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# ROLE OF p16 IN ORAL SQUAMOUS PREMALIGNANT AND MALIGNANT LESION

Pathology

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## ABSTRACT

The objective of this study was to determine the presence of p16 positivity in oral squamous premalignant and malignant lesions. A total of 200 biopsies were analysed, out of which, After applying chi-square test, significant correlation was seen between p16 positivity and malignant lesions.

## KEYWORDS

squamous cell carcinoma, premalignant lesions, malignant lesions, p16

## INTRODUCTION

Oral squamous cell carcinoma (OSCC) constitutes the sixth most common cancer worldwide and the third most common cancer n the developing countries [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]**.** Guthka (mixture of crushed areca nut, tobacco, catechu, slaked lime, paraffin wax and flavourings) and tobacco chewing are the two most important known risk factors for development of oral and oropharyngeal cancer. 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]. Histologically, squamous cell carcinoma is the most common cancer in the oral cavity (90%) and oropharynx. Although representing 2-4% of the malignancies in the West, oral carcinoma accounts for almost 40% of all cancers in the Indian subcontinent [5]. Among the premalignant lesions are leukoplakia, erythroplakia and oral submucous fibrosis [6]. Histological examination of tissue remains the gold standard for diagnosis and identification of malignant oral lesions. Molecular level changes in the lesion occur before clinical and histopathological changes [5]. Identification of high risk oral premalignant stages could constitute one of the keys in reducing the mortality and cost of treatment associated with OSCC.

The Human Genome Organisation nomenclature committee assigned the designation CDKN2A (for cyclin dependent kinase inhibitor 2A) for p16INK4a. This gene is located on chromosome 9p21 and lies on three exons [7]. Commonly, p16 is inactivated by deletions, mutations or promoter methylation in the absence of HPV in Head and Neck Squamous Cell Carcinoma [8]. Therefore, screening for both cyclin D1 and p16 aberrations in OSCCs may be useful for identifying aggressive tumours, disease recurrence and in patients with a poor prognosis [9].

## MATERIALS AND METHOD

Specimen received in 10% formalin. Paraffin sections of 5µm thickness were stained by haematoxylin and eosin (H & E) for histopathological study.

Immunohistochemical staining was carried out by means of an avidin-biotin complex (ABC) immunoperoxidase method. We used similar protocols to those used previously for the study of p16 expression. The specimens were cut at 5 µm thickness, dewaxed in xylene for 15 minutes, and rehydrated with ethanol. The slides were treated with 3% hydrogen peroxide for 30 minutes at room temperature. After three washes in phosphate buffered saline, antigen retrieval was performed by microwaving in citrate buffer (pH 6) for five minutes. We used a 1/50 dilution of the monoclonal anti-p16 antibody and incubation was carried out overnight at 4°C. The sections were then incubated with secondary antibody for 30 minutes. Staining was performed using ABC reagents and 3,3`-diaminobenzidine as substrate. The sections were counterstained with Mayer's haematoxylin for 30 seconds and were run in tap water for three minutes.

## OBSERVATIONS

A total of 200 cases of oral squamous cell lesions were included in this study. Male female ratio was 6:1 and most of the cases belonged from rural areas (72.5%). Maximum number of cases were in their 4$^{th}$ and 5$^{th}$ 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% |
| Verruous hyperplasia | 20 | 10% | Grade III carcinoma | 20 | 10.0% |

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

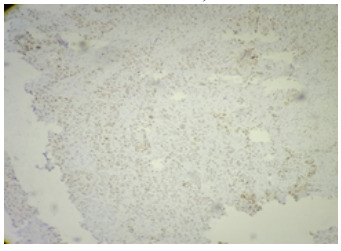
| RESULT | PREMALIGMANT | | MALIGNANT | |
|---|---|---|---|---|
| | N | % | N | % |
| Absent | 40 | 53.3% | 32 | 25.6% |
| Present | 35 | 46.6% | 93 | 74.4% |

(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)

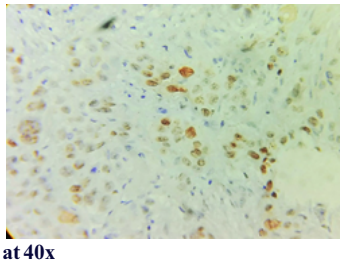
**Table VIII- Distribution of cases according to intensity of staining for p16 antibody**

| 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 in24% cases and maximal in 31% cases)



**p16 positivity at 10x**



**p16 positivity at 40x**

## 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 and 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. It was found that the peak incidence was in the 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. There were no normal tissues used in this study. However, in various studies, p16 expression in normal mucosa varied from 0% to 23% [12,13,14,15]. Within pathological groups the percentage of p16 positivity increased from 53.3% in oral premalignant lesions to 74.4% in malignant lesions. A study reported that the percentage of p16 expression in the oral cavity was 100% in normal tissues normal (n=20), 88.1% in dysplasia (n=42) and 30.8% in carcinomas (n=117) [16]. These results seem to contradict our current findings, but we could argue the discrepancies observed could be attributed to differences in sample size and the scoring scheme used for p16 assessment. Few studies reported a significantly lower percentage of p16 positivity, 43-58% compared to our study in benign oral lesions [17,18].

## CONCLUSION

Chi-square statistic for the positivity of p16 in premalignant and malignant cases is 15.6481. Significant p-value is obtained as 0.000076 (i.e. p-value <0.05), thus confirming the p16 positivity in maximum of the malignant squamous cell lesions. Hence we conclude that p16 is a useful marker to assess the grade and prognosis of oral squamous cell carcinoma.

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