10.5005/jp-journals-10051-0058

ORIGINAL ARTICLE

Immunohistochemical Expression of Epidermal Growth Factor Receptor in Different Grades of Oral Squamous Cell Carcinomas

[1]Niraj Patil, [2]Harish H, [3]Surabhi Sinha, [4]Vandana Shah, [5]Pratik B Kariya, [6]Usha Sharma

ABSTRACT

Introduction: Epidermal growth factor receptor (EGFR) is a tyrosine kinase receptor of the ErbB family, which is expressed or highly expressed in a variety of solid tumors, including oral cancers.

Aims and objectives: The aim of this study was to evaluate the immunohistochemical (IHC) expression of EGFR in different grades of oral squamous cell carcinoma (OSCC).

Materials and methods: A series of 50 paraffin blocks with different grades of previously diagnosed OSCC were analyzed for EGFR, which was determined by standardised histopathology. A total of 32 (64%) cases out of 50 showed positivity for EGFR.

Results: A total of 32 cases (64%) showed positivity for EGFR, although statistically, a nonsignificant p-value of 0.814 was obtained for EGFR.

Conclusion: We noticed the correlation of increased membrane and cytoplasmic expression of EGFR in poorly differentiated grades of OSCC that can be utilized to predict the prognosis of the patients.

Keywords: Epidermal growth factor receptor, Oral cancer, Squamous cell carcinoma.

How to cite this article: Patil N, Harish H, Sinha S, Shah V, Kariya PB, Sharma U. Immunohistochemical Expression of Epidermal Growth Factor Receptor in Different Grades of Oral Squamous Cell Carcinomas. Int J Oral Care Res 2016;4(4):259-262.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the commonest head and neck malignancy, representing almost 95% of the head and neck carcinomas. Of all the cancers worldwide, its prevalence rate is 5%.[1] Globally, about two-thirds of oral cancers occur in developing countries, and it is a major health hazard. The highest prevalence of oral cancer has been observed in the Indian subcontinent,[2] where huge populations are open to the elements, exterior irritants, and carcinogens, such as smoke, tobacco, and betel nut extracts. Usually, cancer develops through rising grades of oral epithelial dysplasia to fatal invasive malignancy.[1]

Intense research has started a new age of cancer treatment for the duration of the last decade, that of molecular therapeutics. Promising preclinical studies have prompted the expansion of clinical trials testing molecules, such as the epidermal growth factor receptor (EGFR) with conventional cytotoxic therapy.[3]

The EGFR has a significant role in the differentiation and morphogenesis of numerous organs and proliferation and continued existence of mammalian cells.[4] The EGFR is a proto-oncogene, which if activated at the cell surface by transforming growth factor-α serves to advance cellular proliferation.[4] It is a transmembrane receptor expressed in an array of human tumors of epithelial origin; higher expressions of EGFR are seen in 80% of squamous cell carcinomas (SCCs), which explains that an unrestrained growth may be aided by abnormal EGFR expression.[4] The higher expressions of EGFR have been seen to be of prognostic value in head and neck SCC (HNSCC).[5,6]

This study may help in indicating that EGFR expressions correlate with the severity of OSCC, and it is also possible that the immunohistochemical (IHC) demonstration of these markers may serve to be a useful prognostic tool for a more precise clinical outcome of OSCC.

MATERIALS AND METHODS

A total of 50 cases of OSCC were evaluated immunohistochemically for EGFR expression. Normal buccal mucosa has been taken as a control for EGFR.

Inclusion Criteria

Formalin-fixed paraffin-embedded blocks with adequate tissue size (a minimum of 4–5 mm of lesional tissue) for sectioning and IHC staining with representative histological features.

