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ORIGINAL ARTICLE

A Cross Sectional Study on the Expression of MIB-1 and P16INK4a in Oral Carcinoma in a Tertiary Care Hospital

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Abstract

Background: Oral cancer is one of most prevalent cancer with high morbidity and mortality rate in India. Around 90% are squamous cell carcinomas among the oral cancers. Uncontrolled proliferation of cell is considered to be the key processes of oncogenesis. MIB-1 or Ki67 is expressed in all proliferating cells and used as a tool for estimating the percentage of dividing cells within a tumor. In addition to it, MIB-1 acts as a potentially prognostic marker in oral malignancies. **Materials and Methods:** Around 100 cases of oral squamous cell carcinoma were assessed for nuclear atypia, mitotic activity and necrosis. For Ki-67, strong nuclear positivity was seen as favorable. There were three grades: 1 for 10–30%, 2 for 30–50%, and 3 for 50% and above. The number of positive cells and staining intensity for p16 (nuclear or/and cytoplasmic staining) were computed. **Results:** Ki-67 and p16 expressions were seen in oral squamous cell carcinomas that are well, moderately, and poorly differentiated. Ki-67 expression increased when grades rose from well differentiated to poorly differentiated carcinoma. p16 had a better expression in well differentiated carcinoma compared to poorly differentiated carcinoma. The mean mitotic count, were increased in high grade neoplasms. **Conclusions:** Therefore, the expression of p16 and Ki-67 in malignant lesions of the oral squamous cell carcinoma can be used in conjunction with the histomorphological attributes to research their capacity for proliferation of oral malignancies.

Keywords: Head and neck, Human papilloma virus, MIB-1 antigen, p16INK4A gene, Squamous cell carcinoma

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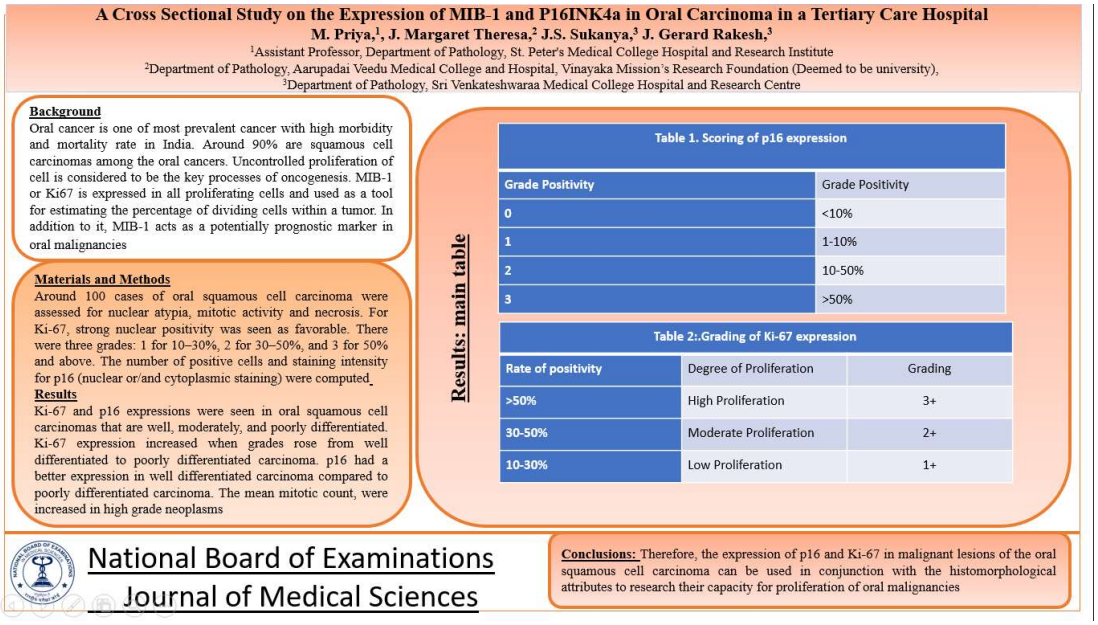
Graphical Abstract



Introduction

Squamous cells carcinoma of oral cavity is the most frequent type of head and neck neoplasm. Annually more than 500,000 new cases of squamous cell carcinoma of oral cavity reported worldwide [1]. Incidence of oral cancer in gradually increasing every year and being a major health problem in India [2]. Oral and oropharyngeal cancers is the 6th most common cancer. Most common histological type of oral and oropharyngeal cancer is squamous cell carcinoma accounting around 90% among the neoplasm [3]. Various studied have proved that squamous cell carcinoma of oral cavity is associated with human papillomavirus (HPV). Squamous cell carcinoma associated with HPV believed to have better prognosis. They exhibit a distinct tumor morphology, with different demographics and unique characteristic and molecular profiling [4]. Various methods are available to detect the HPV, but the single best method to diagnose HPV remains controversial. In recent days highly preferred and recommend method to identify HPV in biopsy is In Situ Hybridization (ISH) and p16 immunohistochemistry in combination for detecting high-risk HPV.

