IJPRP

Indian Journal of Pathology: Research and Practice
Volume 7 Number 8, August 2018
DOI: http://dx.doi.org/10.21088/ijprp.2278.148X.7818.6

Original Research Article

Immunohistochemical Expression of Epidermal Growth Factor Receptor in Head and Neck Tumours

Monnappa Gitanjali M.[1], Kolakkadan Hasaf M.[2], Sulata Kamath[3], Monnappa Priyadarshini[4], Sunitha V.P.[5]

[1,2]Assistant Professor, Department of Pathology, DM Wayanad Institute of Medical Sciences, Wayanad, Kerala 673577, India. [3]Associate Professor, Department of Pathology, M.S. Ramaiah Medical College, Bengaluru, Karnataka 560054, India. [4]Assistant Professor, Department of Pathology, Kodagu Institute of Medical Sciences, Madikeri, Karnataka 571201, India. [5]Pathologist, K.C General Hospital, Bengaluru, Karnataka 560003, India.

Corresponding Author:

Dr. Hasaf Mubeen K, Assistant Professor, Department of Pathology, DM Wayanad Institute of Medical Sciences, Wayanad, Kerala 673577, India.

E-mail: hasafmubeen@gmail.com

(**Received on** 26.07.2018, **Accepted on** 06.08.2018)

Abstract

Background: Head and neck squamous cell carcinoma (HNSCC) is an aggressive epithelial malignancy that is the sixth most common neoplasm in the world today. Overexpression of Epidermal Growth Factor Receptor (EGFR) is one of the most common molecular alterations in HNSCC. Promising preclinical studies have prompted the development of clinical trials testing EGFR inhibitors as single-agent therapy or in combination with conventional cytotoxic therapy.

Objectives: This study was aimed to evaluate the immunohistochemical expression of EGFR in malignancies of the upper aerodigestive tract.

Methods: A retrospective and a prospective study was conducted by light microscopy and immunohistochemical examination of 80 resected specimens of malignancies of the upper aerodigestive tract at the Department of Pathology, M.S. Ramaiah hospital, Bangalore over a period of three and a half years between January 2010- June 2013.

Results: All the cases were Squamous cell carcinomas (SCC) of which 72/80 cases were conventional SCC and the remaining were verrucuous carcinoma. Majority were Grade I tumours (43/80). 72 of the 80 cases showed EGFR positivity on IHC with >50% of the tumour cells showing EGFR positivity in 60% of the cases and 53% of cases showing strong intensity staining of EGFR. In majority of stage IVA tumours, >51% of the tumour cells showed EGFR positivity (84%) in comparison with stage I, II and III tumours.

Conclusion: The expression of EGFR protein in HNSCC can be used as a marker for targeted therapy.

Keywords: Epidermal Growth Factor Receptor; Head And Neck Squamous Cell Carcinoma; Immunohistochemistry.

Introduction

Head and Neck Squamous Cell Carcinoma (HNSCC) is an aggressive epithelial malignancy that is the sixth most common neoplasm in the world today, arising most commonly in the oral cavity. Despite numerous advances in treatment, taking advantage of the most recent protocols for surgery, radiation therapy, and chemotherapy, the overall long-term survival has remained at less than 50% for the past 50 years [1].

Over the last few years, many biological markers

have been reported in head and neck tumors. However, in recent studies, prognostic value has been attributed to the following markers: mutations of the p53, over-expression of the epidermal growth factor receptor (EGFR), over expression of the cyclin D16 and the Transforming growth factor (TGF-α). EGFR plays an important role in numerous processes that affect tumor development, growth, progression, and differentiation, inhibition of apoptosis and development of metastasis [2].

Several lines of evidence support EGFR as a molecular target for therapy of HNSCC. Treatment with EGFR-targeted therapy such as the chimericmonoclonal antibody cetuximab (C225) or the quinazoline gefitinib inhibits EGFR signaling and potentiates the effects of chemotherapy or radiation [3].

Promising preclinical studies have prompted the development of clinical trials testing EGFR inhibitors as single-agent therapy or in combination with conventional cytotoxic therapy. The clearest benefit of EGFR-inhibitor treatment to date is noted when it is combined with radiotherapy (RT) to treat locally advanced head and neck cancer [4].

The present study is done to assess the immunohistochemical expression of Epidermal Growth Factor Receptor in malignancies of the upper aerodigestive tract.

Materials and Method

Study was conducted in the Pathology department of M.S.Ramaiah Medical Teaching Hospital on the Mandibulectomy/ maxillectomy/ laryngectomy/ pharyngoesophagectomy/ with or without neck dissection specimens received for routine histopathological evaluation,from January 2010 to June 2013.

