ORIGINAL RESEARCH PAPER

Oncology

EVALUATING THE USE OF P16 EXPRESSION IN ORAL SQUAMOUS CELL CARCINOMA

KEY WORDS:

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ABSTRACT

Background: Wide variations in incidence and factors associated with the expression of p16 were reported in the literature. In order to understand the better insight on the same, this study was planned to assess the exact prevailing incidence of p16 expression and clinic-pathological parameters associated with it among the cases with Oral SCC. **Methods:** This study was conducted as a cross sectional study in the department of Surgical Oncology at Yenepoya Medical College Hospital a tertiary care teaching hospital located in Mangalore, Karnataka. Patients diagnosed with Oral Squamous Cell Carcinoma in Yenepoya Medical College Hospital Mangalore, who were admitted during study period were included in the study. A total of hundred and thirty three patients, with oral squamous cell carcinoma were included in this study. After taking detailed history and examination, P16 IHC was done on formalin-fixed, paraffin-embedded tissues of proven OSCC. **Results:** In our study p16 marker was positive among 8.3% of cases with oral SCC. Age, gender, side and site of pathology, recurrent disease, grade, Clinical TNM staging, lymphovascular invasion, perineural invasion, treatment modalities, and addiction behaviors were not significantly associated with the positive expression of p16. **Conclusion:** There is no significant correlation between p16 expression and clinicopathological parameters.

INTRODUCTION:

Squamous cell carcinoma of the head and neck is still a leading source of morbidity and mortality globally[1]. Squamous cell carcinomas (SCCs) account for 90% of head and neck malignancies, with the oral cavity accounting for more than half of all tumours[2]. Oral SCC (OSCC) is frequently preceded by potentially cancerous conditions like oral lichen planus (OLP).

Despite significant advancements in medical research, the prognosis for oral SCC appears to have remained same, with a 5-year overall life expectancy of around 40%[3-5]. Cervical nodal metastasis is the most important predictive marker; if there is only single positive lymph node, survival drops by half[6].

A lot of work has gone into developing a valid neck lymph node categorization that can be used to better guide treatment and predict prognosis. When defining cervical nodal status, the latest edition of the AJCC classification considers the size, number, extracapsular spread (ECS), and laterality of positive nodes[7].

The specific cause of mouth cancer is unknown, most likely due to the variable quality of cancer statistics and the multifaceted nature of the malignancy's genesis[8]. Tobacco use, consumption of alcohol, human papillomavirus (HPV), dietary factors (e.g. inadequate intake of fruits and vegetables, iron deficiency, lack of vitamins, decreased immunity, and potentially malignant illnesses) are some of the aetiological risk factors for oral cancer[9].

Lip cancer is linked to excessive sun exposure, while buccal mucosa carcinoma appears to be linked to betel quid eating. Chronic irritation has not been linked to the development of oral cancer.

Tumour suppressor genes play an important role in physiological processes like cell proliferation, differentiation, and programmed cell death. P16 and p53 are tumour suppressor genes that play a role in cell cycle regulation[10].

p16 antibody immunohistochemistry (IHC) investigations in OSCC and pre - malignant lesions (PML) have yielded mixed findings. There have been reports of both reduced expression[11-13] and overexpression[14,15]. Although p16 expression cannot distinguish nondysplastic from dysplastic oral mucosa, a recent study found lower expression of p16 in oral dysplastic lesions[13].

IHC analysis of p16 in biopsies from head and neck SCC has been found to act as a surrogate marker for detecting HPV in head and neck SCC samples[16]. p16 is a protein involved in cell cycle control. According to Lewis et al[17] and Harris et al[18] p16 expression is associated with positive outcomes in head and neck SCC. Positive p16 expression was found in only 28% of head and neck SCC patients, according to Antonsson et al[19].

Considering these variations in incidence and factors associated with the expression of p16, this study was planned to assess the exact prevailing incidence of p16 expression and clinic-pathological parameters associated with it among the cases with Oral SCC.

