoring system. Results: EGFR expression in occult metastasis was associated with higher T stage (p=<0.001) and higher tumor grade (p=0.001). EGFR expression in occult metastasis was correlated with primary LSCC cartilage invasion (p=0.05), lymphovascular invasion (p=0.028) & muscle invasion (p=0.05). Laryngeal expression of EGFR and cervical lymph node metastasis as evidenced by EGFR immunohistochemical staining (p=0.001). Conclusion: EGFR immunohistopathological analysis of the lymph nodes and the primary tumour is a highly valuable tool for the detection of the cervical metastatic status and the N stage. Key words: Epidermal Growth Factor Receptor (EGFR), Larynx, N0, Squamous cell Carcinoma, Metastasis

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Conclusion: EGFR immunohistopathological analysis of the lymph nodes and the primary tumour is a highly valuable tool for the detection of the cervical metastatic status and the N stage.

Key points:

Epidermal Growth Factor Receptor (EGFR), Laryngeal squamous cell carcinoma (LSCC), N0, Squamous cell Carcinoma, Lymph node metastasis, First echelon node

Level of evidence: 2

Introduction

Increased expression of the epidermal growth factor receptor (EGFR), which is linked to the early phases of neoplastic transformation, is linked to the tumor's aggressiveness, invasiveness, and treatment failure (1). Additionally, it supports angiogenesis, cellular proliferation, and the metastatic process (2).

Metastasis is one of the main causes influencing the treatment outcome in laryngeal cancer and EGFR expression analysis may assist identify individuals who are at a high risk of developing metastasis (2-3). Early identification of patients at higher risk of recurrence using EGFR in conjunction with tumor necrosis factor alpha (TNF- α) may be beneficial in directing treatment (3-4).

Additionally, EGFR expression has an impact on the course of treatment; the literature discusses how overexpression of EGFR leads to the failure of radiotherapy and chemotherapy with increased resistance to oncological treatment. EGFR expression can be detected even before the diagnosis of a primary laryngeal mass by using immunohistochemical analysis on the biopsy taken during the initial histopathological diagnosis (2).

Because of this, the goal of chemotherapy is to inhibit EGFR function. Tyrosine kinase inhibitors and monoclonal antibodies are used to achieve the blockage, and these monoclonal antibodies' main targets are to inhibit ligand binding, cause the degradation of some types of receptors, and activate the antitumor immune response (5).

Tyrosine kinase inhibitors' main function is to prevent the phosphorylation of the EGFR. The combination of radiation and the monoclonal antibody cetuximab produced the best outcomes for head and neck cancers in their advanced stages (2).

Based on all of these facts, employing this biomarker may change how LSCC and associated lymph node metastases are diagnosed, categorised, and prognosed, based on the metastatic status indicated by the biomarker's expression.

Our study aimed to determine and document the EGFR over-expression in laryngeal carcinoma primary tumor and in 1st echelon node in cases with clinically and radiologically N0 neck.

Materials and Methods

Study design: A Cross sectional retrospective study. The reporting guideline is for an observational study.

Setting: Otolaryngology and Pathology Departments.

Participants: Patients who were diagnosed with LSCC and their clinical and radiological neck lymph nodes status was N0 were included into our study while patients had irresectable tumors (T4b) or comorbidities interfering with surgery were excluded.

Study plan

Twenty patients with documented laryngeal squamous cell carcinoma without lymph node enlargement as demonstrated clinically or radiographically who had surgery were included in our study and had their paraffin-preserved primary tumour and nodal tissues analysed immunohistopathologically. The following immunohistochemistry protocol was used to perform an immunohistopathological investigation.

Histopathological evaluation:

The samples were paraffin-embedded after being 10% formalin-fixed. Sections of 3 μm thickness from each block were submitted, mounted on a glass slide, and stained with hematoxylin and eosin (H&E).

Immunohistochemical staining:

For immunohistochemistry (IHC) staining, slices from the chosen paraffin blocks were cut into 4 micrometers thick sections. Primary anti-EGFR antibody (ABclonal® polyclonal, rabbit source, EGFR antibody, Cat. No. A11351) was produced and incubated on slides. After that, the secondary antibody of choice (ABclonal® anti rabbit IgG, Cat. No. Ab214880) was incubated. Prior to dehydration and mounting, all slides were lightly counterstained with hematoxylin for 30s.

