ISSN: 0976-3376

Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology
Vol. 08, Issue, 09, pp.5592-5595, September, 2017

RESEARCH ARTICLE

ASSESSMENT OF EGFR EXPRESSION IN DIFFERENT GRADES OF LEUKOPLAKIA – A CROSS SECTIONAL STUDY

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ARTICLE INFO

Article History:
Received 14th June, 2017
Received in revised form
29th July, 2017
Accepted 18th August, 2017
Published online 15th September, 2017

Key words:
Oral leukoplakia,
EGFR, Dysplasia.

ABSTRACT

Introduction: Oral carcinogenesis is a multistage molecular and histological process displaying a number of genetic and phenotypic changes at each stage. The progression towards malignancy therefore includes a series of histopathologicalalterations ranging from reactive hyperkeratosis to hyperplasia progressing to dysplasia, eventually into carcinoma insitu and invasive carcinoma.

Material and Methodology: A total of 42 participants clinically diagnosed with leukoplakia 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 leukoplakias 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: 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 oral leukoplakia with dysplasia, transforming into oral squamous cell carcinoma.

INTRODUCTION

Oral carcinogenesis is a multistage molecular and histological process displaying a number of genetic and phenotypic changes at each stage. It may involve an increased function of various tumour proto-oncogenes or/and cause deactivation of genes involved in tumour suppression. This results in the loss of checkpoints of the cell cycle ultimately leading to inhibition of normal apoptotic cycle progressing towards malignancy. This progression towards malignancy is exhibited by a series of histopathological alterations ranging from reactive hyperkeratosis to hyperplasia progressing to dysplasia, eventually into carcinoma in situ and invasive carcinoma. (Jyothi Meka *et al*., 2015) Oral Squamous cell carcinoma often develops in pre-existing lesions known as potentially malignant disorders. Leukoplakia is one such known potentially malignant disorder very common because of prevalent tobacco habits. It is also known that not all the potentially malignant disorders transform into malignancy. This can be explained on the basis that individual variations exist in the susceptibility at the genetic level and other immune & pathogenic pathways in the body (Yardimci *et al*., 2014). Hence, a need arises for determining specific biomarkers at cellular and genetic levels which diagnose or predict dysplastic oral epithelium transforming into oral squamous cell carcinoma. EGFR is one such biomarker. Epidermal Growth Factor Receptor (EGFR) is a member of the ErbB family of receptor tyrosine kinases. It is also known as ErbB1 and HER1 The EGFR gene is mapped to chromosome 7p11.2 and it encodes a 170-kDa transmembrane glycoprotein (Wieduwilt and Moasser, 2008). EGFR expression is normally associated with proliferative capacity of cell and also an indicator of cell maturation (Rajeswari and Saraswathi, 2012). Malignant oral keratinocytes display five to fifty times more EGFR expression than their normal epithelium (Jurel *et al*., 2014). It is seen at abnormally high levels on the surface of several types of cancer cells, thereby concluding that these cells may divide excessively because of the effect of epidermal growth factor. 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 (Chiang *et al*., 2006). Researchers have reported that the over-expression of EGFR and other growth factors with similar structural and functional capacities is associated with several malignancies of breast, ovary,

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stomach, lung, colon and pancreas (Olapade-Olaopa *et al*., 2000; Garcia *et al*., 2001; Porebska *et al*., 2000; Baekelandt *et al*., 1999; Nankivell *et al*., 2013). EGFR over-expression has been correlated with poor prognosis in some human cancers and is apparently predictive of disease-free survival independent of cervical lymph node status. Studies documenting correlation of EGFR with oral squamous cell carcinoma have been reported, while there are quite a few studies elaborating the association between oral potentially malignant lesions and the upregulation of EGFR receptor. Also over the past decade several studies have attempted to identify specific biomarkers to predict the malignant potential of more widely prevalent oral PMDs. (Ries *et al*., 2013) However, only few studies have addressed the molecular markers of malignant transformation in OL. The present study was undertaken to study the immunoexpression of EGFR in various grades of dysplasia in Oral Leukoplakia and find out if its expression could be correlated to dysplasia.

MATERIALS AND METHODS

This cross sectional study was conducted with 42 participants clinically diagnosed of Leukoplakia. 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 (Branes *et al*., 2005). 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 ×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). (Jyothi Meka et al., 2015) The evaluation was done by three independent observers.

