Collation of EGFR (Her1) expression with clinical features in sinonasal squamous cell carcinoma

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Abstract. Prognosis of sinonasal squamous cell carcinoma (SNSCC) depends on TNM (Tumor, Node, Metastasis) staging, histologic categories and activity of cell-surface receptors (p53, Ki67, EGFR − Epidermal Growth Factor Receptor (ErbB-1; Her1). EGFR is the best- studied transmembrane tyrosine kinase receptor. The intensity of tumor cells division and the rate of its metastasis depend on it. It also regulates the processes of angiogenesis. All this determines the severity of the course of the disease and the ability to predict its outcome. Depending on the speed of EGFR expression, targeted therapy may or may not be prescribed. Dependence of the clinical picture and prognosis of SNSCC on the rate of EGFR expression. Prospective case study on thirty-two SNSCC patients treated at National ENT Hospital in two years (2011-2012). Clinical presentations, CT (computed tomography) scan imaging were analyzed and biopsy samples were categorized to investigate EGFR activity (Her1). Semi quantification technique has been used in this study. The level of EGFR expression was explored and result was collated with disease staging. The pace of EGFR expression in SNSCC was 53.1%, with 25% was scored 3+. EGFR was positive in 50% (16/32) stage III patients (21,4% was scored 3+) and 75% (3/4) stage IV patients (50% was scored 3+). The EGFR-positive rate was higher in exophytic, advanced stage with neurological deficits and lymphadenopathy of neck. The EGFR was positive in 53,1% of sinonasal squamous cell carcinoma. Patients with more severe manifestations, the presence of neurological symptoms, facial deformities and exophytic tumor growth have a significantly higher EGFR expression rate.

Key words: Sinonasal cancer, EGFR, Epidermal Growth Factor Receptor (ErbB-1; Her), squamous cell carcinoma, SNSCC

INTRODUCTION

Sinonasal cancer is relatively uncommon, accounting for less than 1% of all neoplasms and about 3% of those arising in the head and neck region (Đoàn Phước Thi et al., 2018). Until now, the literature search shows approximately 2000 articles with diverse analysis corresponding to sinonasal cancer. Prognosis depends on many factors (Piccirillo and Feinstein, 2016) such as clinical staging, histologic types (undifferentiated carcinoma is more aggressive than differentiated type, malignant lymphoma has worse prognosis than that of squamous tumors) and the degree of malignancy evaluated by measurements of biomarkers (p53, Ki67 and EGFR (Her1)). In Vietnam, EGFR is being actively studied as a marker of cancer processes. It becomes an object of study not only for sinonasal cancer, but also for lung cancer (Phạm Mai Thủy Tiên et al., 2018), lymphoma (Đoàn Phước Thi et al., 2018), maxillary sinus (Maemondo, 2010), colorectal cancer. But meta-studies have not been conducted, and, unfortunately, no systematics of all information. Many materials are published exclusively in Vietnamese and are not submitted for publication in international journals.

The aims of this study were:

1. Determine the rate of EGFR expression in biopsy samples.
2. Collate the rate of EGFR activity with clinical features in SNSCC.

MATERIAL AND METHODS

Thirty-two SNSCC patients were treated at National ENT (Ear Nose Throat) Hospital in two years (2011-2012). All patients were informed about the study and gave their consent to participate in it. They also agreed to publish this data in this article.

The material was created in accordance with the standards of the committee on experiments with people in Vietnam and the Helsinki Declaration of 1975 (revision of 2000).

Prospective case study was conducted.

a. Data of clinical presentations and CT scan images were collected.

b. Fixed biopsy samples in 10% neutral buffered formalin were transferred to Department of Pathology Hanoi Medical University for HE (hematoxylin and eosin) and PAS (Periodic acid–Schiff) stains (PAS stain for adenocarcinoma and follicular carcinoma to identify mucous substance).

c. Tumors were categorized according to the WHO (World Health Organization) histological classification 2005.

d. Immunostaining for EGFR (Her1) was performed in all SNSCC cases.

e. Her1-status was scored as follow: negative = no staining of tumor cells, (1+) = light brown staining 10–30% tumor cells, (2+) = brown staining >30% tumor cells, (3+) = strong brown staining >30% tumor cells. This classification helped evaluate the pace of EGFR expression in each group.

f. Finally, all data was analyzed using common statistic algorithm.

