



IMMUNOHISTOCHEMICAL ASSESSMENT OF EGFR EXPRESSION IN ORAL SUBMUCOUS FIBROSIS - A CROSS SECTIONAL STUDY

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ABSTRACT

Introduction: Oral submucous fibrosis (OSMF) is a chronic disorder characterized by fibrosis of the lining mucosa of the upper digestive tract involving the oral cavity, oropharynx and frequently the upper third of the oesophagus.

In India, OSMF affects between 0–2% and 1.2% of an urban population attending dental clinics.¹ There is a positive association between the incidence of leukoplakia and oral cancer with OSMF. The frequency of malignant change has been reported from 3% to 6%. In a long-term follow-up study, the annual malignant transformation rate was approximately 0.5% or 7.6% over 17 years.

Material and methodology: A total of 18 participants clinically diagnosed with OSMF were histopathologically categorised in two groups : with and without epithelial dysplasia. Furthermore biopsy tissue from each participant was subjected to immunohistochemistry for EGFR expression. EGFR expression was correlated to dysplasia and compared within two groups.

Results : EGFR was over expressed in OSMF with dysplasia. A statistically significant difference was seen in the expression of EGFR in dysplastic cases compared to that of non dysplastic cases ($p < 0.001$).

Conclusion: On performing immunohistochemistry, EGFR expression was observed to be positive in all 18(100%) samples. A statistically significant difference was seen in the expression of EGFR in dysplastic cases compared to that of non dysplastic cases in OSMF ($p < 0.001$).

KEYWORDS : Oral submucous fibrosis, EGFR, Dysplasia

Introduction :

Oral submucous fibrosis (OSMF) is a chronic disorder characterized by fibrosis of the lining mucosa of the upper digestive tract involving the oral cavity, oropharynx and frequently the upper third of the oesophagus.

In India, OSMF affects between 0–2% and 1.2% of an urban population attending dental clinics.¹ There is a positive association between the incidence of leukoplakia and oral cancer with OSMF. The frequency of malignant change has been reported from 3% to 6%. In a long-term follow-up study, the annual malignant transformation rate was approximately 0.5% or 7.6% over 17 years.²

Oral carcinogenesis is a multistage molecular and histological process displaying a number of genetic and phenotypic markers at each stage. It may involve an increased function of various tumor proto-oncogenes or/and cause deactivation of genes involved in tumor suppression. This results in the loss of checkpoints of the cell cycle ultimately leading to inhibition of normal apoptotic cycle. The progression towards malignancy therefore includes a series of histopathological alterations ranging from reactive hyperkeratosis to hyperplasia progressing to dysplasia, eventually into carcinoma in situ and invasive carcinoma.³

The epidermal growth factor receptor (EGFR) is a member of the ErbB family of receptor tyrosine kinases. The EGFR gene is mapped to chromosome 7p11.2 and it encodes a 170-kDa transmembrane glycoprotein. Alterations in the activity and behaviour of EGFR has been linked to oncogenic transformation, autonomous growth of the cell, increased invasion potential, angiogenesis and increased incidence of metastases in various cancers and are key features of tumors.⁴

Materials And Methods:

This cross sectional study was conducted with 18 participants clinically diagnosed of OSMF (Oral Submucous Fibrosis). After recording the demographic details and a brief history with tobacco habits, a biopsy was obtained from the most representative site. It was then subjected to routine tissue processing. Four sections each of 4 micron thickness were obtained, two of these on silanated slides for IHC staining. Two sections stained with H& E were evaluated for epithelial dysplasia and graded as mild, moderate and severe dysplasia.⁵

They were grouped in those with dysplasia and without dysplasia. IHC was done for the other two sections for EGFR evaluation using the clone EP38Y (Thermo Scientific rabbit monoclonal antibody #RM-2111-R7, 7 mL). The EGFR expression was assessed as positive or

negative and further assessed for intensity of staining. The presence of brown-colored end product at the site of target antigen was taken as immunohistochemically positive. Tissue sections of normal oral epithelium were taken as positive control for EGFR. 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 $\times 40$ 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).⁶ The evaluation was done by three independent observers.

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

Results :

Table1: IHC expression of EGFR in total cases of oral submucous fibrosis (OSMF)

S No.	OSMF Cases	EGFR Positive Cases	EGFR Negative Cases	Total	Chi Square (Goodness of fit)	P value
1	With Dysplasia	4 (22.22%)	0 (0%)	4 (22.22%)	5.556	0.018
2	Without Dysplasia	14 (77.77%)	0 (0%)	14 (77.77%)		
	Total	18 (100%)	0 (0%)	18 (100%)		

Graph 1: Bar diagram representing IHC expression scores of EGFR in total cases of oral submucous fibrosis (OSMF)

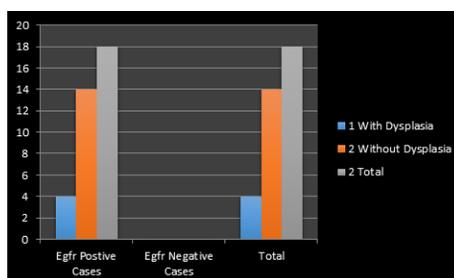


Table 1 shows immunohistochemical expression scores of EGFR in total cases of OSMF. Of the 18 cases of leukoplakia, all 18(100%) showed positive expression. Out of these 18 cases of leukoplakia, 4(22.22% of the total cases) were dysplastic while 14 (77.77 % of the total) cases did not exhibit any dysplasia. This is depicted in bar diagram in **Graph 1**. A statistical significant difference was seen in the expression of EGFR in dysplastic cases compared to that of non dysplastic cases (**p value 0.018**).

