Original Research Paper

Volume - 12 | Issue - 04 | April - 2022 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Histopathology

EXPRESSION OF EGFR AND p53 IN ORAL PRE-MALIGNANT AND MALIGNANT LESIONS

Dr Lubna Khan	Professor, Department of Pathology, GSVM Medical college, Kanpur.
Dr Suman Lata Verma	Professor , Department of Pathology, GSVM Medical college, Kanpur.
Dr Kuldeep Singh	Associate Professor , Department of Pathology, GSVM Medical college, Kanpur.
Dr Gaurav Singh*	JR 3, Department of Pathology, GSVM Medical college, Kanpur. *Corresponding Author

ABSTRACT Oral cancer is one of the 10 most common cancers in the world, with a delayed clinical detection, poor prognosis, without specific biomarkers for the disease and expensive therapeutic alternatives. This study aims to present the fundamental aspects of this cancer, focused on squamous cell carcinoma of the oral cavity (OSCC), moving from its definition and epidemiological aspects, addressing the oral carcinogenesis, oral potentially malignant disorders, epithelial precursor lesions and experimental methods for its study, therapies and future challenges. Oral cancer is a preventable disease, risk factors and natural history is already being known, where biomedical sciences and dentistry in particular are likely to improve their poor clinical indicators.

MATERIAL AND METHODS: A total of 200 cases were taken for study.

RESULT: Maximum number of cases were in their 3rd (30%) and 4th (23.5%) decades of life. The most common site of oral mucosal lesions was buccal mucosa which accounted for 37.5% of total cases followed by tongue (20%). There were 155 males (77.5%) and 45 females (22.5%) with male:female ratio of 3.5:1. p53 was found positive in 69% pre-malignant lesions and 89% of malignant lesions while EGFR was positive in 73% of pre-malignant lesions and 79% of malignant lesions.

CONCLUSION: In oral squamous cell carcinoma expression of p53 is increased and its intensity of expression is related with clinical severity of the disease. In pre-malignant lesions also expression of p53 was found raised. Expression of p53 depends on grading and expression increases with higher grade.

The intensity of EGFR expression increased with increasing grades of dysplasia. This may again be possibly explained due to increased EGFR expression with increasing grades of dysplasia. Similarly, the overexpression of EGFR correlated with poor tumor differentiation in our study.

KEYWORDS :

INTRODUCTION:

Oral cancer ensues with a small, unfamiliar, unexplained growth or sore in the oral cavity that include lips, cheeks, sinuses, tongue, hard and soft palate, the base of the mouth extended to the oropharynx. India has the largest number of oral cancer cases and one-third of the total burden of oral cancer globally. Oral cancer poses a serious health challenge to the nations undergoing economic transition [1]. In India, around 77,000 new cases and 52,000 deaths are reported annually, which is approximately one-fourth of global incidences [2]. The increasing cases of oral cancer are the most important concern for community health as it is one of the common types of cancers in India [3]. As compared to the west, the concern of oral cancer is significantly higher in India as about 70% of the cases are reported in the advanced stages (American Joint Committee on Cancer, Stage III-IV). Because of detection in the late phase, the chances of cure are very low, almost negative; leaving five-year survival rates around 20% only [4].

Oral squamous cell carcinoma (OSCC) contributes remarkably i.e. 84-97% to all oral malignancies. OSCC commonly results from potentially malignant lesions or normal epithelium linings. Potentially malignant disorders (PMDs) such as inflammatory oral submucosa, fibrosis, erythroplakia, leukoplakia, candidal leukoplakia, dyskeratosis congenital, and lichen planus are indicators of the preclinical phase of oral cancer [5]. Tobacco consumption including smokeless tobacco (SLT), betel-quid chewing, excessive alcohol consumption, poor oral hygiene, nutrient-deficient diet, and sustained viral infections, i.e. human papillomavirus (HPV) are some of the risks associated with the occurrence of oral cancer. Inflammation plays an important role in tumorigenesis and inflammation produced by viral and bacterial infections, and inflammatory bowel diseases may cause malignancy. Tobacco consumption (in any form) is a prime cause of cancer, prominently in developing nations. Apart from tobacco, chewing paan containing leaves of piper betel with areca nut, lime, catechu etc., is a leading source of oral malignancy, especially in the north-eastern parts of India that contributes the highest incidence of cancer in India [6]. The continued activity of chewing paan causes prolonged exposure of oral mucosa along with abrasion of epithelium linings.

