



## ROLE OF P16 IN ORAL PREMALIGNANT AND MALIGNANT SQUAMOUS CELL LESIONS

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### ABSTRACT

**Context:** An upward trend is observed in the incidence and prevalence of oral premalignant and malignant squamous lesions in India, hence study on the role of p16 in such lesions was undertaken to assess whether p16 can be effective tool in early diagnosis and management of oral squamous lesions

**Aims:** To determine the presence of p16 positivity in oral squamous premalignant and malignant lesions

**Settings and Design:** A prospective observational study

**Methods and Material:** 200 cases of oral squamous cell lesions were analysed, out of which 76 cases were premalignant and 124 cases were malignant. Immunohistochemical staining was carried out and presence of p16 positivity was seen.

**Results:** 76 cases (38%) were premalignant and 124 cases (62%) were malignant. Male to female ratio was 6:1 and the most common site involved was buccal mucosa. Approximately 72.5% patients belonged to rural areas. Majority of the cases were in their 4<sup>th</sup> and 5<sup>th</sup> decades of life. Tobacco usage was seen in 85% of the patients. Out of 200 cases, p16 was positive in 128 cases and negative in 72 cases. 46.6% premalignant cases and 74.4% of the malignant cases were positive for p16.

**Conclusions:** Chi-square statistics in our study for p16 positivity is 15.6481 in premalignant and malignant cases. Significant p-value is obtained as 0.000076 (i.e. p-value <0.05) in malignant squamous cell lesions. Hence we conclude that to access the prognosis and grade in oral squamous cell carcinoma p16 is a useful marker.

**KEYWORDS :** squamous cell carcinoma, premalignant lesions, malignant lesions, p16

### INTRODUCTION:

Oral squamous cell carcinoma has been reported as the sixth most common cancer world and in developing countries it is third most common cancers [1]. Oral malignancies occur in about twice as many men as women and 95% are found in persons more than 40 years of age [2]. The two most important known risk factors for development of oral and oropharyngeal cancer are guthka and tobacco chewing. Tobacco consumption is in the form of smoking bidi, cigarettes, guthka, chillum, khaini, chuttah (Reverse smoking), hookah and inhalation in the form of snuff or chewing betel quid which consists of betel leaf, areca nut, lime and tobacco [3,4]. Squamous cell carcinoma is the most common cancer in oral cavity and accounts for almost 40% of all cancers in the Indian subcontinent [5]. Leukoplakia, erythroplakia and oral submucous fibrosis are among the premalignant lesions[6].

Histopathology is the gold standard for the diagnosis of oral malignancy. Molecular changes occur before histopathological changes [5]. Identification of premalignant lesions is important in reducing the mortality.

This study was conducted to study p16 expression in oral premalignant and malignant lesion.

To correlate the expression of p16 with grading and staging of oral premalignant and malignant lesion and to perform a detailed clinicohistological study of oral premalignant and malignant lesions

### SUBJECTS AND METHODS:

This study was carried out in department of pathology GSVM Medical College and associated LLR hospital a total of 200 cases diagnosed between 2017-2018 were included in the study

Specimen were received in 10% formalin. For histopathology 5µm paraffin sections were prepared and stained with (H&E).

For Immunohistochemistry avidin-biotin complex (ABC)

immunoperoxidase method was used.

### RESULTS:

200 cases of oral squamous cell lesions were included in the study. Male female ratio was 6:1 and most of the cases belonged from rural areas (72.5%). Majority of cases were in their 4<sup>th</sup> and 5<sup>th</sup> decades of life. The most common site involved was buccal mucosa (24.5%) followed by soft palate (21%) and tongue (20%). Tobacco usage was seen in 85% of the patients. Out of 200 cases, 76 cases (38%) were premalignant lesions and 124 cases (62%) were malignant lesions. p16 positivity was seen in 46.6% of the premalignant lesions and 74.4% of the malignant lesions. p16 staining was negative in 36% cases, mild in 9% cases, moderate in 24% cases and maximal in 31% cases.

**TABLE I-** Sex wise distribution of total cases studied (n=200)

SEX	NO. OF CASES	PERCENTAGE
Male	172	86.0%
female	28	14.0%

**TABLE II-** Geographical distribution of total cases studied (n=200)

URBAN/RURAL	NO. OF CASES	PERCENTAGE
Urban	55	27.5%
Rural	145	72.5%

**TABLE III-** Age-wise distribution of total cases studied (n=200)

AGE GROUP	NO. OF CASES	PERCENTAGE
21-30 Yrs	05	2.5%
31-40 Yrs	30	17.5%
41-50 Yrs	54	27.0%
51-60 Yrs	94	44.5%
61-70 Yrs	15	7.5%
71-80 Yrs	02	1.0%