[1,3]Postgraduate Student, [2]Reader, [4]Professor and Head [5,6]Senior Lecturer

[1-4,6]Department of Oral Pathology, KM Shah Dental College & Hospital: Sumandeep Vidyapeeth, Vadodara, Gujarat, India

[5]Department of Paedodontics and Preventive Dentistry, KM Shah Dental College & Hospital: Sumandeep Vidyapeeth Vadodara, Gujarat, India

Corresponding Author: Usha Sharma, Senior Lecturer Department of Oral Pathology, KM Shah Dental College & Hospital: Sumandeep Vidyapeeth, Vadodara, Gujarat, India e-mail: ushasharma7@gmail.com

Exclusion Criteria

The tissue and blocks without representative area of any grade of SCC were excluded.

About 4 µm sections were taken, and EGFR was detected by IHC staining using the clone EP38Y (Thermo Scientific rabbit monoclonal antibody #RM-2111-R7, 7 mL). All instances of OSCCs were subjected to histopathological evaluation and sorted as well-differentiated SCC (WDSCC), moderately differentiated SCC (MDSCC), and poorly differentiated SCC (PDSCC) as per Broder's classification of SCC.

ANALYSIS OF IMUNOHISTOCHEMICAL STAINING

Positive immunoreactivity was pointed out by the appearance of brown color as the final result at the antigen target spot. The nonpositive control tissue exhibited lack of staining. Tissue sections of normal oral epithelium were taken as positive control for EGFR. The evaluation of study cases was done subsequently in a similar way, and they were graded as positive or negative. The positive results were assessed further for intensity of staining. The presence of brown-colored end product at the site of target antigen was taken as immunohistochemically positive and the negative control tissue demonstrated absence of staining. Presence of immunostaining in the cell membrane of various layers of epithelium was evaluated in randomized six fields/intensity of positively stained cells as percentage expression at ×40 and graded as 0 (under 10% positively stained cells), 1+ (10–25% positively stained cells: Weak expression), 2+ (25–50% positively stained cells: Mild-to-moderate expression), 3+ (50–75% positive cells: Moderate-to-strong expression) (Table 1).

Although judging the depth of staining was looked upon as biased, care was taken to decrease the bias by three autonomous investigators (two other investigators who were senior faculty associates in the branch), which demonstrated intensely positive staining in EGFR.

Statistical Methods

The EGFR expression in different grades of OSCC is compared by using the Pearson correlation test and Student's t-test. Mann–Whitney and the Kruskal–Wallis tests were employed for comparison of continuous variables.

RESULTS

The gender distribution of the cases was, male:female ratio being 2.1:1 (34:16). In males, 19 (55%) cases out of 34 were diagnosed as WDSCC, 12 (35.5%) cases out of 34 as PDSCC, and 3 (8.8%) cases out of 34 as MDSCC. In females, 12 (75%) cases out of 16 were diagnosed as WDSCC, 3 (18.8%) as MDSCC, and 1 (6.2%) as PDSCC (Table 2).

Table 1: Ranks of grading for staining evaluation[7]

0	No Labeling	<10% tumor cells, patchy/ homogeneous
1	Weak labeling	10–25% tumor cells, patchy/ homogeneous
2	Moderate labeling	25–50% tumor cells, patchy/ homogeneous
3	Intense labeling	>50% tumor cells, patchy/ homogeneous

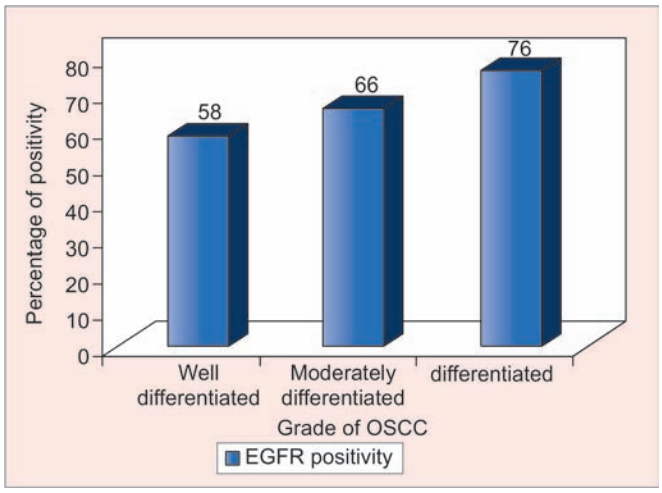
Table 2: Age of the OSCC cases considered for this study, with age range being 29–70 years (mean 48 ± standard deviation 12.04 years)

	n (%)	*Minimum age*	*Maximum age*	*Mean age*	*Std. deviation*
Age (years)	50 (100)	29.00	70.00	48.5200	12.04624