OBJECTIVES:

Objectives of this study is to study assess

- The Incidence of p16 expression in Oral squamous cell carcinoma
- Association between p16 expression with clinic-pathological parameters

METHODOLOGY:

Study Design:

This study was conducted as a cross sectional study, to evaluate the use of p16 expression in Oral Squamous Cell Carcinoma.

Study Area
Department of Surgical Oncology at Yenepoya Medical College Hospital a tertiary care teaching hospital located in Mangalore,Karnataka.

Study Population:
Patients diagnosed with Oral Squamous Cell Carcinoma inYenepoya Medical College Hospital Mangalore ,who are admitted during study period were taken as study population.

Study Period:
August 2020 to December 2021

Inclusion Criteria:
- Both male & female patients.
- Age 20- 80 years
- All stages of Oral squamous cell carcinoma.

Exclusion criteria:
- Patients who have not consented for the study
- Inadequate tissue specimen present in the paraffin block

Information Received:
First Article From Review Literature ----- "p16 - a Possible Surrogate Marker for High-Risk Human Papillomaviruses in Oral Cancer?" By "Thanun Sritippho"

Z=1.96 (Type-I error at 5% percentage)

Precision (d) at 7%

Over expression of p16 was found in 21.6% of the cases, hence the Proportion considered to be (p) =0.216 (21.6%)

$$n=\frac{z_{\alpha}^2 * p * (1-p)}{d^2}$$

$$n=\frac{1.96^2 * 0.216 * 0.784}{0.07^2}$$

n=132.76

Approximately 133 subjects

Hence the sample size for the present study recommended to be n=133 subjects to study the incidence of p16 as surrogate marker for HR-HPV.

Study Design: prospective study

Sampling Design: Purposive Sampling (Non-probability sampling)

Statistical Analysis Suggested:

Descriptive statistics is used to describe the data. Chi-square test is used to test the correlation between p16 expression and histological grade of OSCC. ROC curve analysis will be done to analyze the sensitivity, specificity, false positive predictive and false negative predictive. Incidence will be assessed with 5% level of significance and sensitivity will be tested with 80% power.

Hence, a total of hundred and thirty three patients, with oral squamous cell carcinoma were included in this study.

Data Collection:
Patients attending the Department of Surgical Oncology with oral squamous cell carcinoma from the months of August 2020- December 2021 were included in the study. The individual participants were explained in detail regarding the study and they were also assured that, their identity would be kept strictly confidential and they have the option to refuse participation in the study at any cost of time.Written informed consent was obtained from the study participant's prior to the interview.

P16 IHC was done on formalin-fixed, paraffin-embedded tissues of proven OSCC (biopsy and resection specimens). All the patients had undergone a routine evaluation which included a biopsy for histological confirmation of cancer, along with a comprehensive history and physical examination.

Detailed Clinical Data Includes
1.Patient History
- Age
- Sex
- Chief complaints

2.Habit history
- Smoking
- Tobacco chewing
- Alcohol

3.Site of lesion
- Buccal mucosa
- Floor of mouth
- Gingivobuccal sulcus
- Lower alveolus
- Retromandibular trigone
- Tongue

4.Size of the Tumor

5.Clinical staging
- T stage
- N stage
- M stage

The mode of treatment, surgical procedure, recurrence was also noted in the clinical history.

Pathological data includes histology, grade, differentiation, lymphovascular invasion, perineural invasion. P16 IHC: p16 expression was performed on five-micron sections of paraffin blocks. The sections were deparaffinised in xylene and rehydrated in absolute ethanol. Endogenous peroxidase activity was blocked by incubation in 0.3% hydrogen peroxide in Phosphate- buffered Saline (PBS) for 30 minutes and subjected to antigen retrieval in Tris-EDTA buffer (pH-8) by autoclaving at 121°C for 10 minutes.