**TABLE IV-** Incidence in relation to tobacco chewing

ADDICTION	NO. OF CASES	PERCENTAGE
Tobacco users	170	85%
Non-tobacco users	30	15%

**TABLE V-** Anatomical distribution of total cases studied

SITE	NO. OF CASES	PERCENTAGE
Buccal mucosa	49	24.5%
Floor of mouth	26	13.0%
Gingiva	14	07%
Hard palate	5	2.5%
Soft palate	42	21%
Upper lip	3	1.5%
Lower lip	12	06%
Retromolar trigone	9	4.5%
tongue	40	20.0%

**TABLE VI-** Distribution of total cases studied(n=200)

PREMALIGNANT CASES(76)			MALIGNANT CASES(124)		
TYPE	N	%	TYPE	N	%
Submucosal fibrosis	4	02%	Verrucous carcinoma	7	3.5%
leukoplakia	24	12%	Grade I carcinoma	39	19.5%
Leukoplakia with dysplasia	28	14%	Grade II carcinoma	58	29.0%
Verrucous hyperplasia	20	10%	Grade III carcinoma	20	10.0%

**TABLE VII-** p16 positivity in total cases studied (n=200)

RESULT	PREMALIGNANT		MALIGNANT	
	N	%	N	%
Absent	40	53.3%	32	25.6%
Present	35	46.6%	93	74.4%

(Expression of p16 was found in 46.6%(35/75) of the premalignant cases and 74.4%(93/125) of the malignant lesions or oral cavity)

**TABLE VIII-** Distribution of cases according to intensity of staining for p16 antibody

INTENSITY	PREMALIGNANT		MALIGNANT	
	N	%	N	%
Negative	40	53.3%	32	25.6%
Mild(+)	08	10.6%	10	08%
Moderate(++)	09	12.0%	39	31.2%
Maximal(+++)	18	24.0%	44	35.2%

(p16 staining was negative in 36% cases, mild in 9% cases, moderate in 24% cases and maximal in 31% cases)

**DISCUSSION:**

Present study comprises an analysis of 200 oral squamous premalignant and malignant lesions. The data for gender was 86% for males and 14% for females. The sex ratio in the present group of patients was 6:1. Various studies in India have found a male to female ratio ranging from 2.3:1 to 3.27:1 [5, 10]. Cases from rural areas predominated over urban population. The higher prevalence in rural area can be attributed to higher tobacco and alcohol consumption, illiteracy, poor oral hygiene and paucity of availability of medical facilities in remote areas. Hence patients present at a late stage in progression of the disease. Peak incidence was observed in fourth and fifth decade of life.

Tobacco addiction has been widely implicated in the aetiology of oral cancers. Tobacco chewers are at 8 times more risk than non-tobacco chewers [11]. In the present study, 85% cases were tobacco users, which strongly suggest close relationship between tobacco use and cancers of oral cavity. In our study, chewing tobacco had 5.6 times more risk than non tobacco chewers. The commonest site for oral cancer has been buccal mucosa (24.5%) followed by soft palate (21%) and tongue (20%).

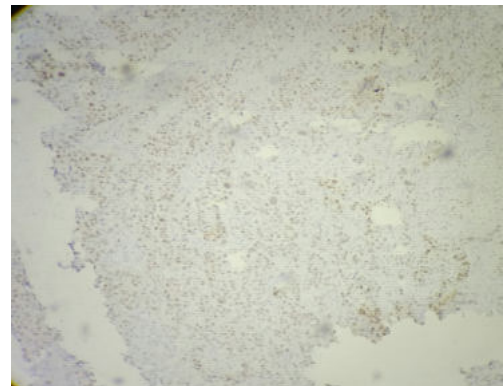
Incidence of premalignant lesions in our series was 37.5% and common premalignant lesions observed were in the form of

leukoplakia which accounted for 32%, leukoplakia with dysplasia 36%. Incidence of malignant lesions in our study was 62.5%. Common malignant lesions observed were in the form of squamous cell carcinoma grade II (46.4%) followed by squamous cell carcinoma grade I (31.2%) and squamous cell carcinoma grade III (16.8%). Verrucous carcinoma constituted about 5.6% of all malignant lesions.

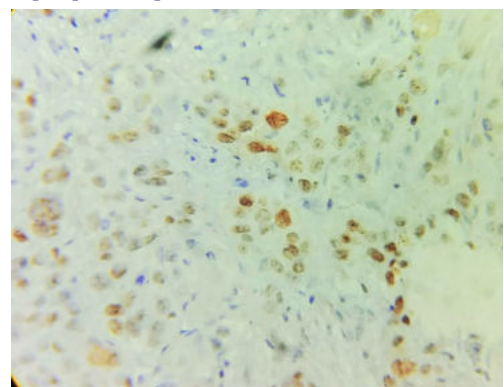
In our study of 200 cases, 64% cases were p16 positive. Whereas in various studies, normal mucosa expression of p16 was from 0% to 23% [12,13,14,15] p16 positivity in pathological groups increased from 53.3% in premalignant lesions to 74% in cancerous lesions. In a study p16 expression was 100% in normal tissue (n=20), indysplasia 88.1% (n=42) in carcinoma 30.8% (n=17)[16]. These results are different from the result observed in our study which can be attributed to sample size difference and p16 assessment scoring scheme, few studies observed p16 positivity with much less percentage 43.58% in comparison to our study in benign oral lesions.

