ORIGINAL ARTICLE

Expression of epithelial growth factor receptor in oral epithelial dysplastic lesions

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ABSTRACT

Aim: The aim of the study was to assess the expression of epithelial growth factor receptor (EGFR) in normal oral mucosa and varying grades of oral epithelial dysplasia (OED) and to correlate these findings, with clinicopathologic features and findings on routine hematoxylin and eosin–stained (H and E) sections. **Materials and Methods:** Twenty-nine formalin-fixed, paraffin-embedded blocks of various grades of OED and 10 normal mucosa were stained with routine H and E and immunostained with EGFR by avidin–biotin method. **Results:** The results showed a significant increase in the staining reactions in varying grades of dysplasia as compared with normal mucosa. **Conclusion:** EGFR can be considered as an early marker of a cell proliferation and maturation as well as early marker of epithelial dysplasia and onset of cancer in oral dysplasia. However, further studies with a larger sample size and continuous followup is suggested to determine its role and significance precisely.

Key words: Dysplasia, EGFR, oral squamous cell carcinoma

INTRODUCTION

In the oral cavity some of the potentially malignant lesions metamorph to malignancy and some regress.[1-3] Various research works are undertaken to locate the biochemical changes in the dysplastic cells so as to identify these high-risk lesions.

Epidermal growth factor receptor (EGFR) 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. Increased expression of EGFR is found in oral squamous cell carcinomas and premalignant lesions.[4] It is hypothesized that only those potentially malignant lesions that express high levels of EGFR, progress to frank malignancies during tumorigenesis.[5] So, by finding the expression of EGFR in these suspicious lesions, it would be possible to assess the potentiality of these lesions to become malignant, hence the patient is available for early treatment.

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MATERIALS AND METHODS

Twenty-nine, formalin-fixed, paraffin-embedded blocks of various grades of dysplasia were retrieved from the archives of two dental colleges in Chennai. Patient demographics and details regarding personal habits were noted. Healthy individuals without any oral lesions, habits, or systemic diseases served as controls. Incision biopsies were obtained from 10 controls with written consent. Biopsies were taken from pericoronal region near third and second molars. Tissues were routinely processed and embedded in formalin. Four consecutive sections of 4 μm thickness were cut from each block and 4 sets of slides were prepared.

One set of slides (both normal and dysplastic lesions) were stained with hematoxylin and eosin (H and E) and was evaluated by an Oral Pathologist and graded according to criteria used by Arne Burkhardt.[1] The 29 cases of oral epithelial dysplasia (OED) consisted of 13 cases of mild, 8 cases of moderate, and 8 cases of severe epithelial dysplasia.

The next set of slides was immunostained by avidin–biotin method[6-8] with mouse monoclonal antibodies to EGFR (Sigma, St. Louis, MO, USA) in 1:100 dilution (primary antibody). The secondary antibody was biotinylated horse antimouse antibody. (Vector Laboratories, Burlingame, CA, USA). The peroxides conjugated streptavidin–biotin method was used. Positive controls were sections of breast

carcinoma that overexpress EGFR. Negative controls consisted of phosphate-buffered saline instead of the primary antibody.

In the epithelium, the membrane- and/or cytoplasm-associated brownish red staining was taken as positive. The staining intensity of basal and suprabasal epithelial cells was assessed and scored on a 4-point scale.[9] Five areas from each tissue specimen were analyzed for positive staining at a magnification of ×400 using light microscope. Following scores were given: 0 for negative staining, + for consistent weak staining, ++ for moderate staining, and +++ for consistent heavy staining. The results were subjected for necessary statistical analysis.

RESULTS

Out of the 29 cases of epithelial dysplasia, 23 were males and 6 were females. Most of the patients had a history of either pan masala chewing or keeping snuff in the buccal mucosa and labial vestibule. Duration of the habit varied from 4 months to 16 years. Eight patients had bilateral lesions. Lesions were predominantly found in the buccal mucosa (21/29 cases) followed by commissural area (4/29), tongue (2/29), and gingiva (1/29). There was a single patient with floor of the mouth lesion but was not associated with habits. Most of the patients were asymptomatic, especially those who had smoking habit. Others complained of burning sensation on taking food.