p16 is a protein that suppress tumor and is a cyclin-dependent kinase inhibitor. p16 is usually mutated or deleted in squamous cell carcinoma. p16 overexpression is observed in tumors with biologically active HPV. HPV E7 protein functionally inactivates the retinoblastoma protein (Rb). Human papilloma virus driven malignancies are identified with a strong and diffuse pattern of p16 immunostaining and is thought to be an extremely sensitive diagnostic marker [4]. However p16 overexpression can also result from other mechanisms [4-6]. Tumorogenesis is a abnormal cell proliferation; the expression of the Ki-67 protein is strongly correlated with cell proliferation and may serve as a biomarker [7]. Proliferative markers may be useful in enhancing the prognostic assessment of Oral Squamous cell carcinoma. Ki-67

antigen may be employed as a marker for Oral Squamous Cell Carcinoma and Oral Epithelial Dysplasia [7]. Epithelial dysplasia is a premalignant disease that act as a risk factor for oral carcinoma, which is a multistage process. Significant variation exists when it comes to the diagnosis and grading of oral epithelial dysplasia. Given the close correlation between oral carcinogenesis and the human papillomavirus (HPV), the use of P16 act as a biomarker could aid in identifying the cells that may be undergoing malignant transformation. Nevertheless, dual labeling test P16INK4/Ki67 may be a more promising marker for detecting the transformed cells due to P16 limited specificity [8].

So, in the present study we examined the expression of proteins that regulate the cell cycle P16INK4a and proliferative marker MIB-1 (Ki-67) immunohistochemistry in oral carcinoma.

Materials and Methods

This study is cross-sectional and observe the expression of p16INK4a and MIB-1 in oral malignancies in a tertiary care hospital. This study comprised 100 cases of oral squamous cell carcinoma in total. The clinical data including patient's age, clinical staging were obtained from the pathology records. Incisional and excisional biopsy taken from oral cavity like buccal mucosa, tongue, lips, tonsils were routinely processed after being fixed in 10% buffered formalin. Sections having a thickness of 4-5 μm were cut and stained using Hematoxylin and Eosin stains. The slides were examined using light microscopy, and the information was recorded. Immunohistochemistry of p16INK4a and MIB-1 were also performed on 3-4 μm-thick sections on a poly-L-lysine-coated slides. The data were collected, complied and analysed. Scoring of p16 expression shown in Table 1. Grading of Ki-67 expression shown in Table 2.

Table 1. Scoring of p16 expression

Grade Positivity	**Grade Positivity**
0	<10%
1	1-10%
2	10-50%
3	>50%

Table 2. Grading of Ki-67 expression

Rate of positivity	**Degree of Proliferation**	**Grading**
>50%	High Proliferation	3+
30-50%	Moderate Proliferation	2+
10-30%	Low Proliferation	1+

Ki-67 labelling index was calculated as follows:

$$\frac{\text{No of cells showing positive nuclear staining for Ki67} \times 100}{\text{Total. No. of cells}}$$

The percentage of mitotic count is determined for every 100 cells. In all the cases, the whole epithelium has been surveyed. Approximate calculation of 1000 cells were examined under high power view.

Results

The present study on expression of p16INK4a and MIB-1 in oral malignancies analyses the demographic attributes of the population under investigation. The Demographic details are shown in Table 3.

Table 3. Demographics

Parameter	Value
Age (Mean ± SD)	57 Years
Male	13%
Female	12%
Alcohol use	40%
Tobacco use	92%
Diabetes	20%
Hypertension	24%
Radiation exposure	0%
Family history	0%