Various conventional clinical techniques such as physical and histopathological examination, staining, biopsy, spectroscopic and radiological techniques, etc. are used routinely to detect oral cancer. Upon early diagnosis, timely and proper treatment can be initiated that may improve the survival rate up to 90%.

MATERIAL AND METHODS:

STUDY SAMPLE: 100 cases each of premalignant lesions and squamous cell carcinomas (SCC) were randomly selected for the study.

INCLUSION CRITERIA:

- Only histopathologically diagnosed cases were included.
- Patients who have undergone surgical therapy as a primary mode of treatment.

EXCLUSION CRITERIA:

- Patients with known primary other than oral cavity.
- Inadequate biopsies were excluded from the study.
- Patient who have undergone radiotherapy as a primary mode of treatment.
- Histopathologically ulcerative and necrotic areas will be excluded.

IMMUNOHISTOCHEMICAL EVALUATION

The slides were stained for p53 and EGFR observed under the light microscope. The tissue samples were thoroughly examined. A brown precipitate seen with in the nucleus confirmed the presence of p53 protein. EGFR was evaluated on the basis of extent and intensity of EGFR immunolabelling in tumor cell membrane.

The p53 positive samples were graded as follows;[7]

Negative : < 10 %of the cells showed positive staining
Positive : > 10 % of the cells showed positive staining.
1+: 10 to 30 % of cells showed positive staining.
2+: 30 to 50 % of cells showed positive staining.
3+ : > 50 % of cells showed positive staining.

EGFR positive samples were graded as follows;[8]

Negative <10 % of tumor cells showed labelling
Positive >10 % of cells show labelling

1+ weak labelling, homogenous or patchy in >10 % of tumor cells
2+ moderate labelling, homogenous or patchy in >10 % of tumor cells.
3+ intense labelling, homogenous or patchy in >10 % of tumor cells.

RESULTS:

On histopathology, Leukoplakia, 30 cases (15%) was the most common lesion among pre-malignant category (Table-1)

Table-1 Premalignant Lesion (100)

Epithelial lesions	Total no. of cases	Percentage
Leukoplakia	30	15%
Mild dysplasia	20	10%
Moderate dysplasia	26	13%
Severe dysplasia	24	12%

In malignant category majority of the cases were well differentiated squamous cell carcinoma, 45 cases (22.5%) (Table-2).

Table-2 Malignant Lesion (100)

WDSCC	45	22.5%
MDSCC	30	15%
PDSCC	15	7.5%
Verrucous carcinoma	10	5%

*WDSCC-Well differentiated squamous cell carcinoma.
*MDSCC- Moderately differentiated squamous cell carcinoma.
*PDSCC- Poorly differentiated squamous cell carcinoma.

The study included 200 cases of oral cavity. Maximum number of cases were in their 3rd (30%) and 4th (23.5%) decades of life. There were 155 males (77.5%) and 45 females (22.5%) with male to female ratio of 3.5:1. Most common site of oral mucosal lesions was buccal mucosa which accounted for 37.5% of total cases followed by tongue (20%). Expression of p53 was found in 69% of the pre-malignant cases and 89% of the malignant lesions of the oral cavity (Table-3).

Table-3 Presence of p53 in epithelium of different groups

S.No	Result	Premalignant		Malignant	
		N	%	N	%
1.	Absent	31	31%	11	11%
2.	Present	69	69%	89	89%

It was found that 48% malignant and 36% premalignant lesions showed strong p53 positivity. Intensity of p53 increases with degree of dysplasia and grade of carcinoma (Table-4).