Immunohistochemical staining

All specimens of normal mucosa, epithelial dysplasia of all grades stained positively with various intensity of staining. Staining was observed in the cell membrane and cytoplasm of keratinocytes.

In normal mucosa [Figure 1; Graph 1], the basal layer showed intense staining in 55% of cases, moderate staining in 30% of cases, and 15% of cases showed negative staining. In the deep spinous layer, there was moderate staining observed in 60% of cases and no staining reaction in rest of the cases. Also no staining was observed in superficial spinous layer. Keratin stained as streak.

In mild dysplasia [Figures 2 and 3; Graph 2], 57.5% cases showed moderate staining reactions in the basal layer and 10% of cases showed intense staining reactions in the deep spinous layer.

In moderate dysplasia [Figures 4 and 5; Graph 3], intense staining reaction was observed in basal layer in 37.5% of the cases and in deep spinous layer in 45% of the cases. Intense staining was observed in the superficial spinous layer in one case.

Severely dysplastic lesions [Figures 6 and 7; Graph 4] showed an intense staining reaction not only in the basal, deep spinous layer but also in 50% of cases of superficial spinous layer.

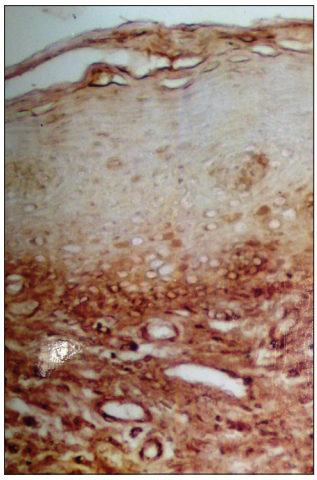


Figure 1: Photomicrograph of normal mucosa showing immunoreactions of epidermal growth factor receptors in basal cells and a few suprabasal cells

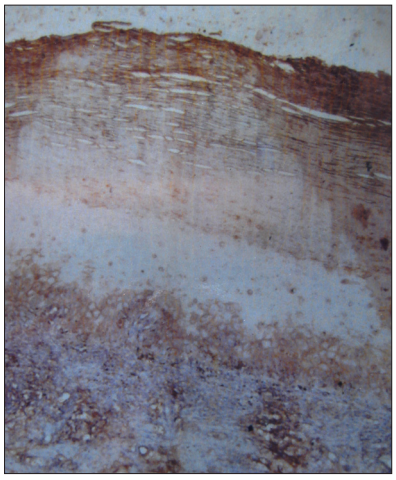


Figure 2: Photomicrograph of epithelium with mild epithelial dysplasia showing immunoreaction of membrane and cytoplasm of basal and suprabasal cells and keratin layer (×40)

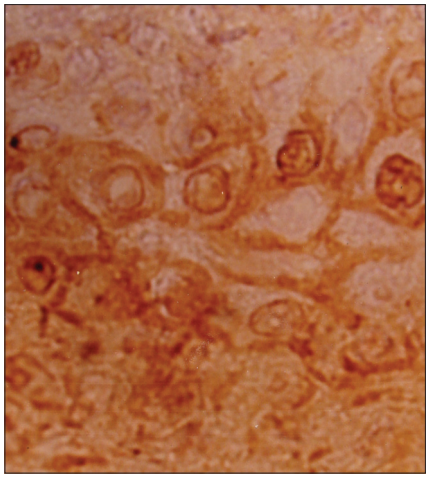


Figure 3: High-power photomicrograph showing thickened cell membrane in mild dysplasia (×400)

The staining intensity of the basal cells of normal mucosa and all cases of dysplasia showed a progressive increase with the degree of dysplasia and a slightly higher percentage of staining of the cell membrane than in the cytoplasm.

In general, the intensity of staining EGFR in oral epithelium increased significantly in mild, moderate, and severely dysplastic lesions as compared with control mucosa.

DISCUSSION

Histopathologic categorization of epithelial changes of routine H and E–stained section as mild, moderate, and severe dysplasia distinguishes the cellular changes associated with the abnormal epithelium from the normal. Yet, some cases of even mild dysplasia progress to malignancy.[1] This necessitates the need for identifying changes at a molecular level.