Sections was pre-incubated in 2% Bovine Serum Albumin (BSA) for 30 minutes and then incubated with mouse monoclonal antibody against p16 in 1:150 dilution, overnight at 4°C. p16 expression was observed using the SuperSensitive™

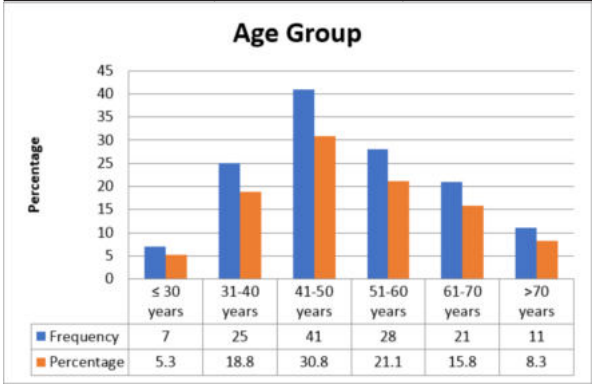
Polymer-HRP IHC Detection System . Sections were counterstained with hematoxylin, dehydrated, and mounted in DPX. Primary antibody was replaced with 2% BSA in negative control. Briefly, each sample was given a cytoplasmic as well as nuclear intensity score on a scale of 0-3. The percentage of tumor cells with positive nuclei was determined by scoring 10 microscopic fields of 100 tumor cells each.

A semiquantitative approach was opted to give the p16 scores based on percentage of tumor cells expressing p16. Scale 1-faint or low cytoplasmic and nuclear staining (LS) in less than 20% of tumor cells; Scale 2-High cytoplasmic and low nuclear staining (HC) in less than 50% of tumor cells & Scale -3-High nuclear and high cytoplasmic staining in greater than 50% of tumor cells. We considered Scale 3 tumors showing intense nuclear and cytoplasmic staining as positive for p16 expression. All the reports were entered in the same proforma where socio demographic and clinical data was entered by the principal investigator.

RESULTS:
In this study to evaluate the p16 expression in oral squamous cell carcinoma there were 5.3% of the participants below 30 years of age, 18.8% of the participants between 31-40 years of age and 30.8% of the cases were in the age group of 41-50 years. In the age range of 51-60 years, 61-70 years and above 70 years of age the proportion of study participants were 21.1%, 15.8% and 8.3% respectively.

Table 1: Age Wise Distribution Of Study Participants

Age group	Frequency	Percentage
≤ 30 years	7	5.3
31-40 years	25	18.8
41-50 years	41	30.8
51-60 years	28	21.1
61-70 years	21	15.8
>70 years	11	8.3
Total	133	100.0



Graph 1: Age Wise Distribution Of Study Participants

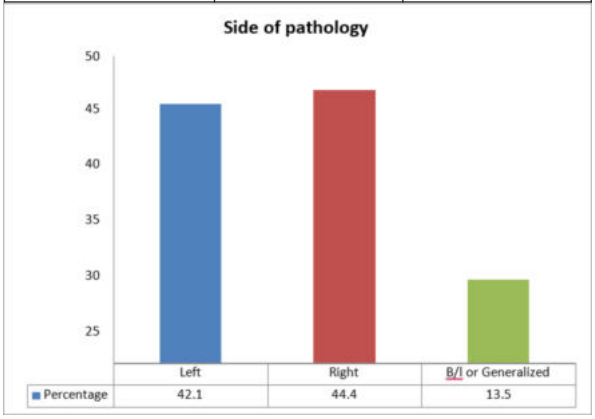
Among the total 133 cases maximum of 72.2% of the cases were males and 27.8% of the cases were females in this study

Table 2: Proportion Of Participants Based On The Gender

Sex	Frequency	Percentage
Female	37	27.8
Male	96	72.2
Total	133	100.0

Table 3: Side Of Oral Pathology Among The Study Participants

Sideofpathology	Frequency	Percentage
Left	56	42.1
Right	59	44.4
B/l or Generalized	18	13.5
Total	133	100.0

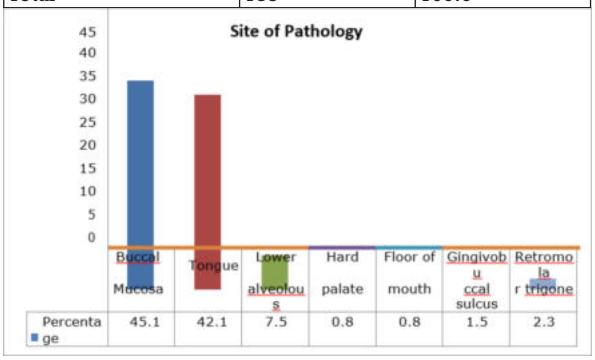


Graph 3: Side Of Oral Pathology Among The Study Participants

The most common side of oral pathology was found to be on right side (44.4%) while on left side the pathology was seen among 42.1% of the cases. Bilateral or generalized lesion was noted among 13.5% patients