Table 2. IHC expression scores of EGFR in different grades of oral submucous fibrosis

ORAL SUBMUCOUS FIBROSIS	EGFR IHC SCORING				TOTAL
	SCORE 0	SCORE 1	SCORE 2	SCORE3	
NO DYSPLASIA	0 (0%)	4 (22.22%)	2 (11.11%)	8 (44.44%)	14 (77.77%)
MILD DYSPLASIA	0 (0%)	2 (11.11%)	1 (5.55%)	1 (5.55%)	4 (22.22%)
TOTAL	0 (0%)	6 (33.33%)	3 (16.66%)	9 (50%)	18 (100%)

Graph 2: Bar diagram representing IHC expression scores of EGFR in different grades of oral submucous fibrosis

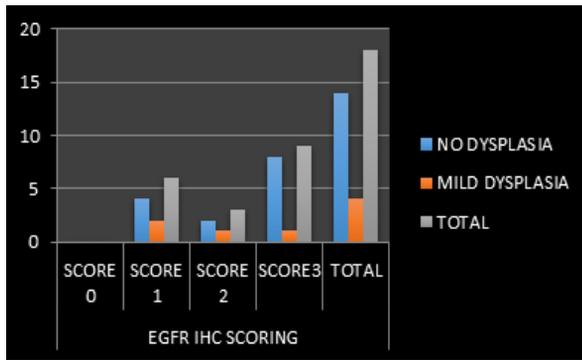


Table 2 shows IHC expression scores of EGFR in different grades of OSMF. Out of the 18 cases of OSMF, all 18 (100%) cases showed positivity. Of these 18 cases of OSMF, 14(77.77%) cases which were non dysplastic showed a mild expression in 4(22.22% of total cases of OSMF).

A total of 4(22.22%) cases of OSMF showed mild dysplasia, in which 2(11.11% of total cases of OSMF) showed mild expression, 1(5.55% of total cases of OSMF) showed moderate staining intensity while 1(5.55% of total cases of OSMF) showed intense expression of EGFR. This data is represented in a bar diagram in **Graph 2**.

Table 2a: Spearmans correlation test between IHC expression scores of EGFR in different grades of oral submucous fibrosis

Correlations			EGFR_Epre	Grades of OSMF
Spearman's rho	EGFR_Epre ssion score	Correlation Coefficient	1.000	.254
		Sig. (2-tailed)	.	.310
		N	18	18
Grades of OSMF	Grades of OSMF	Correlation Coefficient	.254	1.000
		Sig. (2-tailed)	.310	.
		N	18	18

Table 2 a shows Spearmans correlation test between the IHC expression scores of EGFR and grades of OSMF. A positive negative correlation coefficient of 0.254 was obtained, which shows that as the grade of dysplasia is increasing, the intensity of EGFR also increases, although the correlation is not statistically significant.

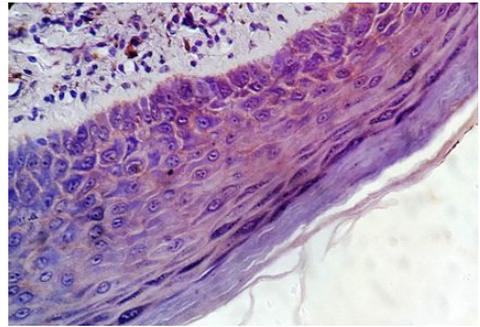


Figure 1: showing mild expression of EGFR in OSMF

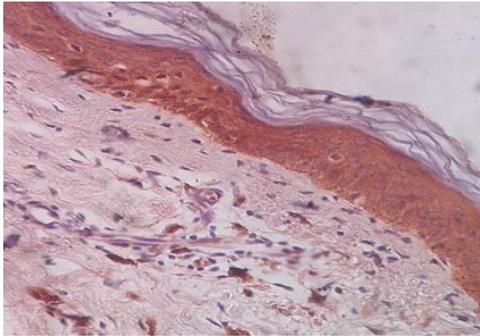


Figure 1: showing intense expression of EGFR in OSMF

Discussion :

Of 18 cases of OSMF in the present study, out of which 4(22.22%) showed dysplasia, while 14(77.77%) showed no dysplasia. All the 18 (100%) were positive for EGFR. Out of the 4 dysplastic cases, 2 showed mild expression (fig.1) while the other two showed moderate and intense expression (fig.2) each. There was a statistical significant difference found in the expression of EGFR amongst the dysplastic and non dysplastic group (p value 0.018). A high expression of EGFR in the OSMF cases could be attributed to increased loss of cells from the surface despite the increased proliferative activity. Also, according to McAlinden RL and Teni T the bcl-2-induced block of the apoptotic process is absent in case OSMF, which contributes to the increased proliferative activity and hence an increased expression of EGFR.^{7,8}

*Srinivasan M (2001)*⁹ also reported a statistically significant (p<0.05) increase in the EGFR expression in the dysplastic cases of OSMF. These results were also in concordance with the study of *Jyothi MN et al (2015)*³, who observed a statistically significant (P<0.05) difference in the EGFR expression in the OSMF compared to normal mucosa.

Conclusion :

On performing immunohistochemistry, EGFR expression was observed to be positive in all 18(100%) samples. A statistically significant difference was seen in the expression of EGFR in dysplastic cases compared to that of non dysplastic cases in OSMF (p<0.001). Hence, EGFR over-expression can be one of the useful diagnostic markers. High risk subgroups can be recognised using this biomarker and can also be used for predicting the potential biologic behaviour of OSMF transforming into oral squamous cell carcinoma.

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