Immunohistochemical scoring:

The primary mass EGFR expression results were compared to a negative control (benign vocal cord polyp). By combining the two scores mentioned above, an immunostaining score was created that ranged from 0 to 6. The immunostaining score was then used to categorize the immunohistological expression of EGFR: immunostaining scores of 0 indicate no expression, 1-2 indicate weak expression, 3-4 indicate moderate expression, and 5-6 indicate strong expression. Strong and moderate EGFR expression was considered positive in the results, but no or weak EGFR expression was regarded as negative.

The membranous expression of EGFR in tumor cells was observed. The intensity of the staining was determined using a semi-quantitative method, and the number of labeled cells with malignant features was determined in accordance with both the intensity and the proportion of stained cells. Scores were determined based on:

1- Expression of membranous EGFR

2- Intensity of staining

The intensity scale was determined using semi-quantative method in which 4 grades were obtained as follow: 0: no staining; 1: weak, 2: moderate; and 3: intense staining. The proportion of cells staining was also scored on a 4-point scale: 0: no cells staining; 1: less than one-third of cells staining; 2: between one-third and two-thirds of cells staining; 3: more than two-thirds of cells staining.

Statistical analysis

Data collected were processed using IBM SPSS® software package version 20.0. (Armonk, NY: IBM Corp)

Results

Twenty patients (19 males and 1 female) with mean age of 60.95 ± 9.23 years diagnosed with LSCC were included in the study. 70% of the cases (14 patients) showed positive membranous expression of EGFR. Notably, 60% of the studied lymph nodes which were regarded as clinically and radiologically negative revealed to harbor occult metastasis, micro-metastasis or isolated tumor clusters (ITC) by showing malignant features and positive expression to EGFR immunohistochemical polyclonal antibody.

Notable association between T stage of the primary and the presence of occult metastasis as evidenced with immunohistochemical membranous expression of EGFR in neck lymph nodes of patients with N0 laryngeal squamous cell carcinoma (p=<0.001). Also, it was found that there is a correlation between the tumour grade and the immunohistochemical expression of EGFR in neck lymph nodes of patients with laryngeal squamous cell carcinoma (p=0.001) (table 1, figure 1).

In addition, it was found that there is a significant correlation between the neck nodal metastasis as detected by EGFR immunohistochemical staining and tumour cartilage invasion (p=0.05), lymphovascular invasion (p=0.028) & muscle invasion (p=0.05) detected by histopathological analysis of the total laryngectomy specimen (table 2, figure 1).

Our study showed a remarkable correlation between the laryngeal expression of EGFR and cervical lymph node metastasis as evidenced by EGFR immunohistochemical staining (p=0.001), as it was revealed that all the patients who had cervical node metastasis had positive EGFR expression in their primaries (table 3, figure 1).

Discussion

Our research found a significant correlation between lymph node metastasis and the stage of the LSCC tumor (p=0.001). Redaelli et al. studied the occult neck lymph node metastasis in LSCC patients and found an incidence of 39.2% (6). This result was significantly correlated with the stage of the tumor (P= 0.04). Yilmaz et al. study results are also consistent with those of ours (7).

Our results showed that 91.6% of the subjects that had cervical lymph node EGFR expression in their metastasis had a primary with moderate to poor differentiation. It was also revealed that there is a strong association between tumour grade of differentiation and the cervical metastasis (p=0.001). Magnano et al. reported that the poorer the differentiation was, the higher the possibility of cervical node metastasis would be (8).

On the other side, we did not find evidence of statistical nor clinical correlation between the age and the gender with the EGFR expression state in the lymph nodes. Similarly, (Čelakovský, Kalfeřt, Smatanová, et al., 2015) did not postulate age nor gender correlation with the cervical metastasis in laryngeal cancer patients. [(9)]

Also, we did not reveal any statistically significant correlation between the site of the primary and the cervical. This was in consistency with the study results of Čelakovský et al. who did not postulate a relation between tumor sub-site and EGFR expression in the cervical metastasis [(9)]. In contrast, Mayers & Allvi reported that transglottic and supraglottic carcinoma cause early cervical lymph node metastasis that they recommended lateral neck dissection with the laryngectomy surgery having a desirable effect on the prognosis [(10)].