Statistical Methods

The EGFR expression in different grades of leukoplakia 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 AND OBSERVATIONS

Table 1 shows immunohistochemical expression scores of EGFR in total cases of leukoplakia (hyperkeratotic lesion). Of the 42 cases of leukoplakia (hyperkeratotic lesion), (42)100% showed positive expression. Out of these 42 cases of leukoplakia, 37 (88.09%) cases were dysplastic while 5 (11.9%) 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 ($p<0.001$). Table 2 shows IHC expression scores of EGFR in different grades of leukoplakia (hyperkeratotic lesion). All the 42 (100%) cases showed positivity. Out of these 42 cases of leukoplakia (hyperkeratotic lesion), 5 cases were non dysplastic but showed mild staining in 1(2.38% of total cases of leukoplakia) case, moderate in

Table 1. Immunohistochemical expression scores of EGFR in total cases of leukoplakia (hyperkeratotic lesion)

S No.	Leukoplakia (hyperkeratotic lesion) Cases	EGFRPositive Cases	EGFR Negative Cases	Total	Chi Square test (Goodness of fit)	p value
1	With Dysplasia	37(88.09%)	0(0%)	37(88.09%)		
2	Without Dysplasia	5(11.9%)	0(0%)	5(11.9%)	24.381	0.000
	Total	42(100%)	0(0%)	42(100%)		

Table 2. IHC expression scores of EGFR in different grades of leukoplakia (hyperkeratotic lesion)

Oral leukoplakia (hyperkeratotic lesion)	EGFR IHC scoring				TOTAL
	SCORE 0	SCORE 1	SCORE 2	SCORE3	
NO DYSPLASIA	0(0%)	1(2.38%)	1(2.38%)	3(7.14%)	5(11.90%)
MILD DYSPLASIA	0(0%)	4(9.52%)	6(14.28%)	17(40.47%)	27(64.28%)
MODERATE DYSPLASIA	0(0%)	1(2.38%)	2(4.76 %)	4(9.52%)	7(16.66%)
SEVERE DYSPLASIA	0(0%)	0(0%)	0(0%)	3(7.14%)	3(7.14%)
TOTAL	0(0%)	6(14.28%)	9(21.42%)	27(64.28%)	42(100%)

Table 3. Spearmans correlation test between the IHC expression scores of EGFR and grades of Leukoplakia (hyperkeratotic lesion)

Correlations				
			Hyperkeratotic lesion grades	EGFR Expression scores
Spearman's rho	Hyperkeratotic lesion grades	Correlation Coefficient	1.000	.098
		Sig. (2-tailed)	.	.537
		N	42	42
	EGFR Expression scores	Correlation Coefficient	.098	1.000
		Sig. (2-tailed)	.537	.
		N	42	42

1(2.38% of total cases of leukoplakia) and intense in 3(7.14% of total cases of leukoplakia) cases.