Table 4: Site Of Oral Pathology Among The Study Participants

Site of Pathology	Frequency	Percentage
Buccal Mucosa	60	45.1
Tongue	56	42.1
Lower alveolous	10	7.5
Hard palate	1	0.8
Floor of mouth	1	0.8
Gingivobuccal sulcus	2	1.5
Retromolar trigone	3	2.3
Total	133	100.0



Graph 4: Site Of Oral Pathology Among The Study Participants

Regarding the site of oral lesion the most common site was Buccal mucosa (45.1%) followed by Tongue (42.1%), Lower alveolous (7.5%) and Retromolar trigone (2.3%). However Gingivobuccal sulcus, hard palate and Floor of mouth were the site of lesion for 1.5%, 0.8% and 0.8% of the cases with oral squamous cell carcinoma.

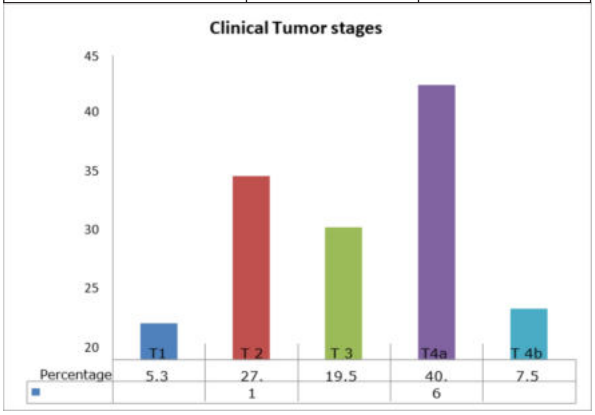
Table 5: Proportion Of Cases With Recurrence Of Oral Carcinoma

Recurrence	Frequency	Percentage
Yes	11	8.3
No	122	91.7
Total	133	100.0

In this current study recurrence of carcinoma was recorded among 8.3% of the cases after treatment.

Table 6: Clinical Tumor Staging Of Oral Squamous Carcinoma Among The Cases

Clinical Tumor stages	Frequency	Percentage
T1	7	5.3
T2	36	27.1
T3	26	19.5
T4a	54	40.6
T4b	10	7.5
Total	133	100.0



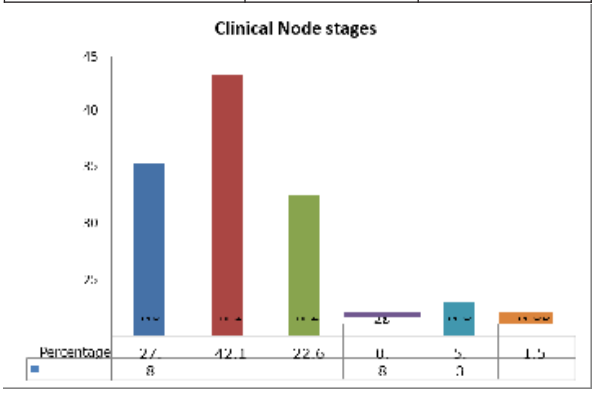
Graph 6: Clinical Tumor Staging Of Oral Squamous Carcinoma Among The Cases

There were 40.6%, 27.1%, 19.5%. 7.5% and 5.3% of the cases in the clinical tumor stage T 4a, T 2, T 3, T 4b and T1 respectively in this current study.