A total of 80 cases was studied. The detailed clinical history and results of relevant investigations done was collected from the patients' case files. The upper aero digestive tract tumor specimen was received in the Pathology department in 10% formalin. In every case the standard protocol for surgical grossing of the specimens was followed. After a detailed specimen description, multiple sections were taken from the tumor; mucosal, soft tissue and bony surgical margins and all the lymph nodes.

After conventional processing, paraffin sections of 5µm thickness were stained by haematoxylin and eosin (H & E) for histopathological study. In addition, 4µm sections was cut from a paraffin block of tumour tissue and taken on glass slides coated with adhesive (silane) for immunohistochemistry (IHC) to detect EGFR expression.

EGFR Immunohistochemistry:

The technique for IHC included antigen retrieval in TRIS buffer in a microwave oven, blocking endogenous peroxidase with 3% hydrogen peroxide, incubating with primary mouse monoclonal antibody (Novocastra, UK), linking with rabbit anti mouse secondary antibody (Novocastra, UK), enzyme labelling with streptavidin- horseradish peroxidase (Novocastra, UK), developing chromogen with deaminobenzidine (DAB) and counterstaining with haematoxylin. Positive and negative controls were taken with each batch of slides.

Specimens were evaluated microscopically. The extent of EGFR immunostaining was graded and scored as follows [3]:

Grading of EGFR

Staining of the tumour cells: Negative, <10%, 10-50%, 51-80%, >81%

The intensity of staining was scored as:0 - No staining, 1 – Weak, 2 –Moderate, 3 – Strong

Statistical Analysis:

The data was represented in terms of frequency distribution tables. The proportion of subjects revealing the expression was estimated along with 95% confidence interval.

Comparison of percentage of staining of cells by EGFR with intensity of staining was tested for statistical significance by employing chi-square/ Fischer exact test. $p \leq 0.05$ was considered statistically significant.

Results

Of the 80 specimens, majority were females 52 (65%), and 28 (35%) were males. The F: M ratio was 2:1 showing a clear female preponderance. Of the 80 cases, age ranged from 23 to 80 years with a mean age of 55 (±12SD) years. Peak incidence was seen in the age group of 41-50 years followed by 51-60 years. 34% patients were known alcoholics and 70% of the patients gave history of tobacco intake. 76/80 (95%) cases had tumour in the oral cavity. Majority of the tumours were ulceroinfiltrative on gross examination (54%).

Table 1: Comparison of percentage of staining of cells by EGFR with tumour stage

Percentage of Staining of Tumour Cells	Tumour Stage. Frequency (Percentage %)				
	I	II	III	IV A	Total
Negative	1(11.1)	4(44.4)	0(0)	4(44.4)	9
<10	1(100.0)	0(0)	0(0)	0(0)	1
10-50	6(43)	2(14)	1(7)	5(36)	14
51-80	3(10)	4(13)	11(35)	13(42)	31
>81	1(4)	6(24)	7(28)	11(42)	25

'p'. value = 0.013 (Significant)

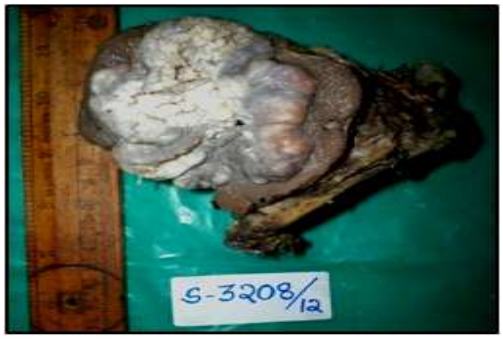

Fig. 1: Left Hemimandibulectomy: Ulceroproliferative gray white growth with irregular margins, everted edges and a necrotic floor.

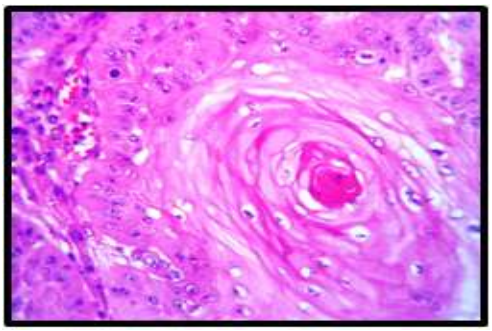

Fig. 2: Photomicrograph of a well differentiated Squamous cell carcinoma showing a keratin pearl. (H&E, x400)

All the cases in the present study were squamous cell carcinoma of which 8/80 (10%) cases were verrucous carcinoma and the remaining 72/80 (90%) cases were conventional squamous cell carcinoma. Majority of the cases presented with grade I tumour, 43/80 cases (54%); followed by 36/80 (45%) cases of grade II tumours. There was only one case (1%) of grade III carcinoma. In majority of the cases, i e. (39%), 51-80% of the tumour cells showed positive EGFR staining followed by 31.3%, where >81% of tumour cells showed a positive EGFR staining. 9/80cases (11%) were negative for EGFR staining.

