# IMMUNOEXPRESSION PATTERN OF P16 & P53 IN ORAL EPITHELIAL DYSPLASIA AND ORAL SQUAMOUS CELL CARCINOMA

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**ABSTRACT**

**BACKGROUND**

Oral squamous cell carcinoma (OSCC) has become one of the leading causes of death mostly in developing countries with worldwide estimated incidence of around 3,00,000 and accounts for more than 95% of oral cancers. Oral epithelial dysplasia (OED) is a modified epithelial tissue that shows cytological changes, specifically severe dysplasia, related to increased risk of developing OSCC. The present study is undertaken to evaluate the role played by p16 and p53 on oral carcinogenesis, their use as biomarkers of malignant transformation of OED and as predictor of biological behaviour of OSCC. We wanted to determine the immune expression of p16 and p53 in the tissue obtained from squamous dysplasia and squamous cell carcinoma lesion of oral mucosa and establish the possible relation of p16 and p53 expression to histologic grading of OED and OSCC.

**METHODS**

Materials consisted of 14 histologically diagnosed cases of hyperplasia without dysplasia oral mucosa, 20 cases of dysplastic lesions (3 mild, 5 moderate and 12 severe dysplasia) and 40 cases of SCC lesions (28 well differentiated, 9 moderately differentiated and 3 poorly differentiated) of oral mucosa. These cases are subjected to routine H & E staining and p53, p16 immunochemical staining.

**RESULTS**

p16 positivity found in 50% of hyperplastic, 35% of dysplastic and 10% of carcinoma lesions of oral mucosa and this association was to be significant (p value= 0.004). Intensity of p16 gradually decreased with increase in severity of lesion with 42% of benign, 29% of premalignant and 25% of malignant cases showed strong intensity. p53 positivity was seen in 21% of hyperplastic, 60% of dysplastic and 75% of carcinoma lesions showing a significant association (p value= 0.001). p53 staining confined to basal layer was observed in 66% of hyperplastic and 17% of dysplastic lesions but none of carcinoma lesions showed this pattern.

**CONCLUSIONS**

p16 and p53 could not be specific enough to identify patients suffering from OED with high risk of malignancy. However, the evaluation of their presence can be used as an effective tool to evaluate the progression of dysplastic changes in the development of OSCC in conjunction with histological parameters.

**KEYWORDS**

Oral Cancer, Oral Epithelial Dysplasia, Oral Squamous Cell Carcinoma, p16, p53

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## BACKGROUND

Oral squamous cell carcinoma (OSCC) is an important source of morbidity and mortality worldwide with an incidence rate that varies widely by geographic location. According to the reports of the WHO, oral cancer ranks sixth among all malignancies worldwide.[1] The oral epithelial dysplastic (OED) lesion also called as a pre-cancer lesion is among the most intricate topics of head and neck pathology.[2] The concept of a stepwise development of cancer in the oral mucosa, i.e., the initial presence of a precursor (pre-malignant/precancerous) lesion subsequently developing into cancer is well established. Oncogenes are altered growth promoting regulatory genes that govern the cells' signal transduction pathways, and mutation of these genes leads to either overproduction or increased function of the excitatory proteins.[3] Mutations in tumour suppressor genes namely p53, pRb, p16 and pro-apoptotic genes namely bcl2, bax have been variously attributed to the development and transformation of precancer to cancer.

CDKN2A is a cyclin dependent kinase inhibitor (CKI), a tumour suppressor gene, and is the second commonly affected gene next to p53 in OSCC, located on chromosome 9p21, encodes a cell-cycle protein (p16) which is a strong and specific inhibitor of cyclin-dependent kinases (CDK) 4 and 6 and down-regulates cyclin D-dependent The main function of p16 is to control the phosphorylation of