**CONCLUSION:**

From the present study we observed a M:F ratio 6:1 with male predominance, rural : urban ratio 2.6 : 1, 82.5% cases (max) cases were over the age of 40 years. Commonest risk factor observed was tobacco chewing, most common site (24.5%) was buccal mucosa. Leukoplakia (31.6%) was the most common pre malignant lesion. P16 was expressed in 75% malignant and 47% premalignant lesions and showed both nuclear and cytoplasmic positivity in basal location in both lesions, in chi-square statistics the p-value was <0.05 hence the result of positivity in malignant cases is highly significant (<0.05). In present study we confirm the p16 positivity in most of the malignant squamous cell lesions. Hence we conclude that p16 can be used as important tool for early diagnosis of high grade oral squamous cell lesions.



**Fig. 1 – p16 positivity at 10x**



**Fig. 2 – p16 positivity at 40x**

**REFERENCES:**

- 1) Argiris A., Karamouzis M., Raben D. and Ferris R.L. Head and neck cancer- *Seminars Lancet* 2008;371:1695-709
- 2) Silverman S, Gorsky M, Kaugars GE. Leukoplakia, dysplasia and malignant

- transformation. *Oral Surg, Oral Med, Oral Pathology, Oral Radial Endod*; 1996;82:11
- 3) Mehta FS, Pindborg Ji, Gupta PC: Epidemiologic and Histologic study of oral cancer and Leukoplakia among 50, 91.5 villagers in India, *Cancer*. 1969;24:832-49
  - 4) Gupta PC., Mehta F.S. and Daffary D.K. Incidence rate of oral cancer and natural history of oral precancerous lesions in a 10-year follow-up study of Indian villagers. *Community Dent Oral Epidemiol* 1980;8:287-333
  - 5) Mehrotra R, Singh M, Kumar D, Pandey AN et al : Age specific incidence rate and pathological spectrum of oral cancer in Allahabad, *Ind J Med Sci* 2003;57:400-04
  - 6) Shafer W.G. and Waldron C.A. Erythroplakia of the oral cavity. *Cancer* 1975;36(3):1021-28
  - 7) Foulkes W.D., Flanders T.Y., Pollock P.M. and Hayward N.K. The CDKN2A (p16) gene and human cancer. *Mol Med* 1997;3:5-20
  - 8) Shintani S., Nakahara Y., Mihara M., Ueyama Y. et al, Inactivation of the p14ARF, p15INK4B and p16INK4A genes is a frequent event in human oral squamous cell carcinomas. *Oral Oncol* 2001;37:498-504
  - 9) Uzawa N., Sonoda I., Myo K., Takahashi K-I et al Fluorescence in situ hybridization for detecting genomic alterations of cyclin D1 and p16 in oral squamous cell carcinomas. *Cancer* 2007; 110:2230-9
  - 10) Wahi P.N. Oral and Oropharyngeal tumour. Gann monograph on cancer. Res 1976;218: 19-26
  - 11) Wahi P.N. Epidemiology of oral and oropharyngeal cancers. A report to study in Mainpuri Distt. Uttar Pradesh, India. *WHO Bull* 1968;38:495
  - 12) Angiero F, Berenzi A., Benetti A., Rossi E. et al Expression of P16, P53 and Ki-67 proteins in the progression of epithelia dysplasia of the oral cavity. *Anticancer Res* 2008;28:2535-40.
  - 13) Angiero F, Farronato G., Dessy E., Magistro S. et al Evaluation of the cytotoxic and genotoxic effects of the orthodontic bonding adhesives upon human gingival papillae through immunohistochemical expression of p53, p63 and p16. *Anticancer Res* 2009;29:3983-88
  - 14) Buajeab W., Poonsawat S., Punyasingh J. and Sanguansin S. Expression of p16 in oral cancer and premalignant lesions. *J Oral Pathol Med* 2009;38:104-108
  - 15) Bilde A., von Buchwald C., Dabelsteen E., Therkildsen M.H. et al Molecular markers in the surgical margin of oral carcinomas. *J Oral Pathol Med* 2009;38:72-78
  - 16) Shintani S., Mihara M., Nakahara Y., Kiyota A. et al Expression of cell cycle control proteins in normal epithelium, premalignant and malignant lesions of oral cavity. *Oral Oncol* 2002;38:235-243
  - 17) Cao D.F., Begum S., Ali S.Z. and Westra W.H. Expression of p16 in benign and malignant cystic squamous lesions of the neck. *Hum Pathol* 2010;41:535-39
  - 18) Fregonesi P.A.G., Teresa D.B., Duarte R.A., Neto C.B., et al p16 INK4A immunohistochemical overexpression in premalignant and malignant oral lesions infected with human papillomavirus. *J HistochemCytochem* 2003;51:1291-97