Cytogenetic analysis[10] of head and neck tumors have revealed the activation of oncogenes, inactivation, or mutation of tumor suppressor genes, and expression of growth factors, such as EGF, TGF-α, TGF-β, and FGF and receptors, such as EGFR.

EGF in oral epithelium exerts its biologic effect by binding to its receptor EGFR.[4] These are transmembrane proteins having 3 zones that are external, transmembrane, and intercellular zones. Upon signal transmission there is elevated phosphorylation of tyrosine residues of several proteins *in vitro* and *in vivo*.[11] EGFR gene located on chromosome 7p.12–13 shows an increased expression in majority of head and neck cancer.[12]

EGFR expression had been studied by many authors. [10,11,13-20] Although majority of them are of the opinion that EGFR expression is indicative of proliferative capacity of cell, some consider that it is indicative of cell maturation also.[17-19,21]

The present study was undertaken to observe the EGFR

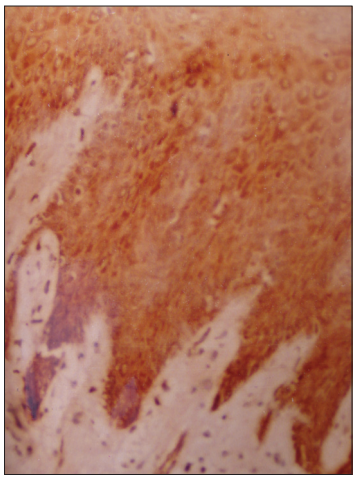


Figure 4: Photomicrograph of epithelium with moderate epithelial dysplasia showing immunoreaction of membrane and cytoplasm of basal and suprabasal cells and focal staining of epithelial cells (×100)

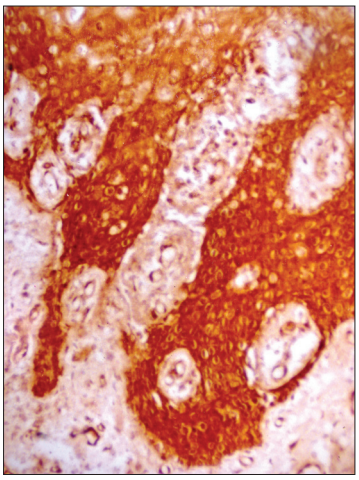


Figure 5: High-power photomicrograph of epithelium in moderate dysplasia showing membrane and cytoplasmic immunostaining and variation in intensity of staining between basal and suprabasal cells (×200)

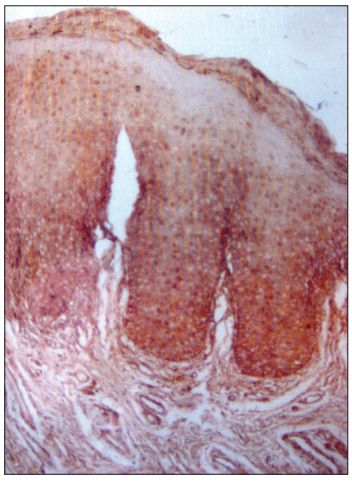


Figure 6: Photomicrograph of epithelium with severe dysplasia showing immunoreaction in all the layers (×40)

