Comparative Evaluation of EGFR Expression in Oral Normal Epithelium, Dysplastic Epithelium and Squamous Cell Carcinoma – An IHC Study

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ABSTRACT

Introduction: The epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases (TKs), control many developmental, metabolic and physiological processes.

Objectives: To evaluate different aspects of tumour dynamics through the immunohistochemical evaluation of EGFR expression in OSCC and its correlation with proliferation.

Methods: A total number of 35 cases are included in the study, comprising of 5 histologically proven normal cases, 10 histologically proven cases of epithelial dysplasia, 10 histologically proven cases of well-differentiated squamous cell carcinoma and 10 histologically proven cases of moderately differentiated squamous cell carcinoma. Serial sections of 3 µm were obtained from the archival blocks using a standard microtome. They were evaluated by histologic examination.

Results: There is a significant association was found between the level of intensity of staining and group. Since the p-value is less than 0.05 there is a significant association was found between the grading of overall staining and group.

Conclusion: Based on our observation, it may be concluded that EGFR can be considered as an early marker of cell proliferation on cell differentiation as well as an early marker of epithelial dysplasia and onset of cancer.

Key Words: Dysplasia, Squamous cell carcinoma, Epidermal growth factor receptor

INTRODUCTION

Oral cancer is the 6th communal cancer in the globe. In current years, EGFR has been measured as an encouraging target for monoclonal antibody treatment. Great EGFR appearance has been associated with tumour size, metastasis, and existence. It is of great importance to conduct studies to determine the spectrum of mutations in the human EGFR-2 gene to gain a better insight into the mechanisms.1-3

Epidermal growth factor receptor is one among the group of regulatory agents that acts to control cell viability and proliferation in a hormone-like receptor-dependent fashion under normal circumstances. The bigger appearance of EGFR is establishing in oral squamous cell carcinomas and premalignant lesions. It is assumed that only those potentially malignant lesions that express high levels of EGFR, advancement to frank malignancies during tumorigenesis. So, by finding the appearance of EGFR in these doubtful lesions, it would be probable to evaluate the potentiality of these lesions to become malignant, hence the patient is presented for early treatment.4,5

The epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases (TKs), mentioned to as the HER or ErbB family contains four members—EGFR (HER1/ErbB1), HER2 (ErbB2), HER3 (ErbB3) and HER4 (ErbB4)—that regulate many developmental, metabolic and physiological processes.6 Epidermal growth factor receptor (EGFR) is one amongst the group of regulatory mediators that acts to control cell viability and production in a hormone-like receptor-dependent fashion under normal conditions. All normal cells require stimulation based on signals to experience proliferation, growth, and differentiation many of which carried by growth factors. EGFR theatres a significant role in the morphogenesis and differentiation of many organs and proliferation and existence in mammalian cells. EGFR has been reported to be expressed in a variety of human tumours of

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epithelial origin; overexpression of EGFR has been documented in 80% of SCC.5,6, 7 This research related to different aspects of tumour dynamics through the immunohistochemical assessment of EGFR expression in OSCC and its association with proliferation.

MATERIALS AND METHODS

The present study was carried out in archival blocks taken from the Department of Oral Pathology, Vinayaka Mission’s Sankarachariyar Dental College and Hospital, Salem, Tamil Nadu. A total number of 35 cases are included in the study, comprising of:

- 5 histologically proven normal cases
- 10 histologically proven cases of epithelial dysplasia
- 10 histologically proven cases of well-differentiated squamous cell carcinoma
- 10 histologically proven cases of moderately differentiated squamous cell carcinoma

**Inclusion criteria**

_**Normal Epithelium:**_
- Patient above 40 years of age.
- Patient without systemic diseases like diabetes etc. Patient without deleterious habits

_**Dysplastic Epithelium:**_
- All grades of dysplasia without any other pathology.
- All areas of oral cavity
- Above 40 years of age.

_**Carcinoma:**_
- Well and moderately differentiated squamous cell carcinoma.
- All areas of the oral cavity.
- Above 40 years of age.

**Exclusion criteria**

- Patients with immunocompromised diseases like diabetes, HIV infections were excluded.
- Other forms of carcinoma like verrucous, adipogenic, basaloid, and spindle types were not included.
- Poorly differentiated squamous cell carcinoma also not included.
- Patients with other mucosal diseases and developmental disorders were excluded.

**Preliminary Procedure**

Serial sections of 3 µm were obtained from the archival blocks using a standard microtome. One set of segments were stained with routine eosin and haematoxylin. The other set of sections were used for Immunohistochemical study. Routine processing of all the specimens was done with the help of alcohol, xylene etc and finally embedded in paraffin. Sectioning was done using rotary microtome and sections of 3 microns thick were cut and carefully fixed on the positivity charged poly-L-Lysine coated microscopic slides. The sections were dried at room temperature (37°C) followed by 1 hour incubation at 60°C. The tissues were de-paraffinized by giving 2 dips lasting 10 minutes each in fresh xylene. Rehydration of tissue were carried out by giving 2 dips for 10 minutes each in absolute alcohol and placed in distilled water bath and not allowed to dry.