Table 7: Clinical Node Stage Of Oral Squamous Carcinoma Among The Patients

Clinical Node stages	Frequency	Percentage
N0	37	27.8

N1	56	42.1
N2	30	22.6
N2b	1	0.8
N3	7	5.3
N3b	2	1.5
Total	133	100.0

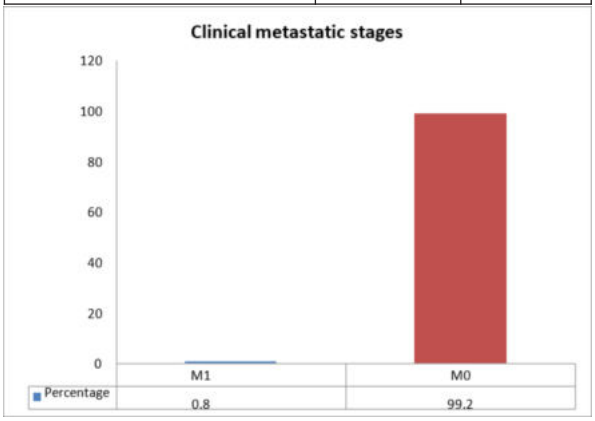


Graph 7: Clinical Node Stage Of Oral Squamous Carcinoma Among The Patients

Likewise the Clinical Node stage among the study subjects was noted to be N0, N1, N2, N2b, N3 and N3b among 27.8, 42.1, 22.6, 0.8, 5.3 and 1.5% respectively.

Table 8: Proportion Of Cases Based On Clinical Metastatic Stage

Clinical metastatic stages	Frequency	Percentage
M1	1	0.8
M0	132	99.2
Total	133	100.0



Graph 8: Proportion Of Cases Based On Clinical Metastatic Stage

Among all the cases in this present study 0.8% of the patients were in M1 clinical metastatic stage while the rest 99.2% of the patients were in M0 clinical metastatic stage.

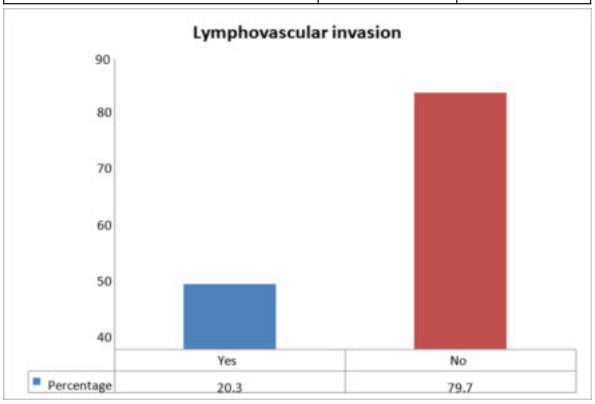
Table 9: Histo-pathological Findings Among The Study Participants With Oral SCC

Histo-pathological findings	Frequency	Percentage
Well differentiated SCC	41	30.8
Moderately differentiated SCC	87	65.4
Poorly differentiated SCC	05	3.8
Total	133	100.0

Based on the Histo-pathological findings 65.4% of the cases had moderately differentiated SCC which was found to be predominant in our study, 30.8% of the cases had well differentiated SCC and 3.8% of the patients were found with poorly differentiated SCC.

Table 10: Proportion Of Cases With Lympho Vascular Invasion

Lymphovascular invasion	Frequency	Percentage
Yes	27	20.3
No	106	79.7
Total	133	100.0

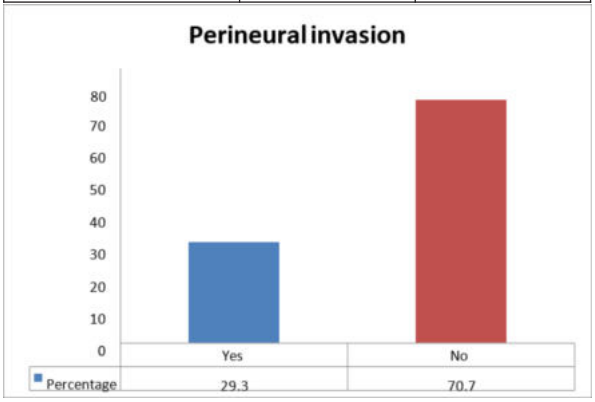


Graph 10: Proportion Of Cases With Lympho Vascular Invasion

In this present study Lympho vascular invasion was noted among 20.3% of the subjects with oral SCC.