We found a positive correlation between lymphovascular invasion and EGFR expression in the cervical metastasis (p=0.028). This is in congruity with the results of Ozdek et al which found that lymphovascular invasion is a risk factor for occult metastasis and is a histological feature that indicates an aggressive behavior of the carcinoma as tumor cells access the blood and lymph vessels causing lymphatic and distant spread altering the prognosis of the patients [(11)].

We examined the membranous expression of EGFR in the LSCC primary tumor masses using immunohistological analysis. It was revealed that 70% of the specimens had positive reaction to the EGFR antibody. Zimmermann et al. asserted that nearly 80% of the head and neck carcinomas are associated with an elevated expression of EGFR [(12)].

We noticed that 100% of the study sample who revealed to have positive EGFR expression in their cervical lymph node metastasis had positive membranous EGFR expression in their primary tumor with statistically significant correlation (p=0.001). Şimşek H et al correlated the aggressive behavior the tumor expresses in the matters of poorer differentiation, increased tumor volume and cartilage, lymphovascular and muscle invasion and metastasis to tumor EGFR expression[(13)].

Conclusion

EGFR immunohistopathological analysis of the lymph nodes and the primary tumor is a highly valuable tool for the detection of the cervical metastatic status and determine the N stage and plan for proper management.

Conflict of Interest

We conclude that there is no conflict of interest in writing this manuscript.

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Tables

Table (1): Show the relation between EGFR expression in cervical lymph node metastasis and T stage & tumor grade (n = 20).

	EGFR IHA in Cervical Lymph Node	**EGFR IHA in Cervical Lymph Node**	**EGFR IHA in Cervical Lymph Node**	**EGFR IHA in Cervical Lymph Node**	χ^2	**p**
(n =8)	**Negative**					
(n =8)	**Negative**					
(n = 12)	**Positive**					
(n = 12)	**Positive**					
	No.	**%**	**No.**	**%**		
Depth (T stage) of the primary						
T2	7	87.5	0	0.0	16.667	^{MC}p= <0.001
T3	1	12.5	4	33.3		
T4a	0	0.0	8	66.7		
Grading of the tumor						
I	6	75.0	1	8.33	10.873	^{MC}p=0.001

	EGFR IHA in Cervical Lymph Node	**EGFR IHA in Cervical Lymph Node**	**EGFR IHA in Cervical Lymph Node**	**EGFR IHA in Cervical Lymph Node**	**χ^2**	**p**
II	2	25.0	4	33.3		
III	0	0.0	7	58.3		

χ^2 : Chi square test FE= Fisher Exact test

P: value for association between EGFR expression in cervical lymph node metastasis and T stage & tumor grade.

Table (2): Show the relation between EGFR expression in cervical lymph node metastasis and primary cartilage invasion, muscle invasion and lymphovascular invasion (n = 20).

	EGFR IHA in Cervical Lymph Node	**EGFR IHA in Cervical Lymph Node**	**E**
	Negative		
(n =8)	**Negative**		
(n =8)	**Positive**		
(n = 12)	**Positive**		
(n = 12)			
	No.	%	**N**
Cartilage invasion	1	12.50	7
Lymph vascular invasion	1	12.50	8
Muscle invasion	1	12.50	7

χ^2 : Chi square test FE= Fisher Exact test

P: p value for association between EGFR expression in cervical lymph node metastasis with primary lesion cartilage invasion, muscle invasion and lymphovascular invasion.

Table (3): show the relation between EGFR expression in cervical lymph node metastasis and laryngeal primary mass immunohistochemical EGFR analysis (n = 20).