RESULTS

Histologic characteristics

We found three histological types tumors with the total cases of 32 (Table 1).

a) Among the 32 cases, 28 was SCC (occupy 87.6%), 3 was adenocarcinoma (occupy 9.3%), and one was undifferentiated squamous cell carcinoma (occupy 3.1%).

b) Whereas, 24 cases of highly differentiated tumor occupy 75%, and eight cases of

poorly differentiated tumors occupy 25%.

In South Asia, head and cervical cancers are the third leading cause of cancer- related morbidity and mortality (Naz et al., 2015; Bhayekar et al., 2016; Gupta et al., 2017). Approximately 90 to 95% is oral squamous cell carcinoma (SCC). AC and USCC have significantly less weight in the incidence structure and are less common. The frequency of pathology in this study confirms the general statistics.

Table 1. Histologic characteristics

	SCC	AC	USCC	Total
Highly differentiated	22 (68.8%)	2 (6.2%)	0 (0.0%)	24 (75.0%)
Poorly differentiated	6 (18.8%)	1 (3.1%)	1 (3.1%)	8 (25.0%)
Total	28 (87.6%)	3 (9.3%)	1 (3.1%)	32 (100.0%)

(SCC: squamous cell carcinoma, AC: adenocarcinoma, USCC: undifferentiated squamous cell carcinoma).

These kinds of cancer show varied degrees of epithelial dysplasia and malignancy. The rate of EGFR expression is also different.

EGFR status

An EGFR expression was detected in 17 cases (53.1%). Each eight cases (8/17), occupy 47.05% of all EGFR-positive cases, were scored 3+ (Table 2).

Table 2. EGFR status

Status	n	%
Negative	15	46.9
1+	4	12.5
2+	5	15.6
3+	8	25.0
Total	32	100.0

Expression of EGFR in a number of epithelial cell tumors in humans has been well documented, and 80% of squamous cell carcinomas are marked by overexpression of EGFR, resulting in proliferation and differentiation of keratinocytes. High expression of EGFR in head and neck SSC has been reported by some another studies (Xia et al., 1999; Patricia et al., 2012). In a similar study conducted by Sarkis et al. (2010), the EGFR immunostaining was positive in 87.5% of the cases (Laimer et al., 2007). Likewise, a high expression of EGFR 73.42% was found in another study conducted by Laimer et al. Moreover, 92.3% of cases were positive for EGFR staining in the study conducted by Hiraishi et.al (2006).

Global statistics are confirmed by the results of this study. About half of all head and neck tumors are EGFR-positive.

Collation of EGFR expression with clinical presentations EGFR

with S staging

Among 28 stage III patients, 14 cases (50%) were EGFR-positive, and 6 (6/14) patients were

scored 3+ occupy 42.85%. Among 4 stage IV patients, three (3/4) were EGFR-positive occupy 75%, and two (2/4) were scored 3+ occupy 50% (table 3).

The EGFR level correlates with the degree of the oncological process according to the clinical classification. It opens up opportunities for early staging and quick response to critical situations.

Table 3. EGFR with S staging

	S III	SIVA	n
Negative	14	1	15
1+	3	1	4
2+	5	0	5
3+	6	2	8
Total	28	4	32

EGFR with tumor morphology

The rate of EGFR expression in exophytic and exophytic with polyps were 54.1% and 50.0%, respectively. This difference was not considered statistical significance (p>0,05).

Table 4. EGFR expression with tumor morphology

	Positive	Negative	p
Exophytic (n=24)	13/24 (54.1%)	11/24 (45.9%)	> 0,05
Exophytic with polyp (n=8)	4/8 (50%)	4/8 (50%)	
Total	17/32 (53.1%)	15/32 (46.9%)	

EGFR and facial deformities

Table 5 demonstrates the rate of EGFR expression in sinonasal cancer with facial deformities was higher than that of cases without facial deformities (85.7% and 44.0%, respectively). This difference was considered statistical significance (p<0,05).