Table 11: Perineural Invasion Among The Study Participants

Perineural invasion	Frequency	Percentage
Yes	39	29.3
No	94	70.7
Total	133	100.0



Graph 11: Perineural Invasion Among The Study Participants

Likewise perineural invasion was seen among 29.3% of the subjects with oral SCC while 70.7% of the cases had no perineural invasion.

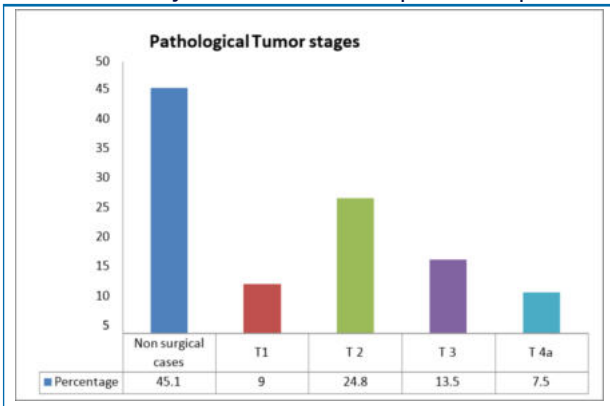
Table 12: Mode Of Treatment For Oral SCC

Treatment given	Frequency	Percentage
Surgical	73	54.9
CTRT	60	45.1
Total	133	100.0

In this study 54.9% of the cases were surgically treated whereas 45.1% of the cases received CTRT.

Table 13: Proportion Of Participants Based On Pathological Tumor Stage

Pathological Tumor stages	Frequency	Percentage
Non-surgical cases	60	45.1
T1	12	9.0
T2	33	24.8
T3	18	13.5
T4a	10	7.5
Total	133	100.0

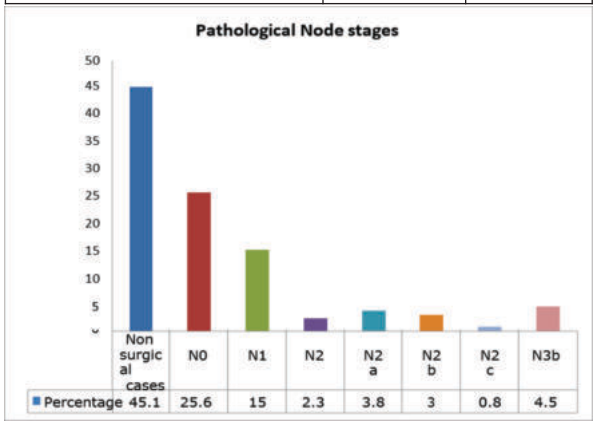


Graph 13: Proportion Of Participants Based On Pathological Tumor Stage

On assessing the Pathological Tumor stage 45.1% of the cases were found to be non-surgical cases, 24.8% of the cases were in T2 stage, 13.5% of the cases were in T3 stage while 9% and 7.5% of the cases were under T1 and T4a pathological tumor stage respectively.

Table 14: Proportion Of Participants Based On Pathological Node Stage

Pathological Node stages	Frequency	Percentage
Non surgical cases	60	45.1
N0	34	25.6
N1	20	15.0
N2	3	2.3
N2a	5	3.8
N2b	4	3.0
N2c	1	0.8
N3b	6	4.5
Total	133	100.0



Graph 14: Proportion Of Participants Based On Pathological Node Stage

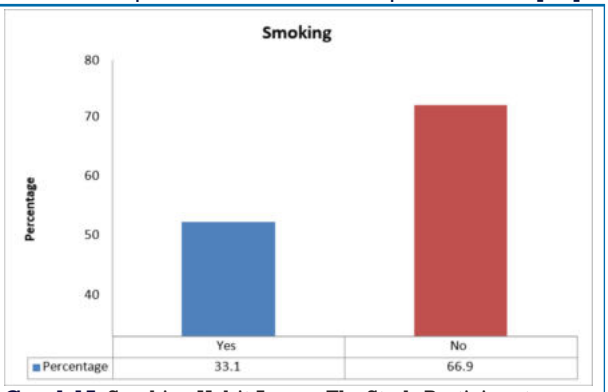
In this present study 45.1%, 25.6%, 15.0%, 2.3%, 3.8%, 3.0%, 0.8% and 4.5% of the cases had non-surgical, N0, N1, N2, N2a, N2b, N2c and N3b nodal pathological stage respectively.