Using pap pen, a circle outside the section was marked to avoid spreading of the reagents over the slides, minimize the quantity of reagent used and to prevent drying of section. All reagents were spread evenly over the sections without any air bubbles. All reagents were brought to room temperature and incubation was done at room temperature in a moist chamber. Photomicrographic evaluation of normal and carcinogenic oral epithelium done and indicates with histological findings as; normal epithelium (Figure-1), grade 1 expression of EGFR (Figure-2) dysplastic oral epithelium (Figure-3), grade 2 expression of EGFR (Figure-4), well-differentiated squamous cell carcinoma (Figure-5), grade 3 expression of EGFR (Figure-6), moderate carcinoma (Figure-7) and grade 4 expression of EGFR (Figure-8). The obtained data were statistically evaluated with SPPS software version 20 using the chi square test at the significance level of less than 0.05.

**RESULTS**

EGFR expression in normal epithelium, dysplastic epithelium, well-differentiated squamous cell carcinoma and moderately differentiated squamous cell carcinoma are calculated in 2 main categories,

1. Intensity of staining
2. Overall staining.

**The intensity of staining:**
It represents specificity and quality of staining, and it is categorized as mild, moderate and strong.

**Overall staining:**
It represents sensitivity and quantity of staining, and it is categorized as grade 1, grade 2, grade 3 and grade 4.

Grade 1 - 0% to 25% areas are sensitive.
Grade 2 - 25% to 50% of areas are sensitive.
Grade 3 - 50% to 75% areas are sensitive.
Grade 4 - 75% to 100% of the areas are sensitive.

A total of 35 cases (n=35), comprising of 5 oral normal epithelium (n=10), 10 dysplastic epithelium (n=10), 10 well-differentiated squamous cell carcinoma (n=10) and 10 moderately differentiated squamous cell carcinoma (n=10), were evaluated for immuno-histo-chemical expression, intensity and overall expression pattern of EGFR (Table 1). Out of 5
normal epithelium, 5(100%) were mild intensity of staining and overall expression exhibits 4(80%) grade1 and 1(20%) was grade 2. Among the 10 cases of the dysplastic epithelium, 6(60%) mild and 4(40%) were moderate intensity of staining and overall expression exhibits 5(50%) grade1, 3(30%) grade2 and 2(20%) were grade 4. Among the 10 cases of well-differentiated squamous cell carcinoma, 2(20%) mild, 5(50%) moderate and 3(30%) strong intensity of staining and overall expression exhibits 5(50%) grade2, 3(30%) grade3 and 2(20%) were grade 4. Among the 10 cases of moderately differentiated squamous cell carcinoma, 4(40%) mild, 2(20%) moderate and 4(40%) strong intensity of staining and overall expression showed 1(10%) grade1, 2(20%) grade 2, 2(20%) grade 3 and 5 (50%) were grade 4. Since the p-value is less than 0.05 there is a significant association was found between the level of intensity of staining and group. Since the p-value is less than 0.05 there is a significant association was found between the grading of overall staining and group (Table 2).

Table 1: Intensity of staining in normal, dysplastic, well and moderately differentiated squamous cell carcinoma

| Intensity of Staining | Mild | Moderate | Strong | Total |
|-----------------------|------|----------|--------|-------|
| Chi                   |      |          |        |       |
| N                     |      |          |        |       |
| %                     |      |          |        |       |
| Normal                | 5    | 100      | 5      |
| Dysplasia             | 6    | 60       | 4      | 10    |
| Well Differentiated   | 2    | 20       | 5      | 3     | 10    |
| Squamous cell Carcinoma | 13.64 | 0.034*    |
| Moderately Differentiated | 4    | 40       | 2      | 4     | 10    |
| Squamous cell Carcinoma | 17   | 49       | 11     | 31    | 7     | 20    | 35    |

Table 2: Overall staining in normal, dysplastic, well and moderately differentiated squamous cell carcinoma

| Overall Staining | Grade | Grade | Grade | Grade | Total |
|------------------|-------|-------|-------|-------|-------|
| Chi              |       |       |       |       |       |
| N                |       |       |       |       |       |
| %                |       |       |       |       |       |
| Normal           | 4     | 80    | 1     | 20    |
| Dysplasia        | 5     | 50    | 3     | 30    |
| Well Differentiated | 5    | 50    | 3     | 30    | 2     | 20    | 10    |
| Squamous cell Carcinoma | 19.96 | 0.018*      |
| Moderately Differentiated | 1     | 10    | 2     | 20    | 2     | 20    | 5     | 50    | 10    |
| Squamous cell Carcinoma | 5     | 29    | 11    | 31    | 5     | 14    | 9     | 26    | 35    |
| Total            | 10    | 29    | 11    | 31    | 5     | 14    | 9     | 26    | 35    |
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Figure 1: H&E stain 10x low power view of normal oral epithelium.

Figure 2: 10x low power view of normal oral epithelium showing mild intensity and grade 1 expression of EGFR.