Table 5. EGFR and facial deformities

	EGFR (+)	EGFR (-)	p
With facial deformities	6 (85.7%)	1 (14.3%)	<0,05
Without facial deformities	11 (44.0%)	14 (56.0%)	
Total	17	15	

EGFR and eye damages

The rate of EGRF-positive cases with and without eye damages were 54.1% and 50.0%, respectively (Table 6). This difference was not considered statistical significance (p>0.05).

Table 6. EGFR expression and eye damages

	EGFR (+)	EGFR (-)	p
With eye damages	4 (50.0%)	4 (50.0%)	>0,05
Without eye damages	13 (54.1%)	11 (45.9%)	
Total	17	15	

EGFR and neurologic deficits

The rate of EGRF-positive cases with and without neurologic deficits were 57.1% and 35.7%, respectively (Table 7). This difference was not considered statistical significance (p>0.05).

Table 7. EGFR and neurologic deficits (ND)

	EGFR (+)	EGFR (-)	p
With ND	12 (57.1%)	9 (42.9%)	> 0.05
Without ND	5 (35.7%)	6 (64.3%)	
Total	17	15	

EGFR and cervical lymphadenopathy

Table 8 demonstrates the rate of EGFR expression in cases with cervical lymphadenopathy was higher than that of cases without cervical lymphadenopathy (100.0% and 46.4%, respectively). This difference was considered statistical significance (p<0.05).

Table 8. EGFR and cervical lymphadenopathy (CL)

	EGFR (+)	EGFR (-)	p
With CL	4 (100.0%)	0 (0.0%)	<0.05
Without CL	13 (46.4%)	15 (53.6%)	
Total	17	15	

DISCUSSION

The rate of EGFR expression

Our study indicated that the rate of EGFR-negative humans was 46.9% and that of EGFR-positive humans was 53.1%. The number of occasions scored 3+ accounted for 8/32 (25%). Thus, our prediction of success with radiotherapy and targeted therapy, represented by EGFR-plus expression, was 53.1%. In oncogenesis, it is well known that atypical cells are transformed from normal cells. These cells bring the altered genome that disrupts the programming regulating the balance between cell division and programmed cell death. These cancer cells need to be activated through various

oncogenic signaling the pathways to growth, otherwise no cell division occurs and therefore the tumor will not develop. One of the well-known pathways is through the epidermal growth factor (EGF) signaling the pathway (Zafar et al., 2017).

This pathway is a family consisting of four closely related transmembrane receptor TK (tyrosine kinase) Her1, Her2, Her3 and Her4. Her1 (also known as EGFR) is one of the identified receptors that exists on the surface of malignant cells in the head and neck squamous cell carcinoma, adenocarcinoma of the lung and some types of colorectal cancer. Kalyankrishna and Grandis (2006) reported that the EGFR overexpression in squamous cell carcinomas, including those in the head and neck region, may reach up to 90%. EGFR plays a very important role in promoting tumor growth, invasion, metastasis and angiogenesis. So far, some drugs targeting at EGFR inhibition have been applied and showed promising clinical benefits. Optimization of the EGFR inhibition therapy can be achieved by identification and selection of the SCCs, which potentially have the greatest response to targeted therapy (Greene et al., 2006; Đoàn Phước Thi et al., 2018). To precisely identify the EGFR mutation, which aims targeting treatment with Figitumumab or Celecoxib, the quantification techniques such as fluorescence or silver in situ hybridization) must be used (Sok et al., 2006; Sundvall et al., 2010; Bernardes et al., 2013). Due to limitation of resources and techniques, only the semi quantification technique had been used to determine the rate of EGFR expression and collate with disease staging. Molecular diagnosis is expensive (approximately 6.000.000 VND/sample), so this test is taken only in EGFR-positive cases detected by immunohistochemistry for the validation of targeted therapy.