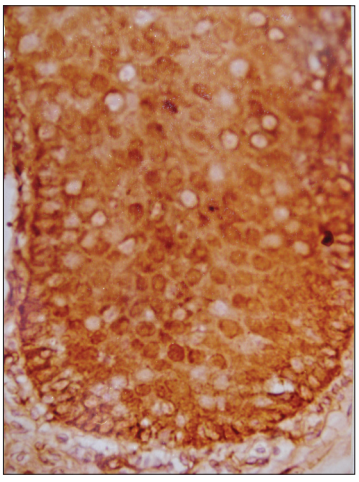
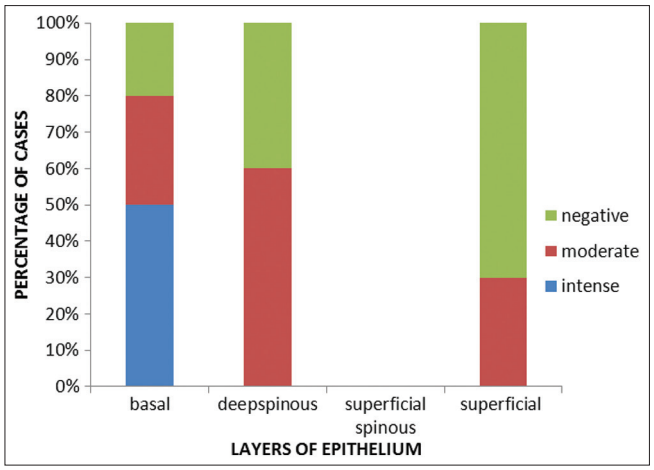
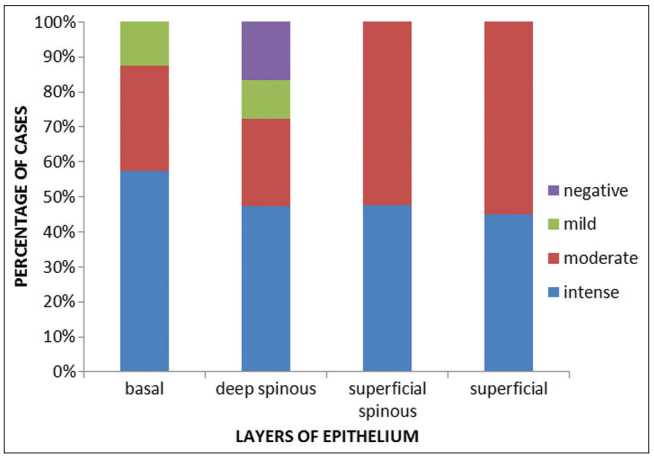


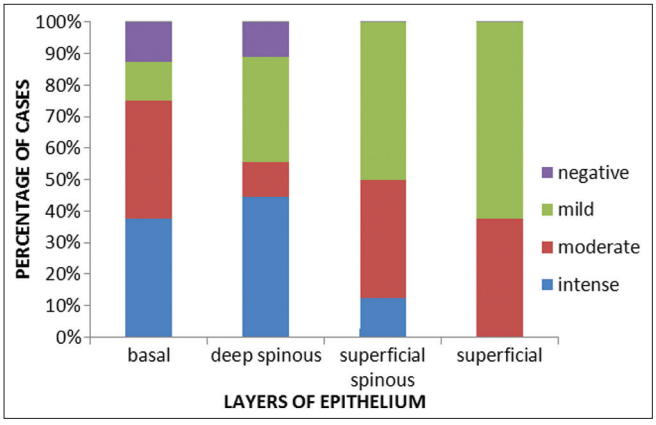
Figure 7: High-power photomicrograph of immunostaining in epithelium of severe dysplasia (×400)



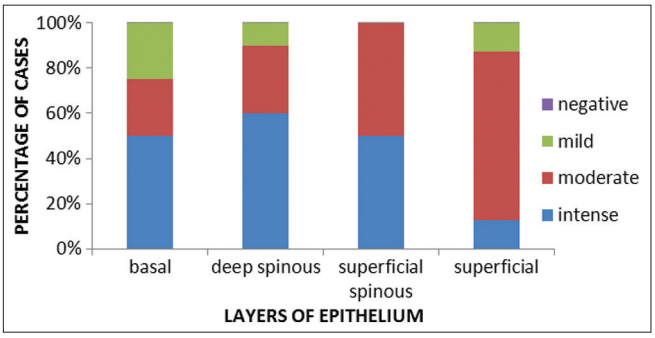
Graph 1: Immunoreaction in cells of the normal epithelium



Graph 2: Immunoreaction in cells of mild displasia



Graph 3: Immunoreaction in cells of moderate displasia



Graph 4: Immunoreaction in cells of severe displasia

expression in normal and dysplastic oral epithelium. Cases that exhibited mild dysplasia belonged to the age group of 20–35 years in contrast to moderate and severe dysplasia in older age group. This observation suggested the time trend involved in carcinogenesis. As per the hypothesis of 2-stage mechanism of carcinomas by Barenhaum (1975), the cells first changed from normal to abnormal by some carcinogenic influence.[22] This is the phase of "Initiation" and in this stage the tissue may be clinically and histologically normal. Continuous exposure of the initiated tissue to same or other agents would transform the dormant tumor cells to active cells so that visible tumor is produced. The effect of promoting agents is usually to cause hyperplasia. Thus a complex situation exists in which the initiating action of carcinogen may not cause morphologic change. A progressive loss of, or diminished effectiveness of normal control mechanism seems a more likely cause. So it is obvious that more severe changes histologically will be revealed in older age group as seen is this study.