Table 15: Smoking Habit Among The Study Participants

Smoking	Frequency	Percentage
Yes	44	33.1
No	89	66.9
Total	133	100.0

Table 16: Tobacco Chewing Among The Study Participants

Tobacco chewing	Frequency	Percentage
Yes	108	81.2
No	25	18.8
Total	133	100.0



Graph 15: Smoking Habit Among The Study Participants

In this study 81.2% of the patients with oral SCC were found to be tobacco chewers while 18.8% of the cases were non tobacco user.

Table 17: Alcohol Consumption Among The Participants

Alcohol consumption	Frequency	Percentage
Yes	12	9.0
No	121	91.0
Total	133	100.0

In this current study 9% of the cases gave history of alcohol consumption.

Table 18: Proportion Of Participants Based On P16 Expression

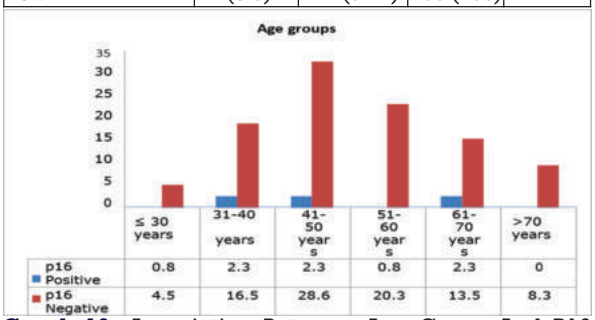
p16	Frequency	Percentage
Positive	11	8.3
Negative	122	91.7
Total	133	100.0

On assessing the p16 surrogate marker 8.3% of the study subjects were found to be positive for p16 while 91.7% of the cases were negative.

In this study p16 marker was found to be positive among 0.8% of the cases below 30 years of age, 2.3% of the cases between 31-40 years of age and 2.3% of the cases between 41-50 years of age. In the age range of 51-60 years, 61-70 years there were 0.8% and 2.3% cases with p16 positive respectively, but there was no significant statistical association noted between age group and p16 marker with p value of 0.601.

Table 19: Association Between Age Group And p16 Expression Among The Participants

Age groups	p16 Positive	p16 Negative	Total	P value
≤ 30 years	1 (0.8)	6 (4.5)	7 (5.3)	0.601
31-40 years	3 (2.3)	22 (16.5)	25 (18.8)	
41-50 years	3 (2.3)	38 (28.6)	41 (30.8)	
51-60 years	1 (0.8)	27 (20.3)	28 (21.1)	
61-70 years	3 (2.3)	18 (13.5)	21 (15.8)	
>70 years	0 (0.0)	11 (8.3)	11 (8.3)	
Total	11 (8.3)	122 (91.7)	133 (100)	



Graph 19: Association Between Age Group And P16 Expression Among The Participants

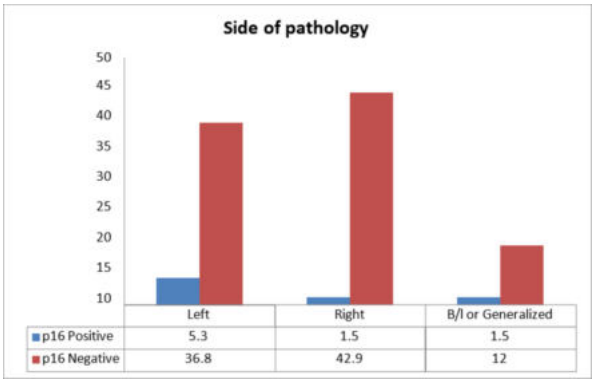
Table 20: Association Between Gender And p16 Marker Among Oral SCC Cases

Sex	p16 Positive	p16 Negative	Total	P value
Female	3 (2.3)	34 (25.6)	37 (27.8)	0.966
Male	8 (6.0)	88 (66.2)	96 (72.2)	
Total	11 (8.3)	122 (91.7)	133 (100.0)	

Among 27.8% of the female participants 2.3% were found with p16 positive marker while among 72.2% of the male participants 6% of them had positive p16 marker. The association for sex of the patient and p 16 marker was insignificant (p value =0.966).