Table-4 P53 Staining Intensity in oral lesions

Epithelial Lesions	Intensity			Total
	Weak	Moderate	Strong	
Premalignant	13 (19%)	20 (29%)	36 (52%)	69
Malignant	10 (11%)	31 (35%)	48 (54%)	89

Similarly, Expression of EGFR was found in 73% of the pre-malignant cases and 79% of the malignant lesions of the oral cavity (Table-5).

Table-5 Presence of EGFR in epithelium of different groups

S.No	Result	Premalignant		Malignant	
		N	%	N	%
1.	Absent	27	27%	21	21%
2.	Present	73	73%	79	79%

It was found that 40% of malignant and 12.3% premalignant lesions showed strong EGFR positivity. Intensity of EGFR increases with degree of dysplasia and grade of carcinoma. (Table-6)

Table-6 EGFR Staining Intensity in oral lesions

Epithelial Lesions	Intensity			Total
	Weak	Moderate	Strong	
Premalignant	48 (65.7%)	16 (21.9%)	9 (12.3%)	73
Malignant	20 (25.3%)	28 (35.4%)	31 (39.2%)	79

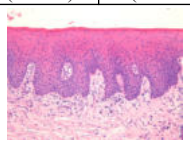

Figure 1. Microphotograph showing normal oral mucosa without dysplasia (H&E, x100)

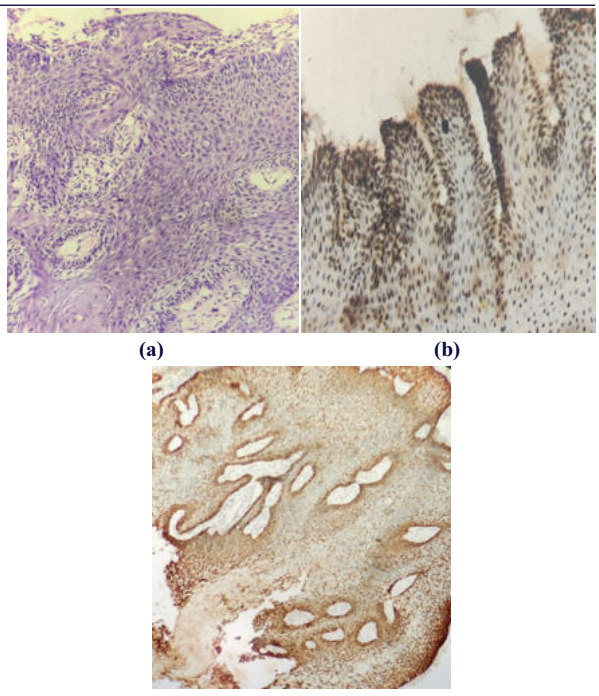

Figure 2. (a) severe dysplasia (H&E)x100, (b) Strong basal & supral basal positivity by p53 (IHC, x100), (c) membranous epidermal growth factor receptor staining with severe intensity in severe dysplasia (IHC, ×100)