A total of 32 cases (64%) showed positivity for EGFR, of which 18 (58%) were WDSCC, 4 (66%) cases were MDSCC, and 10 (76%) were PDSCC. Out of the 18 cases of WDSCC that showed positivity for EGFR, 11 (35.5%) showed mild expression and 7 (22.5%) showed moderate expression. Of the 4 cases of MDSCC showing positivity for EGFR, 1 showed mild expression, 2 showed moderate expression, and 1 showed intense expression. Of the 10 cases of PDSCC, 5 showed moderate expression and 5 showed intense expression; p values obtained for EGFR was 0.814, which was nonsignificant (Graph 1, Figs 1 to 4).

DISCUSSION

A lot of work is currently focused on the recognition of superior biological and clinical factors that can serve as a predictive and prognostic marker. The perception of the biological initiation of tumor development and its progression in the oral cavity has enhanced as a result of the



Graph 1: Expression of EGFR in different grades of OSCC

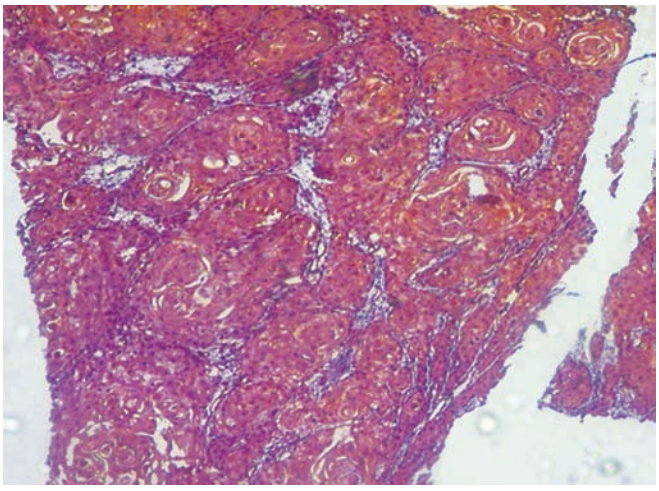


Fig. 1: Epidermal growth factor receptor showing intense expression in WDSCC (10×)

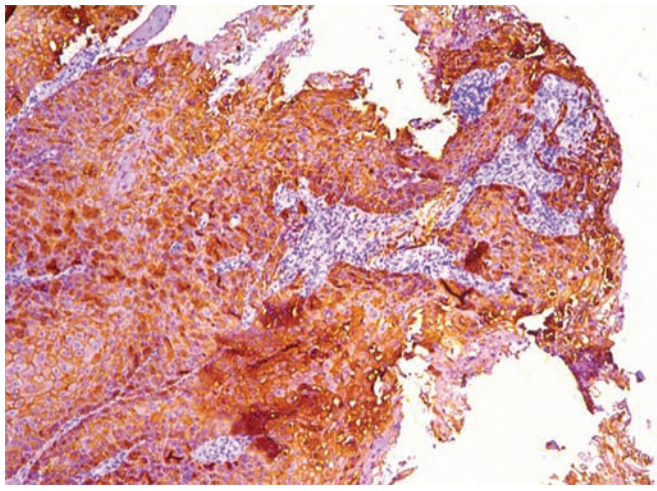


Fig. 2: Epidermal growth factor receptor showing intense expression in MDSCC (10×)