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retinoblastoma (Rb) gene and block the progression of cell cycle from the G1 to S-phase.[4] This may lead to a disappearance of the protein in neoplastic cells.[5] The study of p16 to date been limited to pre-cancerous lesions of the uterine cervix, as a "surrogate" to identify cells infected by high risk human papilloma virus (HR-HPV) carried by the protein E7, which inhibits the anti-oncogene Rb, in its turn linked to p16 through a negative feedback. However, the significance of accumulation of p16 protein in the dysplastic lesions of the upper aerodigestive tract is controversial and poorly understood.[6] The tumour suppressor gene p53, the most frequently mutated gene in human cancers regulates cell cycle progression, DNA repair, cellular senescence and apoptosis. p53 protein blocks cell division at the G1 to S boundary, stimulates DNA repair after DNA damage, and also induces apoptosis. The p53 protein transcriptionally activates the production of the p21 protein which is a inhibitor of cyclin and cyclin dependant kinase complexes that are critical in the progression of cell cycle & ultimately cell division. Mutation of p53 occurs either as a point mutation, which results in a structurally altered protein that sequesters the wild-type protein, thereby inactivating its suppressor activity, or by deletion, which leads to a reduction or loss of p53 expression and protein function.

This study determined the immunohistochemical expression of p16 and p53 in the tissue obtained from squamous dysplasia and squamous cell carcinoma lesion of oral mucosa and establish the possible relation of p16 and p53 expression to histologic grading of OED and OSCC.

## METHODS

The present study was carried out in the department of pathology at MKCG Medical College, Berhampur, Odisha over a period of 2 years from October 2016 to October 2018. Histopathologically diagnosed benign lesions (oral mucosal hyperplasia), oral dysplastic lesions and SCC were included in the study for comparison. The H&E stained sections were thoroughly examined. Dysplastic lesions were categorized as mild, moderate and severe dysplasia and Oral squamous cell carcinoma was graded as well differentiated, moderately differentiated, and poorly differentiated (according to the Broder's Classification). Total 74 cases comprised of 3 groups, Group 1 consisted of 14 cases of hyperplasia without dysplasia oral mucosa, Group 2 consisted of 20 cases of dysplastic lesions and Group 3 consisted of 40 cases of SCC lesion of oral mucosa. These cases are subjected to routine H&E staining and p53, p16 immunochemical staining.

### Immunohistochemistry

Immunohistochemical analysis was performed on paraffin sections within 4-6 weeks of sectioning in order to maintain their antigenicity. Reagents used were p16 monoclonal antibody, BIOGENEX TM Clone: G175 – 405 and p53 primary mouse monoclonal antibody (Monoclonal mouse anti-human p53 protein, clone DO-7, DAKO). Tissue sections of squamous cell carcinoma of cervix and ductal carcinoma of breast were used as positive controls for p16 and p53 expression respectively. The slides stained for p16 and p53 were observed under light microscope and results are expressed in both the number of positive cases and the percentage of immunostained cells after counting 100 cells in 10 consecutive high power (400x) fields. The cells positive for p16 and p53 show brown staining of nucleus plus cytoplasm and nucleus respectively. The intensity of immunohistochemical staining was graded based on subjective evaluation of colour exhibited (brown colour) by antigen, antibody and chromogen complex as[7] -Negative: (0), no colour, Mild: (1+), light brown, Moderate: (2+), dark brown and Strong: (3+), very dark brown. p53 staining pattern were classified according to the relative number of positive cells (brown colour) and the location of positive cells in the different epithelial layers, as described previously by Cruz et al.[8] Statistical analysis was done using SPSS version 17 software and proportion of p16 and p53 staining was compared using 'chi square' test. A p value of $<0.05$ was considered statistically significant.