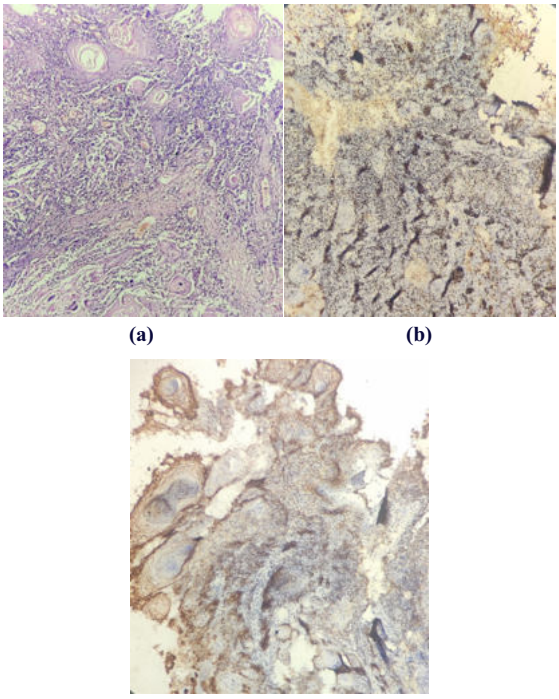

Figure 3. (a) well-differentiated squamous cell carcinoma with formation of squamous pearls (H and E, ×100), (b) p53 nuclear positivity in well-differentiated squamous cell carcinoma (IHC, ×100), (c) membranous epidermal growth factor receptor staining with severe intensity in well-differentiated squamous cell carcinoma (IHC, ×100)

DISCUSSION:

Peak incidence of oral carcinoma was observed in the third and fourth decade of life [9] with male to female ratio of 3.5:1.

The reasons for earlier occurrence of carcinoma among Indian people

might be due to habit of tobacco chewing and smoking started early in the life and prevailing poor socio-economic conditions which affect the general nutritional status of the individual.

Tobacco addiction has been widely implicated in the etiology of oral cancers. In present study 85.5% cases were tobacco (In any form) users, which strongly suggest close relation between tobacco use & oral cancer. Tobacco users have 8 times greater risk of development of oral SCC in comparison to non-tobacco users.[9] The prevalence rate was observed to be closely associated with age at which the patients started tobacco chewing and the frequency of tobacco chewing per day. Majority of cases (96%) had a history of more than 10 years of tobacco use, which indicates that the risk of developing carcinoma increases with the duration of tobacco use.

In present study tobacco (Gutkha) chewing alone is the most common habit accounting 51% of total cases. Tobacco chewing and smoking has been the next most common personal habit accounting for 34% of cases and pan masala accounts 10% of cases**.** These findings corroborate that tobacco chewing, alcohol intake & smoking as the most common identifiable risk for oral & oropharyngeal cancer.[10]

Average duration of presentation was 6 months of onset of symptoms. It was because of early seeking nature of human for any gross discomfort to body, and moreover, easy and cheap availability of health services.

Most common site of oral lesion was buccal mucosa which accounted for 37.5% of cases. The next common site of oral mucosal lesions was tongue (20% of cases). Expression of p53 was found in 69% of the pre-malignant cases and 89% of the malignant lesions of the oral cavity. Our observation that p53 expression increases with severity of dysplasia has been reported in earlier studies as well. [11,12]

Expression of p53 increases from premalignant to malignant oral lesions. 89% cases of OSCC showed staining in basal and supra basal layers at upto 78% intensity, only 11% cases were negative.

Studies by Cruz et al[13] with a follow up of dysplastic lesions for a period of 16 years show that p53 expression pattern could show higher specificity than histopathological assessment of dysplasia (96 % *vs*. 54%) and higher positive predictive value (86 % *vs*. 44 %) for correct prediction of the malignant transformation of the lesions. This suggests that clear expression of p53 above the basal cell layer could be an early event in oral carcinogenesis and an indicator of a developing carcinoma, even preceding morphological tissue alterations.[14]

Nearly all sites of oral epithelium showed similar positive rates of p53 expression and no site specific greater expression was seen.

EGFR expression was found positive in 73% of premalignant and 79% of malignant lesions.

EGFR protein overexpression has been reported in 70–90% of head and neck SCC, and the incidence of gene amplification has been demonstrated in about 17–31%. Some authors have found that EGFR overexpression and amplification were associated with poor tumor differentiation and worse prognosis in HNSCC. [15]

EGFR was evaluated on the basis of extent and intensity of EGFR expression in oral leukoplakias and SCCs. In this study, 79% cases of SCC were EGFR positive. A significant correlation was observed between the EGFR cell staining in leukoplakia and SCC.