The male-to-female ratio was 3.8:1, which was slightly less than the earlier reports[23] in our country. This increase in the number of female cases was probably attributed to the increase in the tobacco habits among the females of the study groups. A 65.6% of the lesions occurred in the buccal mucosa and 9% of lesions in the commissural areas—may be due to the increasing habit of keeping pan masalas and tobacco products as quid in the cheek.[2,23]

In normal controls, EGFR staining was noted in both the cell membrane and cytoplasm of keratinocytes,[19,24,25] which is attributed to its structure. The positive staining seen in the germinative compartment of normal cells is confirmed as with the other earlier workers.[11,14,24-26]

In mild dysplasia, 80% cases showed moderate staining reactions in the basal layer and 15% of cases showed intense staining reactions in the deep spinous layers. Severely dysplastic lesions showed an intense staining reaction not only in the basal, deep spinous layer but also in 50% of cases of superficial spinous layer. It implies increased proliferative activity in the abnormal location, that is, the nonproliferative compartment also.

A comparative study of the staining intensity of the basal cells of all cases of dysplasia and normal mucosa showed a progressive increase with the degree of dysplasia and a slightly higher percentage of staining of the cell membrane than in the cytoplasm. Altered regulation of cell growth is characterized by increased number of normal EGFRs per unit area and formation of abnormal receptors, which may be due to gene mutation or gene rearrangements. In addition, the

viral oncogene product—V.Erb protein, which is homologous with transmembrane and cytoplasmic domain,[27] but lacks the ligand-binding extracellular domain—constituting truncated EGFR, may also attribute. This increased number of normal and abnormal EGFRs may be responsible for the progressive increase in the staining intensity with the severe dysplastic lesion. In contrast to the slight increase in the membrane staining than the cytoplasm seen in normal controls, equal intensity of membrane and cytoplasmic staining is seen in the severe dysplasia. Increased EGFR expressions in severely dysplastic lesions were consistent with finding of many other workers.[14,15,19,20,28]

The faint thin streak of keratin staining in normal and in others is attributed to the pooling effect of degraded complexes of EGFR on the cell membrane[28] giving the positive reaction and is in agreement with the view that EGFR expression is not only due to proliferative activity but also because of the inhibition of apoptosis.[29] Expression of TGF-α in proliferative verrucous leukoplakia, oral submucous fibrosis, observed by many workers[19,30] showed a similar response.

The study also confirms the presence of EGFR in normal oral mucosa and all oral dysplastic lesions with significant increase in the few mild dysplastic lesions. This suggests that this may be taken as an early marker of malignancy. A close followup of those mild dysplastic lesions is suggested. Also one lesion included in the study turned malignant in the 3 months followup period, adds credit to this finding. EGFR levels were relatively low in mild dysplasia and increased with higher degrees of dysplasia. This indicates upregulation of EGFR in the pathogenesis of oral carcinomas.[31]

The development of oral carcinomas is a multistep process where there was initially reactive hyperkeratosis, hyperplasia, in response to chronic exposure to physical or chemical irritants, or viral oncogenes—then the development of various degrees of epithelial dysplasia, intraepithelial carcinoma, and invasive carcinomas. The increased expression of EGFR seen in the proliferative and in the differentiated cells is likely to be a result of paracrine effect on the adjacent nonproliferative cells to increase the expression of cell surface receptors.

CONCLUSION

Based on our observation, it may be concluded that EGFR can be considered as an early marker of a cell proliferation on cell differentiation as well as early marker of epithelial dysplasia and onset of cancer in oral dysplasia. A further study with more number of samples with followup is suggested.

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