## RESULTS

A total of 74 cases (64 male and 10 female) were studied for p16 and p53 expression. Classification of the oral biopsies was as follows: 14 cases of benign (hyperplasia without dysplasia), 20 cases of premalignant (dysplastic) lesions (3 mild, 5 moderate and 12 severe) and 40 cases of malignant (SCC) lesions (28 well, 9 moderately and 3 poorly differentiated) of oral mucosa. The immunohistochemical expression of p16 decreased with the severity of lesion. Positive p16 expression seen in 50% group 1 (hyperplasia), 35% group 2 (dysplasia) and 10% of group 3 (carcinoma) cases and were significantly correlated ($p<0.01$). In respect to histological grading 66% of mild; 60% of moderate; and 17% of severe dysplasia were p16-positive. Only 11% cases of well and moderately differentiated and none of poorly differentiated carcinoma showed p16 positivity. Positive p16 immunostaining showed stained cells arranged in clusters with skip areas in basal and suprabasal epithelial cell layers. From total cases 42% of benign, 29% of premalignant and 25% of malignant cases showed strong p16 positivity.

Of the total number of cases subjected to p53 staining, 45(61%) cases showed positive staining which constitute 21% of group 1,60% of group 2 and 75% of group 3 cases and this relation was statistically significant ($p<0.01$). According to histological grading, 34% of mild, 60% of moderate and 67% of severe dysplasia cases were p53 positive. Out of carcinoma cases, p53 positivity seen in 75% of well differentiated, 78% moderately differentiated and 67% of poorly differentiated carcinoma lesions. In both dysplastic and malignant cases no significant relation could be drawn between p53 expressions and grading of lesion. 66% of hyperplastic and 17% of dysplastic cases showed p53 positivity in basal epithelium while 34% of hyperplastic, 83% of dysplastic and 100% of malignant cases showed p53 staining in suprabasal region. This pattern of p53 staining showed statistically significant relation with p value $<0.01$.58% of premalignant, 53% of malignant and none of benign cases showed strong p53 positivity. Higher number of cases showing p16+/p53- belonged to benign lesion

(36%) with only 20% premalignant lesions and none of the malignant lesion showed this category of expression. Similarly, percentage of cases showing p16-/p53+ gradually increased with increase in progression to malignant lesion with 7% of benign cases, 45% of premalignant and 62.5% of malignant cases and this association was found to be highly significant(p value=0.001).

## DISCUSSION

The present report showed the immunoreactivity to antibodies anti-p16 and anti-p53 in different areas of hyperplastic, OED and OSCC. The inactivation of p16INK4a is an early event in oral carcinogenesis preceding the progression from premalignant to malignant oral lesions.[9] The results obtained support this suggestion since the highest p16-positivity in hyperplastic (50%) compared to dysplastic and carcinoma lesion suggesting that p16 may be associated with increasing risk of malignancy and this association is statistically significant. In dysplastic (group 2), only 7 out of 20 and in carcinoma (group 3), 4 out of 40 cases (10%) showed p16 positivity. These results were in accordance with study conducted by Papadimitrakopoulou et al[10] who demonstrated that there was decreased immunohistochemical expression of p16 in oral premalignant lesions. Study conducted by Muirhead et al. and Shah et al.[11] showed decreased expression of p16 in OSCC when compared with dysplasia and normal epithelium. This study was in accordance with the results obtained in current study. Decreased expression of p16 in dysplasia and OSCC can be explained by p16 gene inactivation which may occur due to homozygous gene deletion, mutation and hypermethylation of promoter region of p16 gene which was observed at high frequency in severe dysplasia and OSCC.[12]

In the present study, 60% (n=12) of dysplastic lesions showed p53 positivity compared to hyperplastic (benign) lesion where only 21% (n=3) came positive. Considering the various grades of dysplasia, 67% of cases of severe dysplastic lesions showed positive expression and there was increased in positive cases for expression of p53 with increase in severity of dysplasia which was in accordance to the study Ralhan et al., 2000.[13] Out of p53 positive benign lesions, 66% (n=2) cases showed expression confined to basal layer while 83% premalignant lesions showed suprabasal layer positivity suggesting that the expression of p53 above the basal layer could be an early event in oral carcinogenesis and an indicator of developing carcinoma. In the present study 30 out of 40 cases of oral SCC expressed p53 protein in comparison to 3 out of 14 hyperplastic lesion and 12 out of 20 dysplastic lesions and this analysis showed a significant relation between p53 positive expression and different severity of lesions (p value-0.001). In current study, 34% of benign lesions, 83% of premalignant lesions and 100% malignant lesions showed p53 positive expression in suprabasal layer and this association was found to be highly significant (p value-0.0003). Thus, suprabasal expression of p53 here would indicate that a larger part of epithelium than normal is dividing.[14] No significant relation was obtained between the percentage of p53 positive cases and grade of carcinoma. A noticeable observation in the present study was the complete absence of p53 positivity in some dysplasia and SCCs which can be explained by Nylander et al[14] as, the tumours lacking detectable p53, could have mutation in p53 gene resulting in production of non-functional and non-detectable protein.