Histologic types and EGFR

Our results showed that the majority of sinonasal cancers were SCCs (87.5%, 28/32). The rest were adenocarcinoma (9.4%, occupy 3/32) and undifferentiated cancer (1/32 occupy 3.1%). Our results were in concordance with other authors (Lê Văn Bích and Phạm Khánh Hoà, 1969; Nguyễn Mạnh Cường, 1978; Nguyễn Công Thành, 1991; Vũ Công Trực, 1996; Trần Thị Hợp, 1996; Phùng Quang Tuấn, 2009; Lê Trung Thọ et al., 2011; Phạm Mai Thủy Tiên et al., 2018). We also noted that squamous pearls were not present in SNSCCs samples, in contrast to SCCs at other sites that have the same embryologic origin (in bronchial cancer) (Maemondo, 2010). This feature warrants for better outcome with radiotherapy of SNSCC than that at other sites.

The rate of highly and poorly differentiated SCC in our study were 68.8% and 18.8%, respectively. Although the squamous pearls were absent, intercellular bridges found in loose tumor cell clusters were the diagnostic morphologic features of SCC. In adenocarcinoma cases, highly differentiated tumors were 2/3. Thus, we assumed that most of the sinonasal cancers were highly differentiated tumors with slow progression and rare metastasis despite its large size.

EGFR and clinical presentations.

Among 28 stage III patients, 50.0% (14/28) of EGFR was positive, in which 6 cases scored 3+ (21.4%). Among 4 stage IV patients, 75% (3/4) of EGFR was positive and 50% (2/4) scored 3+. Our preliminary results showed that stage III patients were more negative with EGFR than stage IV (50% and 25%, respectively) and higher level of EGFR overexpression in stage IV than stage III (50% and 21.4%, respectively). Thus, the rate and level of EGFR overexpression increased with advanced stage of disease. This fact is confirmed by other studies (Sozzi et al., 2018): this result was proved to be similar to Sok et al. (2006), who have conducted a study in 30 SCCs in the head and neck, through the tests on both in vivo and in vitro, and they have demonstrated that tumor cells with EGFR expression grew more rapidly than those without that marker (Greene et al., 2006).

In collating with clinical presentations, our results showed the number of EGFR- positive expression was higher in exophytic than in exophytic with polyp forms, in advanced stage with facial

deformities than localized stage and in stage with neurologic deficits. We also noted that were EGFR-positive all the four patients with cervical lymphadenopathy. But we were unable to compare our results with others due to the lack of data in Vietnam.

CONCLUSIONS

1. The rate of EGFR expression in SNSCC was 53.1%, with 25% scored 3+.
2. The rate of EGFR expression was 50% (14/28) in stage III patients (6/14, scored 3+) and 75% (3/4) stage IV patients (2/4, scored 3+).
3. EGFR expression was higher in exophytic form, advanced stage with facial deformities, significantly higher in cases with neurologic deficits and cervical lymphadenopathy.

First of all, there was founded a significant association of EGFR expression with tumor stage and clinical features, which are the most important prognostic factors in head and neck squamous cell carcinoma, including sininasal. Therefore, EGFR expression can help as a prognostic biomarker in head and neck squamous cell carcinoma.

On the other hand, molecular studies should be performed in squamous cell carcinoma to identify patients that can avail response from anti-EGFR therapy. Understanding of the relationship between the level of EGFR expression and the clinical situation allows to justify the development of new targeted drugs.

The fact of using EGFR inhibitors in a short period of time after being approved them shows the interest and the utility of this therapy in patients with head and neck neoplasia and moreover with sinonasal cancers.

There are a lot of another areas for research in this topic. For example, certain reverse TK pathways are activated by the inhibition of the EGFR. These pathways can escape the influence of EGFR thus we need to consider alternative molecular targets for multimodal therapy. The capacity of cancer cells to adapt to different classes of drugs suggests that additional mechanisms of resistance to EGFR inhibitors may play a key role in regulating tumor response, such as the induction of angiogenesis process, translocation of surface receptors to the nucleus, altered DNA damage response, and all other undiscovered mutations.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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