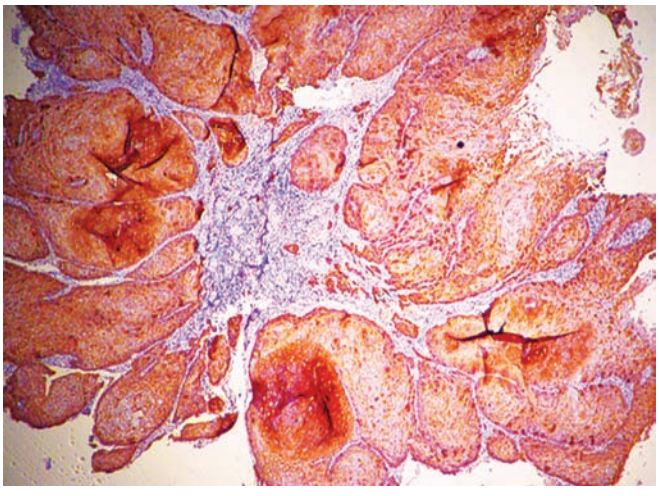


Fig. 3: Epidermal growth factor receptor showing intense expression in PDSCC (10×)

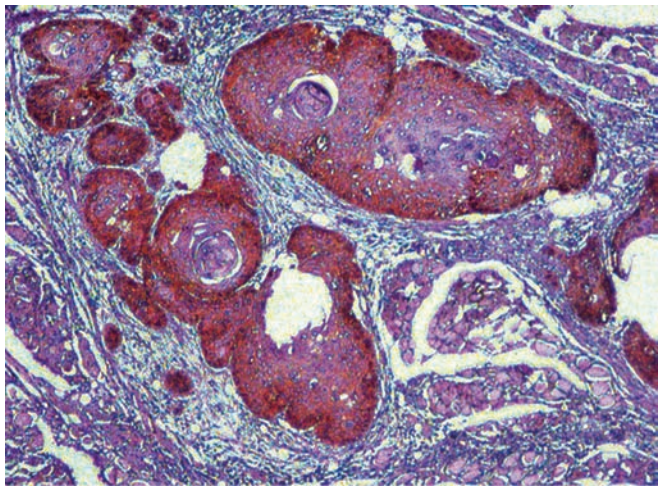


Fig. 4: Epithelial growth factor receptor showing moderate expression in WDSCC (10×)

current advances in fundamental research and genomics. As a result, an array of molecular tumor markers evaluated in the laboratory have been recognized in HNSCC for their potential to predict disease result or response to treatment in patients. According to our present study, we expect to gather further proficiency to either anticipate patients at risk for disease development after standard therapy or identify those who can gain from postoperative radiotherapy and those with radioresistant tumors.[5]

Our study showed moderate-to-intense EGFR-expression in more than 40% of the cases (p-value = 0.814), including all grades of SCC. The status of the lymph node was not recorded for our study. As the grade of the SCC increased, i.e., from WDSCC to PDSCC, the intensity of EGFR increased. It can be summarized that somewhere the expression of EGFR is related to the grade of SCC.

Xia et al,[8] after studying different family members of the EGFR, conclude that there are no significant associations between the expression levels of the EGFR family members and the sex, and histological grade ($p > 0.05$) except that EGFR expression level is significantly associated with age ($p < 0.001$). Our study included 23 cases of WDSCC that were recorded from the below 50 age group with positive EGFR expression as compared with only 4 cases of PDSCC in this age group; about 9 of the 13 PDSCC cases, however, recorded from the above 50 age group support the above-mentioned fact. On the contrary, according to a study conducted by Diniz-Freitas et al,[9] none of the factors, such as age, sex, tumor size and depth, intraoral site, lymph node metastasis, clinical phase, and histological demarcation was concurrent with EFGR expression.

A positive association with cell proliferation and a prognostic significance regarding overall and disease-free survival was found by Temam et al.[10] However, it could not be confirmed by Gröbe et al[11] who studied IHC expression of the EGFR in OSCC.