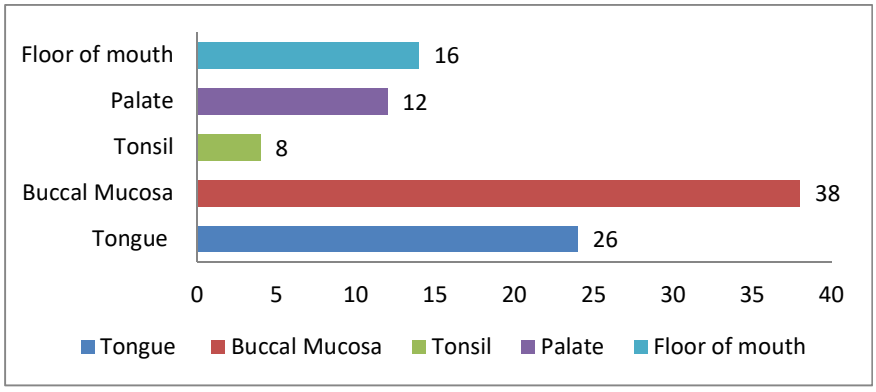


Figure 1. Different sites of distribution of oral cavity malignancies

Table 4. Histopathological grading and typing

Histology Type	Histology grade	Number of cases
Squamous cell carcinoma	Well differentiated	34%
Keratinizing	Moderately differentiated	57%
Squamous cell carcinoma- Non Keratinizing	Poorly differentiated	9%

Table 4 shows displays the different histopathological grades of squamous cell carcinoma. Invasive squamous cell carcinoma histological types are Keratinizing, Non keratinizing and Poorly differentiated. Among the invasive Squamous cell carcinoma, moderately differentiated Squamous cell carcinoma were 57%, followed by the well-differentiated Squamous cell carcinoma (34)% and Poorly differentiated SCC (9%).

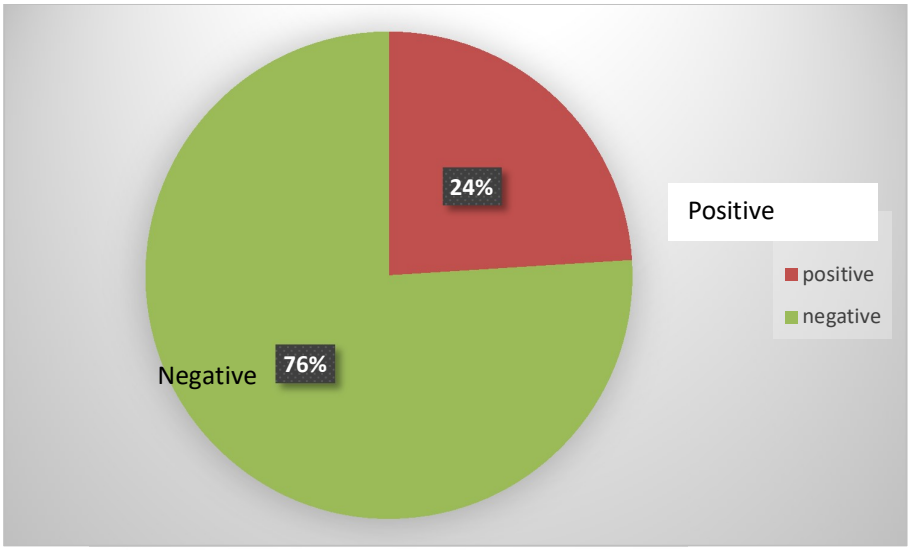


Figure 2. Invasive Oral Squamous Cell Carcinoma with metastatic lymph nodes

Table 5. Expression of Ki-67 and p16 expression in Oral Squamous Cell Carcinoma

MIB-1 (ki67)	p16	
	Positive	Negative
Positive	130	170
Negative	30	30

Table 6. Comparison of p16 in different Histological grades of Oral Squamous Cell Carcinoma

Grade	Grade 0	Grade 1	Grade 2	Grade 3	Total cases
WDSCC	19	9	0	0	28
MDSCC	38	21	4	0	63
PDSCC	6	3	0	0	9

Grade 1 expression of p16 was found to be higher in Moderately differentiated squamous cell carcinoma. (MDSCC) 21 cases followed by Well differentiated squamous cell carcinoma. (WDSCC) 9 cases. Grade 2 expression of p16 was found to be higher in MDSCC with none from WDSCC and Poorly differentiated squamous cell carcinoma (PDSCC). Grade 3 expression of p16 was not found in any type of squamous cell carcinoma.

Discussion

Age of occurrence of squamous cell carcinoma

The mean age of patients involved in the present study is about 57 years (Range from 40-80 years). Omer et al stated in his study, the age ranged from 33 to 89 years, with 64.24 years as the mean age and in which majority of them were males (52.0%) [9]. Saxena et al, in her study found that the patient's age ranged from 31 to 95 years, and the mean was about 53.8 years [3]. Age range of patients with squamous cell carcinoma correlating with both the studies.