We found that the EGFR expression was seen in cases of premalignant (73%) as well as malignant lesions (79%). EGFR is found in an abnormally high number on the surface of cancer cells; hence, these cells may divide excessively in the presence of epidermal growth factor. The intensity of EGFR expression increased with increasing grades of dysplasia. This is due to increased EGFR expression with increasing grades of dysplasia.

A strong correlation was found between p53 and EGFR. This may be due to the fact both p53 and EGFR are linked to each other at a molecular level and may augment each other in cases of dysplasia and carcinogenesis. Mutant p53 binds to promote a sustained EGF-induced extracellular signal–regulated kinase 1/2 activation, thereby facilitating cell proliferation and tumorigenesis.[16]

It was found in the study that p53 and EGFR play an important role in identifying those premalignant lesions which may progress to oral squamous cell carcinoma. These immune-histochemical markers also help in classifying the severity of OSCCs.

CONCLUSION:

In the present study, the expression of mutant p53 was observed in OSCC tissues, suggesting that p53 mutation was present in OSCC. Mutated p53 loses its ability to suppress the function of oncogenes. Furthermore, mutant p53 may function as an oncogene to stimulate cell division and promote the growth of tumor cells [17]. The results of the present study indicated that with the increase of OSCC pathological grading, mutant p53 positive rate was also increased, which was consistent with the results of previous studies [18-24], suggesting that the mutation of p53 may be significant in the pathological differentiation of OSCC.

The present study showed the expression of EGFR in 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. In the present study 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.[25,26]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.

REFERENCES:

1. Gupta B., Bray F., Kumar N., Johnson N.W. Associations between oral hygiene habits, diet, tobacco and alcohol and risk of oral cancer: a case–control study from India. Cancer Epidemiol. 2017;51:7–14. doi: 10.1016/j.canep.2017.09.003.
2. Laprise C, Shahul HP, Madathil SA, Thekkepurakkal AS, Castonguay G, Varghese I, et al Shiraz S., Allison P., Schlecht N.F., Rousseau M.C., Franco E.L., Nicolau B. Periodontal diseases and risk of oral cancer in Southern India: results from the HeNCe Life study. Int. J. Canc. 2016;139:1512–1519. doi: 10.1002/ijc.30201.
3. Sharma S, Satyanarayana L, Asthana S, Shivalingesh KK, Goutham BS, Ramachandra S. Oral cancer statistics in India on the basis of first report of 29 population-based cancer registries. J. Oral Maxillofac. Pathol. 2018;22:18–26. doi: 10.4103/jomfp. JOMFP 113 17.
4. Veluthattil A, Sudha S, Kandasamy S, Chakkalakkoombil S. Effect of hypofractionated, palliative radiotherapy on quality of life in late-stage oral cavity cancer: a prospective clinical trial. Indian J. Palliat. Care. 2019;25:383. doi: 10.4103/IJPC.IJPC_115_18.
5. Ajay P, Ashwinirani S, Nayak A, Suragimath G, Kamala K, Sande A, Naik R. Oral cancer prevalence in Western population of Maharashtra, India, for a period of 5 years. J. Oral Res. Rev. 2018;10:11. doi: 10.4103/jorr.jorr_23_17.
6. Singh M, Prasad CP, Singh TD., Kumar L. Cancer research in India: challenges & opportunities. Indian J. Med. Res. 2018;148:362–365. doi: 10.4103/ ijmr. IJMR 1711 18.
7. Agarwal S, Mathur M, Srivastava A, Ralhan R. MDM2/p53 co-expression in oral premalignant and malignant lesions: Potential prognostic implications. Oral Oncol 1999;35:209-16.
8. 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;13:285-90.
9. Wahi P, Lahiri B, Kehar U. et al. Oral and oropharyngeal cancers in North India. Br J Cancer 1965:19:627–641
10. Agarwal AK, Sethi A, Sareen D, Dhingra S. Treatment delay in oral and oropharyngeal cancer in our population: the role of socio-economic factors and health-seeking behaviour. Indian J Otolaryngol Head Neck Surg. 2011;63(2):145-150. doi:10.1007/s12070-011-0134-9
11. Patel SM, Patel KA, Patel PR, Rasik GR, Hathila N,Gupta S. Expression of p53 and Ki-67 in oral dysplasia and squamous cell carcinoma: an immunohistochemical study. International Journal of Medical Science and Public Health2014;3:10.
12. Shin DM, Ro JY, Hong WK, Hittelman WN. Dysregulation of epidermal growth factor receptor expression in premalignant lesions during head and neck tumorigenesis. Cancer Res 1994;54:3153-9.
13. Cruz I, Napier SS, van der Waal I, et al. Suprabasal p53 immunoexpression is strongly associated with high grade dysplasia and risk for malignant transformation in potentially malignant oral lesions from Northern Ireland. J Clin Pathol. 2002;55:98-104. doi:10.1136/jcp.55.2.98
14. Nylander K, Dabelsteen E, Hall P. The p53 molecule and its prognostic role in squamous cell carcinomas of the head and neck. Journal of Oral Pathology & Medicine. 2000;29:413 - 425. 10.1034/j.1600-0714.2000.290901.x.
15. Ali MAS, Gunduz M, Nagatsuka H, Gunduz E, Cengiz B, Fukushima K et al. Expression and mutation analysis of epidermal growth factor receptor in head and neck squamous cell carcinoma. Cancer Sci. 2008;99:1589–94. doi: 10.1111/j.1349-7006.2008.00861.x.
16. Wang W, Cheng B, Miao L, Mei Y, Wu M. Mutant p53-R273H gains new function in sustained activation of EGFR signaling via suppressing miR-27a expression. Cell Death Dis 2013;4:e574
17. Rybanska I, Ishaq O, Chou J, et al: PARP1 and DNA-PKcs synergize to suppress p53 mutation and telomere fusions during T-lineage lymphomagenesis. Oncogene. 32:1761–1771. 2013.
18. Decastel M, Ossondo M, Andrea AM, et al: Colorectal cancer in patients seen at the teaching hospitals of Guadeloupe and Martinique: Discrepancies, similarities in clinicopathological features, and p53 status. BMC Clin Pathol. 14:122014.
19. Imamura H, Ohishi Y, Aman M, et al: Ovarian high-grade serous carcinoma with a noninvasive growth pattern simulating a serous borderline tumor. Hum Pathol. Jun 16–2015.

20. Jasar Dz, Smichkoska S, Kubelka K, et al: Expression of p53 protein product in triple negative breast cancers and relation with clinical and histopathological parameters. Prilozi. 36:69–79. 2015.
21. Tan PH, Jayabaskar T, Yip G, et al: p53 and c-kit (CD117) protein expression as prognostic indicators in breast phyllodes tumors: A tissue microarray study. Mod Pathol. 18:1527–1534.
22. Reiher F, Ozer O, Pins M, et al: p53 and microvessel density in primary resection specimens of superficial bladder cancer. J Urol. 2002;167:1469–1474.
23. Wang H, Bao W, Jiang F, et al: Mutant p53 (p53-R248Q) functions as an oncogene in promoting endometrial cancer by up-regulating REGγ. Cancer Lett. 2015;360:269–279.
24. Huang K, Chen L, Zhang J, et al: Elevated p53 expression levels correlate with tumor progression and poor prognosis in patients exhibiting esophageal squamous cell carcinoma. Oncol Lett. 2014;8:1441–1446.
25. Chongrui Xu, Qing Zhou and Yi-long Wu, Can EGFR-TKIs be used in first line treatment for advanced non-small cell lung cancer based on selection according to clinical factors ? – A literature-based meta-analysis, J Hemat Onc. 2012; 5(3):62.
26. Chung BM. Endocytic Regulation of EGFR Signaling. Interdis Bio Cent. 2012;4:3:1-7.