CONCLUSION

This study can help in indicating that EGFR expression correlates with the severity of OSCC, and the IHC demonstration of these markers can serve to be a useful prognostic tool for a more precise clinical outcome of OSCC. Increased expression of EGFR in PDSCC may suggest its positive role in the proliferation and differentiation of tumor cells and prognostic significance regarding overall and disease-free survival. However, along with statistically nonsignificant outcome, the sample size taken was also very limited. Hence, further studies with a larger sample size are required to reach a firm conclusion.

REFERENCES

1. Scott IS, Odell E, Chatrath P, Morris LS, Davies RJ, Vowler SL, Laskey RA, Coleman N. Minimally invasive immunocytochemical approach to early detection of oral squamous cell carcinoma and dysplasia. Br J Cancer 2006 Apr;94(8): 1170-1175.
2. Singh RD, Haridas N, Patel JB, Shah FD, Shukla SN, Shah PM, Patel PS. Matrix metalloproteinases and their inhibitors: correlation with invasion and metastasis in oral cancer. Indian J Clin Biochem 2010 Jul;25(3):250-259.
3. Sarkis SA, Abdullah BH, Abdul Majeed BA, Talabani NG. Immunohistochemical expression of epidermal growth factor receptor (EGFR) in oral squamous cell carcinoma in relation to proliferation, apoptosis, angiogenesis and lymphangiogenesis. Head Neck Oncol 2010 Jun;2(1):13.
4. Lim SC, Zhang S, Ishii G, Endoh Y, Kodama K, Miyamoto S, Hayashi R, Ebihara S, Cho JS, Ochiai A. Predictive markers for late cervical metastasis in stage I and II invasive squamous cell carcinoma of the oral tongue. Clin Cancer Res 2004 Jan;10 (1 Pt 1):166-172.
5. Lothaire P, de Azambuja E, Dequanter D, Lalami Y, Sotiriou C, Andry G, Castro G Jr, Awada A. Molecular markers of head and neck squamous cell carcinoma: promising signs in need of prospective evaluation. Head Neck 2006 Mar;28(3):256-269.
6. Carracedo DG, Astudillo A, Rodrigo JP, Suarez C, Gonzalez MV. Skp2, p27kip1 and EGFR assessment in head and neck squamous cell carcinoma: prognostic implications. Oncol Rep 2008 Sep;20(3):589-595.
7. Jyothi Meka N, Ugrappa S, Velpula N, Kumar S, Naik Maloth K, Kodangal S, Ch L, Goyal S. Quantitative immunoexpression of EGFR in oral potentially malignant disorders: oral leukoplakia and oral submucous fibrosis. J Dent Res Dent Clin Dent Prospects 2015 Summer;9(3):166-174.
8. Xia W, Lau YK, Zhang HZ, Xiao FY, Johnston DA, Liu AR, Li L, Katz RL, Hung MC. Combination of EGFR, HER-2/neu, and HER-3 is a stronger predictor for the outcome of oral squamous cell carcinoma than any individual family members. Clin Cancer Res 1999 Dec;5(12):4164-4174.
9. Diniz-Freitas M, García-Caballero T, Antúnez-López J, Gándara-Rey JM, García-García A. Pharmacodiagnostic evaluation of EGFR expression in oral squamous cell carcinoma. Oral Dis 2007 May;13(3):285-290.
10. Temam S, Kawaguchi H, El-Naggar AK, Jelinek J, Tang H, Liu DD, Lang W, Issa JP, Lee JJ, Mao L. Epidermal growth factor receptor copy number alterations correlate with poor clinical outcome in patients with head and neck squamous cancer. J Clin Oncol 2007 Jun 1;25(16):2164-2170.
11. Gröbe A, Eichhorn W, Fraederich M, Kluwe L, Vashist Y, Wikner J, Smeets R, Simon R, Sauter G, Heiland M, et al. Immunohistochemical and FISH analysis of EGFR and its prognostic value in patients with oral squamous cell carcinoma. J Oral Pathol Med 2014 Mar;43(3):205-210.