	EGFR IHA in Cervical Lymph Node	**EGFR IHA in Cervical Lymph Node**
	Negative	
(n =8)	**Negative**	
(n =8)	**Positive**	
(n = 12)	**Positive**	
(n = 12)		
	No.	%
Laryngeal EGFR expression	2	25.0

χ^2 : Chi square test FE= Fisher Exact test

P: value for association between EGFR expressions in cervical lymph node metastasis with primary lesion EGFR expression.

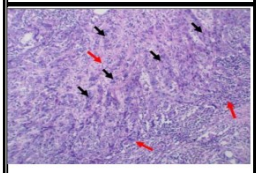
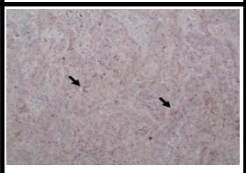
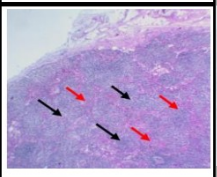
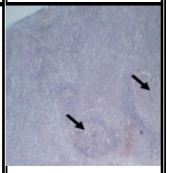
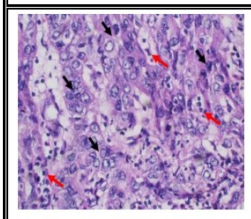
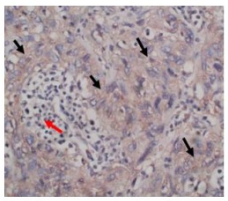
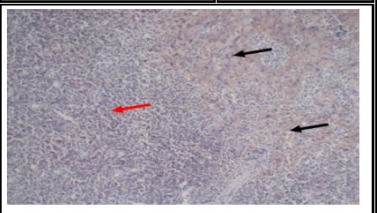
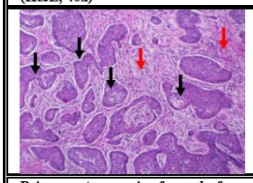
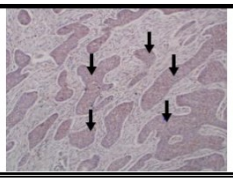
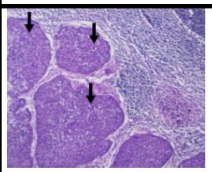
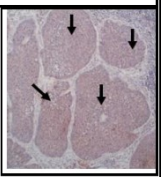
Primary tumor		Related LNd	
H&E examination	**IHC examination of EGFR**	**H&E examination**	**IHC examination of EGFR**
			
Primary tumor is formed from groups and nests of malignant squamous cells (Black arrows) embedded within desmoplastic heavily inflamed stroma (Red arrows) (H&E, 10x)	**The expression of EGFR was weak (1+) in tumor cells (Black arrows) (IHC, 10x)**	**Lymph node show uniform cortex with proliferated lymphoid follicles (Black arrows) and prominent medullary sinuses (Red arrows). There was no detected metastasis (H&E, 4x).**	**No expression to EGFR was noted. Reactive uniform lymphoid follicles are evident (Black arrows) (H&E, 4x)**
			
Higher magnification of the previous figure showing malignant cells with high grade nuclear features and densely eosinophilic cytoplasm (Black arrows) and chronic lymphocytic infiltrate in between tumor cells (Red arrows) (H&E, 40x)	**Higher magnification of the previous figure showing weak membranous expression of EGFR in tumor cells (Black arrows). There is infiltrating lymphocytes (Red arrow) (IHC, 40x)**	**There was a focus of micro-metastasis measuring about 1 mm in greatest dimension, stained weakly positive (1+) (Black arrows); as primary tumor. Rest of lymphoid tissue stains negative for EGFR (Red arrow) (IHC, 10x)**	
			
Primary tumor is formed from groups and nests of malignant squamous cells (Black arrows) embedded within desmoplastic heavily inflamed stroma (Red arrows) (H&E, 10x)	**The expression of EGFR was strong (3+) in tumor cells (Black arrows) (IHC, 10x)**	**Lymph node show deposits of malignant tumor tissue (Black arrows) (H&E, 10x).**	**The expression of EGFR was strong (3+) in deposits of tumor tissue (Black arrows) as primary tumor. (10x)**

Figure 1: Showed pathological correlation in LSCC and related LN.