## CONCLUSIONS

This study evaluated the association between p16, p53 expression in epithelial dysplasia and OSCC. Proportionately decreased expression of p16 and increased expression of p53 was observed with increase in severity of dysplastic lesions and malignancy. Increased expression of p53 in suprabasal cell layer was seen in high risk premalignant lesions (moderate and severe dysplasia) and oral carcinoma in comparison to benign lesion and low risk premalignant lesions (mild dysplasia), which is an early event in oral carcinogenesis. Further studies should ascertain the HPV status, larger sample size to establish and understand the better role of p16 and p53 in oral potentially malignant lesions and OSCC.

## REFERENCES

[1] Landis SH, Murray T, Bolden S, et al. Cancer statistics 1999. CA Cancer J Clin 1999;49(1):8-31.
[2] Zerdoner D. The Ljubljana classification - its application to grading oral epithelial hyperplasia. J Craniomaxillafac Surg 2003;31(2):75-79.
[3] Bishop JM. Molecular themes in oncogenesis. Cell 1991;64(2):235-248.
[4] Liggett WH, Sidransky D. Role of the p16 tumor suppressor gene in cancer. J Clin Oncol 1998;16(3):1197-1206.
[5] Gazzeri S, Gouyer V, Vour'ch C, et al. Mechanisms of p16INK4A inactivation in non-small cell lung cancers. Oncogene 1998;16(4):497-504.
[6] Gologan O, Barnes EL, Hunt JL. Potential diagnostic use of p16INK4A, a new marker that correlates with dysplasia in oral squamoproliferative lesions. Am J Surg Pathol 2005;29(6):792-796.
[7] Patel SM, Patel KA, Patel PR, et al. Expression of p53 and ki-67 in oral dysplasia and squamous cell carcinoma: an immunohistochemical study. International Journal of Medical Science and Public Health 2014;3(10):1201-1204.
[8] Cruz IB, Snijders PJ, Meijer CJ, et al. p53 expression above the basal cell layer is an early event of malignant transformation and has predictive value for developing squamous cell carcinoma. J Pathol 1998;184(4):360-368.
[9] Gonzalez-Moles MA, Gil-Montoya JA, Ruiz-Avila I, et al. Prognostic significance of p21WAF1/CIP1, p16INK4a and CD44s in tongue cancer. Oncol Rep 2007;18(2):389-396.
[10] Papadimitrakopoulou V, Izzo J, Lippman SM, et al. Frequent inactivation of p16INK4a in oral premalignant lesions. Oncogene 1997;14(15):1799-1803.

[11] Shah NG, Trivedi TI, Tankshali RA, et al. Prognostic significance of molecular markers in oral squamous cell carcinoma: a multivariate analysis. Head Neck 2009;31(12):1544-1556.
[12] Vairaktaris E, Yapijakis C, Psyrri A, et al. Loss of tumour suppressor p16 expression in initial stages of oral oncogenesis. Anticancer Res 2007;27(2):979-984.
[13] Ralhan R, Sandhya A, Meera M, et al. Induction of MDM2-P2 transcripts correlates with stabilized wild-type p53 in betel- and tobacco-related human oral cancer. Am J Pathol 2000;157(2):587-596.
[14] Nylander K, Dabelsteen E, Hall PA. The p53 molecule and its prognostic role in squamous cell carcinoma of head and neck. J Oral Pathol Med 2000;29(9):413-425.