A total of 71/80 cases (88.75%) showed positive staining by EGFR. Majority of the cases, 42/80 (52.5%) showed a strong EGFR staining of the tumour cells followed by 24 cases (30.0%) that showed a moderate staining intensity. 9 cases (11.3%) did not show EGFR positivity. 4 (50.0%)

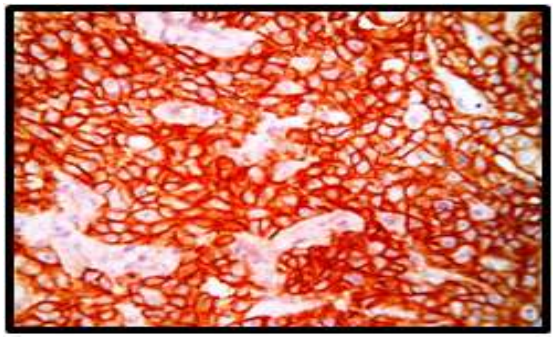

Fig. 3: Photomicrograph showing strong membranous EGFR positivity in >81% of the tumour cells. (Anti-EGFR- poly horseradish peroxidase- DAB chromogen, x400)

cases of verrucous carcinoma showed moderate intensity of EGFR stain. In both grade I and grade II tumours, majority showed moderate to severe intensity of staining. The only case of grade III tumour showed severe intensity of EGFR staining. The 'p' value was not significant (<0.05). Majority of the stage IV A tumours (58%) showed strong intensity of staining of tumour cells by EGFR whereas the Stage I tumours showed moderate intensity of staining. The 'p' value was not statistically significant. Majority of tumours with a size of > 2.1cm showed > 51% staining of tumour cells by EGFR whereas EGFR staining was seen in < 50% of cells in tumours when the size of the tumour was <2cm. The 'p' value was statistically significant. As the size of the tumour increases, the percentage of the cells showing EGFR positivity also increases.

In majority of the stage IV A tumours, > 51% of the tumour cells showed EGFR positivity (84%) in comparison with the other tumours and the difference was statistically significant with a 'p' value of <0.05. There was no significant difference in the percentage of cells stained by EGFR and the tumour grade. The 'p' value was statistically insignificant. There was significant correlation between the percentage of cells showing EGFR positivity and the intensity of staining with a 'p' value of <0.05. A strong intensity of staining of tumours cells was seen when >50% of cells showed EGFR positivity.

Discussion

Epidermal growth factor was one of the first growth factors to be described, and was shown to be mitogenic, with the effect mediated by its binding to the cell-surface receptor known as EGF receptor (HER1/EGFR or erbB1) [5]. In human tumors, high expression of EGFR correlates with a more aggressive clinical course and has been reported to be a useful diagnostic and prognostic marker [6]. Stanley Cohen, Nobel Prize Laureate in Physiology/Medicine, discovered epidermal growth factor (EGF) 25 years ago and elucidated its role in cell growth [7]. Gilbert J and Argiris A have stated that effective targeting of HNSCC and its precursor lesions requires an understanding of the molecular events that occur in the stepwise progression from normal-appearing mucosa to invasive carcinoma and one of the most important discoveries has been the role of the EGFR in HNSCC [8].

In the present study, 80 resected malignant specimens of the upper aerodigestive tract were evaluated by light microscopy to determine the histologic type, histological grade and immunohistochemistry was done to find out the EGFR status of the tumour and a correlation was done between its expression and the type, histological grade and stage of the tumour.

In a study by Issa HI [6], it was seen that the extent of staining in well and moderately differentiated squamous cell carcinomas was seen mainly localized to the undifferentiated cells at the periphery of the tumor nests, while in poorly differentiated squamous cell carcinomas, nearly all the tumor cells were stained. In the present study, the case with grade III tumour showed strong intensity of >80% of the tumour cells by EGFR. However, since there was only one case of grade III tumour, it was not statistically significant.

In the present study, majority of them showed positive EGFR staining (88.7%). This finding is consistent with previous studies regarding the immunostaining and overexpression of EGFR in squamous cell carcinomas of the head and neck. In a study conducted conducted by Issa HI 6, 100% EGFR staining rate was seen with 33.3% of cases exhibiting severe expression, and 43.3% of cases exhibiting moderate expression. It was noted in his study that a stronger intensity of EGFR staining was associated with a greater EGFR extent, with consequently high EGFR expression. This finding is consistent with the present study where a significant relationship was found between greater extent and stronger intensity scores of EGFR staining ($p<0.05$). Issa HI [6] concluded that the high EGFR expression in squamous cell carcinoma of the head and neck was associated with advanced tumor stage, lymph node metastasis, distant metastasis, poor differentiation and invasion.