Figure 3: H&E stain 10x low power view of dysplastic oral epithelium.

Figure 4: 10x low power view of dysplastic oral epithelium showing mild intensity and grade 2 expression of EGFR.

Figure 5: H&E stain 10x low power view of well differentiated squamous cell carcinoma.

Figure 6: 10x low power view of well-differentiated squamous cell carcinoma showing moderate intensity and grade 3 expression of EGFR.

Figure 7: H&E stain 10x low power view of moderately differentiated squamous cell carcinoma.

Figure 8: 10x low power view of moderately differentiated squamous cell carcinoma showing strong intensity and grade 4 expression of EGFR.
DISCUSSION

Epidermal growth factor receptor (EGFR) fits the ErbB receptor family (EGFR or Her-1, Her-2, Her-3, and Her-4). These receptors are made up of a hydrophobic transmembrane segment, an extracellular ligand-binding domain, and an intracellular tyrosine kinase domain. In regular cells, the appearance of EGFR ranges from 40,000 to 100,000 receptors per cell. In HNSCC, EGFR and its ligand, TGF-α, are overexpressed in 80–90% of cases. The nature of the protein overexpression is believed to result from improved transcription, with no obvious change in mRNA stability; gene amplification has been observed less commonly.7,8

EGFR manages numerous physiological functions, including migration, cell proliferation, differentiation and survival. Importantly, abnormal signalling by EGFR has been related to human cancers in which EGFR and its several ligands are regularly overexpressed or mutated. EGFR organizes activation of many downstream factors and is subject of numerous regulatory processes as it facilitates biology of the cell it resides in. Therefore, many types of research have been dedicated to accepting EGFR biology and targeting the protein for the goal of supervising tumour in clinical conditions. Endocytic regulation of EGFR suggests a hopeful area for targeting EGFR activity.9,10 Upon ligand binding, the activated receptor undergoes endocytosis and becomes degraded in lysosome, thereby terminating the signal. Enrooted to the lysosome, the receptor becomes involved in activating numerous signalling pathways counting Phosphatidylinositol-4,5-bisphosphate 3-kinases (PI3Ks), mitogen-activated protein kinase (MAPK) and endocytosis may show both spatial and temporal guideline of downstream target activation. Therefore, endocytosis is an important regulator of EGFR.11

The present study showed the expression of EGFR in normal oral tissues, dysplastic epithelium, well-differentiated squamous cell carcinoma and moderately differentiated squamous cell carcinoma. In the epithelium, the membrane and/or cytoplasm related brownish-red staining was taken as positive. The staining intensity was assessed and scored on a 3 point scale from focal to diffuse as grade 1,2,3,4. The results of the present study showed a significant increase in the staining reactions in dysplasia, well-differentiated squamous cell carcinoma, moderately differentiated squamous cell carcinoma as compared with normal mucosa. In normal mucosa, almost all the cases showed mild intensity and grade 1 staining. Most of the cases of dysplasia showed mild to moderate intensity and grade 1 and 2 stainings. In well and moderate squamous cell carcinoma, most of the cases showed moderate to strong intensity and grade 2,3&4 staining. Similar results were seen in a study, by Ashish Mahendra et al. in 2013, in which a total of 30 patients in which 15 cases of OSCC and 15 cases of epithelial dysplasia were selected for immunohistochemical analysis of EGFR.12

In another study, a total of 52 patients with OSCC was selected for immunohistochemical analysis of EGFR and phosphorylated EGFR(p-EGFR) detection. Compare to this study, EGFR expression was almost equal to the present study, but in the present study phosphorylated EGFR expression was not detected. In another study, authors found negative EGFR expression in normal oral mucosa, 25% expression in epithelial dysplasia, and 40% expression in oral squamous cell carcinoma (OSCC). This proved a significant difference in normal oral mucosa, epithelial dysplasia, and OSCC group (< 0.05). But in the present study positive EGFR expression was seen in normal mucosa in all cases.13,14

The existing study approves the comments of others that high EGFR expression is present in OSCCs which proposes that an uncontrolled growth may be mediated by abnormal EGFR expression. Since the squamous epithelium retains an unremitting physiological regeneration in normal conditions so that it is sensible that the basal cells interpret signals of EGF by binding to EGFR, while its expression beyond basal localization in cancerous tissue suggests that a correlation between EGFR and tumour progress may exist.15,16

CONCLUSION

Depends on our findings, it may be decided that EGFR can be measured as an early marker of cell proliferation on cell differentiation as well as an early marker of epithelial dysplasia and onset of cancer. EGFR expression can be correlated with biologic behaviour of oral normal epithelium, dysplastic epithelium, well and moderately differentiated squamous cell carcinoma.

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Author contribution
1. Dr. C. Ayyadurai- Investigation
2. Dr. B Sekar,- Data collection
3. Dr. Maya Ramesh - Data analysis
4. Dr. Mathew Jacob- Statistical evaluation
5. Dr. P Rajathi- Manuscript writing
6. Dr. M Ambika- Edidting

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