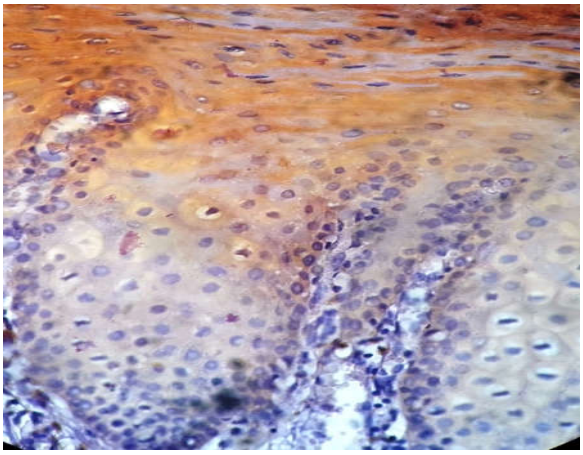

Figure 1. Mild expression of EGFR in hyperkeratotic lesion with mild dysplasia

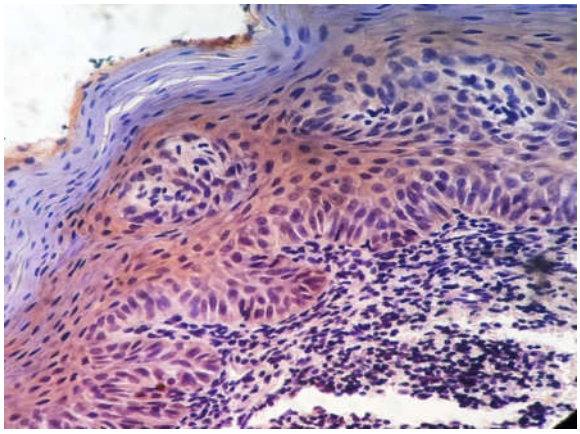

Figure 2. Moderate expression of EGFR in hyperkeratotic lesion with moderate dysplasia

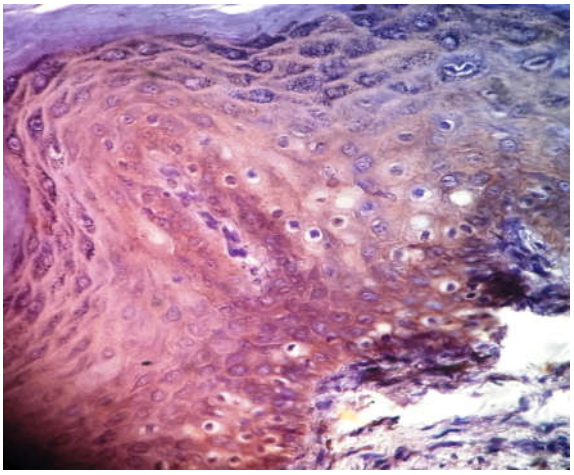

Figure 3. Intense expression of EGFR in hyperkeratotic lesion with severe dysplasia

A total of 27 cases showed mild dysplasia, in which 4(9.52% of total cases of leukoplakia) cases showed mild expression, 6(14.28% of total cases of leukoplakia) showed moderate staining intensity while 17(40.47% of total cases of leukoplakia) showed intense expression of EGFR. Amongst the moderate dysplasia, 1(2.38% of total cases of leukoplakia) showed mild expression, 2(4.76 % of total cases of leukoplakia) showed moderate expression, while 4(9.52% of total cases of leukoplakia) showed intense expression. Out of the 3 cases showing severe dysplasia, all 3(7.14% of total cases of leukoplakia) showed an intense expression. Graph 2 represents this comparative data in the form of bar diagram.

DISCUSSION

Oral Leukoplakia is a clinical diagnosis with a prevalence of 1.7-2.7 %. (Petti, 2003) The histological diagnosis is a hyperkeratotic lesion with or without dysplasia. The malignant transformation rate of oral leukoplakia varies from 0-38% in different variants of oral leukoplakia reported from various studies. (Scheifele and Reichart, 2003) Of the 42 cases of oral leukoplakia diagnosed as hyperkeratotic lesions in the present sudy only 5 showed no dysplasia, 37 were found to have dysplasia. Furthermore the immunoexpression of EGFR was found to be positive in all the cases. A statistically significant difference was seen in the expression of EGFR in dysplastic cases compared to that of non dysplastic cases ($p<0.001$). This could be explained as an altered regulation of cell growth characterised by an increased number of EGFR per unit area and formation of abnormal receptors. Another explanation for this is that there may have been a gene mutation or gene rearrangement. (Rajeswari and Saraswathi, 2012) However, according to Zimmermann M, (Zimmermann *et al*., 2006) EGFR overexpression is thought to result from enhanced transcription, with no apparent change in mRNA stability and gene amplification has been observed less frequently. In the 37 cases of leukoplakia with dysplasia an intense expression was observed in majority (24) of the cases, similar to the findings reported by Jyothi *et al*. (2015) As the degree of dyplasia increased, there was an increase in the staining intensity of EGFR. However, this observation was not statistically significant. Srinivasan (2001) in his study wherein observed an increase in intensity with an increase in dysplasia, which was not statistically significant. On the contrary, Rajeswari (2012) observed that the staining intensity of EGFR in oral epithelium increased significantly in mild moderate and severe lesions as compared to control mucosa.

Conclusion

A significantly high EGFR expression was seen in the dysplastic cases as compared to the non dysplastic cases. 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 oral leukoplakia transforming into oral squamous cell carcinoma.

Financial or other competing interests: None.

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