Maiti GP et al. [10] have conducted a study on the gene amplification, mRNA expression, and protein overexpression of EGFR. It was seen that there was a significant correlation between gene amplification and mRNA expression; protein overexpression did not correlate with mRNA expression. This suggests thatexpression of EGFR is not regulated transcriptionally and mechanism other than gene amplification/mutations might be responsible for observed overexpression of this protein in HNSCC tumors.

Conclusion

Squamous cell carcinomas of the head and neck with advanced primary lesions, with or without regional lymph-node metastases, are challenging to treat effectively while maintaining the function of vital healthy structures. The outcome of patients presenting with advanced stage of the disease still remains poor.

EGFR is a prime target for anticancer therapy,and is beneficial when EGFR-inhibitor treatment is combined with radiotherapy to treat locally advanced head and neck cancer.

Resected malignant specimens of the upper aerodigestive tract from 80 patients was studied by light microscopy and the immunohistochemical expression of EGFR was evaluated. 88.75% of the tumours showed positive EGFR staining. There was a significant correlation between the percentage of tumour cells stained by EGFR with the tumour size and the stage of the tumour.

Table 2: Comparison of intensity of expression of EGFR in various studies

Study	Year of study	Absent	Weak	Moderate	Strong	Total cases
Payne CW et al.[9]	2006	2(8%)	1(4%)	8(33%)	13(54%)	24
Sarkis SA et al[3]	2010	5(12.5%)	15(37.5%)	14(35%)	6(15%)	40
Issa HI [6]	2013	-	5(16.6%)	17(57%)	8(26.6%)	30
Present study	2013	9(11.3%)	5(6.3%)	24(30%)	42(53%)	80

In conclusion, majority of cases of squamous cell carcinomas of the head and neck showed moderate to strong positive EGFR staining with >50% of the tumour cells taking up the stain in most of the cases and therefore the expression of this protein can be used as a marker for targeted therapy in these patients.

References

1. Lingen W. Head and neck, In: Robbins and Cotran Pathologic Basis of Disease, 8th ed. Kumar V, Abbas A K, Fausto N, Aster JC: editors. Philadelphia: Saunders, 2010;740-62.
2. Mariezkurrena XA, Guimera JA, Rodriguez JW, Weisman R, Ongkeko W. Immunohistochemistry study of EGFR expression in head and neck squamous cell carcinoma. Acta Otorrinolaringol Esp 2005;56:143-6.
3. Sarkis SA, Abdullah BH, Majeed BAA and Talabani NG. Immunohistochemical expression of epidermal growth factor receptor (EGFR) in oral squamous cell carcinoma in relation to proliferation, apoptosis, angiogenesis and lymphangiogenesis. Head and neck oncology 2010;2:13-21.
4. Zimmermann M, Zouhair A, Azria D and Ozsahin M. The epidermal growth factor receptor (EGFR) in head and neck cancer: its role and treatment implications. Radiat Oncol 2006;1:11-7.
5. Kim JC, Ali MA, Nandi A, Mukhopadhyay P, Choy H, Cao C et al. Correlation of HER1/ EGFR expression and degree of radiosensitizing effect of the HER1/ EGFR-tyrosine kinase inhibitor erlotinib. Indian Journal of Biochemistry and Biophysics 2005;42:358-65.
6. Issa HI. Immunoexpression of Epidermal Growth Factor Receptor, Ki-67 and P53 Protein in Squamous Cell Carcinoma of the Head and Neck. Research Journal of Medicine and Medical Sciences 2013;8(1):9-15.
7. Seshacharyulu P, Ponnusamy MP, Haridas D, Jain M, Ganti AK, and Batra SK. Targeting the EGFR signaling pathway in cancer therapy. Expert Opin Ther Targets 2012;16(1):15–31.
8. Gilbert J and Argiris A. Emerging Molecular Targeted Therapies in Squamous Cell Carcinoma of the Head and Neck. Clinical Advances in Hematology & Oncology 2006;8(4):611-9.
9. Payne CW, Holden JA and Layfield LJ. Detection of EGFR- and HER2-activating mutations in squamous cell carcinoma involving the head and neck. Modern Pathology 2006;16:634-40.
10. Maiti GP, Mondal P, Mukherjee N, Ghosh A, Ghosh S, Dey S et al. Overexpression of EGFR in Head and Neck Squamous Cell Carcinoma Is Associated with Inactivation of SH3GL 2 and CDC25A Genes. PLoS ONE 2013;8(5):e63440.