Tobacco and Alcohol usage

In the current research, tobacco usage was found in 92% of cases. Alcohol usage noted in 40% of cases [9]. A study done by Mello et al on analysing the results of drinking and smoking on patients with oral SCC had found that the incidence of oral squamous cell carcinoma was positively correlated with synergistic intake with an odds ratio = 5.37 at 95% confidence interval [95%CI] = 3.54-8.14 [10].

Site Distribution

In the present study, commonest tumour site was found to be buccal mucosa (38%) followed by tongue (26%) and the least commonest sites were floor of the mouth (16%), palate (12%) and tonsil (8%). Borse et al. in his study also stated that in recent years, the incidence of the tongue and buccal mucosa cancer increased in India with a higher incidence of carcinoma of buccal mucosa cases [11].

Histopathological grading

In the current research around 34% of cases were diagnosed as well differentiated squamous cell carcinoma, 57% cases were found to be moderately differentiated squamous cell carcinoma, 9% of cases reported as poorly differentiated carcinoma. In our study, moderately differentiated tumors were found to be most common, which varies with the study of Padma et al who stated that in her study 98 (49.5%) accounts for

moderately differentiated carcinoma 32 (16.2%) had poorly differentiated tumors and 68 (34.3%) had well-differentiated carcinomas [12].

Neck Node Metastasis

In the present study around 24% of patients showed metastatic deposits in the regional lymph nodes. Mehta et al in her study found nodal positivity in 33 patients (52.38%) [13].

IHC Expression of Molecular Markers in Oral Squamous Cell Carcinoma

P16 Expression

In our study, on analysing p16 expression, Grade 0 expression is positive in 63 cases, grade 1 expression was seen in 33 cases and 4 cases show grade 2 expression.

Thambiah, et al in their study stated that 45% of high risk potential malignant disorder showed diffuse positivity for p16 [14]. In our study, for p16, none of the Oral squamous cell carcinoma displayed diffuse positivity. Study proposed by Shah et al. found that Oral squamous cell carcinoma with reduced p16 showed expression increasing grades of tumour [15]. Maheswari and Tamilselvi in their study stated that p16 expression was seen in 50% of moderately and poorly differentiated squamous cell carcinomas, while 42.86% of well-differentiated carcinomas of the squamous cell shows expression of P16. Sharada et al. stated that they found a substantial relationship between p16 immunoexpression and dysplasia degree which shows positivity in 16.7% of instances of oral intraepithelial neoplasia I compared to 77.8% and 25% of Oral intraepithelial neoplasia II and III, respectively [2].

Ralli et al stated that in her study, p16 was positive in 78.7% cases and 21.3% cases were negative [16]. Lewis et al., in his study found that of the 239 cases, p16 showed positivity in 187(78%) cases [4].

MIB-1 (Ki-67) Expression

Dysplastic epithelium is characterized by cellular alteration at molecular and genetic level. There is also alteration in the epithelial maturation leading to increased proliferative activity of the suprabasal layer [17].

Pontes et al., study shows in OSCC, the expressions of Ki-67 have demonstrated varying results. According to certain research, the proliferation-associated antigen Ki-67 is one of the most reliable indicators of a patient's prognosis for a number of malignant illnesses, including lung, prostate, and breast cancer [18].

Study conducted by Sharada et al stated that the histological type and grade of the tumor were strongly connected with ki-67 immunoexpression. Malignant cases had higher ki-67 immunoexpression (66.3%) compared to benign cases (10%) and premalignant cases (37%). is seen in poorly differentiated tumours (75%) than well – differentiated tumours (12.2%) [2].

Takkem et al. stated that the expression of Ki-67 antigen may be utilized as a marker for the histological grading of OED and OSCC increases based on how severe the oral epithelial dysplasia [7]. Takkem also stated that in his study the Ki-67 expression was detected in all cases of well-differentiated, moderately and poorly differentiated tumours [7].

Correlation between p16 and MIB-1 (Ki-67)

In our study, expression of p16 and MIB-1 (Ki-67) was significantly correlated. Bharathi et al stated that in her study, dual p16INK4a and in patients below 40 years of age, Ki67 was positive in 5 cases, and 2 cases were positive in patients older than 40. Within the OSCC group, 1 case which was 8 people older than 40 and six patients younger than 40 were positive for Ki-67 [8].

Conclusion

MIB-1 and p16 expression can be used in conjunction with histomorphological features in diagnosis of well differentiated and moderately differentiated oral squamous cell carcinoma and to study their proliferative potential which would provide a significant effect on the therapy.

Statements and Declarations

Conflicts of interest

The authors declares that they do not have conflict of interest.

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