Table 21: Association Between Side Of Pathology And p16 Expression Among The Participants

Side of pathology	p16 Positive	p16 Negative	Total	P value
Left	7 (5.3)	49 (36.8)	56 (42.1)	0.186
Right	2 (1.5)	57(42.9)	59 (44.4)	
B/l or Generalized	2 (1.5)	16 (12.0)	18 (13.5)	
Total	11 (8.3)	122 (91.7)	133 (100.0)	



Based on the side of pathology 5.3%, 1.5% and another 1.5% of the cases with left, right and bilateral or generalized lesion were found to have positive p16 marker but there was no statistical significant association between side of pathology and p 16 expression in our study (p value =0.186).

Table 22: Association Between Site Of Pathology And p16 Marker Among Study Participants

Site of Pathology	p16 Positive	p16 Negative	Total	P value
Buccal Mucosa	4 (3.0)	56 (42.1)	60 (45.1)	0.848
Tongue	5 (3.8)	51 (38.3)	56 (42.1)	
Lower alveolous	2 (1.5)	8 (6.0)	10 (7.5)	
Hard palate	0 (0.0)	1 (0.8)	1 (0.8)	
Floor of mouth	0 (0.0)	1 (0.8)	1 (0.8)	
Gingivobuccal sulcus	0 (0.0)	2 (1.5)	2 (1.5)	
Retromolar trigone	0 (0.0)	3 (2.3)	3 (2.3)	
Total	11 (8.3)	122 (91.7)	133 (100.0)	

P16 marker was found to be positive among 3% of the cases with buccal mucosa pathology, 3.8% of the cases with pathology in the tongue and 1.5% of the cases with lesion in the lower alveolous. There was no significant association found for site of pathology vs p16 expression (p value=0.848).

Table 23: Recurrence Of SCC vs p16 Markeramong The Cases

Recurrence	p16 Positive	p16 Negative	Total	P value
Yes	0 (0.0)	11 (8.3)	11 (8.3)	0.298
No	11(8.3)	111 (83.5)	122 (91.7)	
Total	11 (8.3)	122 (91.7)	133 (100.0)	

In this present study 8.3% of the cases were positive for p16 marker among 91.7% cases without recurrence of oral SCC, but the difference was not statistically significant with p value noted as 0.298.

Table 24: Clinical Tumor Stages Vs P16 Expression Among The Cases

Clinical Tumor stages	p16 Positive	p16 Negative	Total	P value
T1	0 (0.0)	7 (5.3)	7 (5.3)	0.945
T2	3 (2.3)	33 (24.8)	36 (27.1)	
T3	2 (1.5)	24 (18.0)	26 (19.5)	
T4a	5 (3.8)	49 (36.8)	54 (40.6)	
T4b	1 (0.8)	9 (6.8)	10 (7.5)	
Total	11 (8.3)	122 (91.7)	133 (100.0)	

Among 5.3% of the cases with T1 clinical staging no cases was found to be positive for p16 whereas among 27.1% of the cases with T2 staging 2.3% of the cases had p16 positive expression among 19.5% cases with T3 staging 1.5% cases had positive p16 marker. With T4a and T4b stage 3.8% and 0.8% of the cases were positive for p16 marker respectively. The p value was found to be statistically insignificant which indicates there is no significant association for p16 expression based on the clinical tumor stage.

Table 25: Clinical Node Stages Vs p16 Marker Among The Cases

Clinical Node stages	p16 Positive	p16 Negative	Total	P value
N0	2 (1.5)	35 (26.3)	37 (27.8)	0.491
N1	3 (2.3)	53 (39.8)	56 (42.1)	
N2	5 (3.8)	25 (18.8)	30 (22.6)	
N2b	0 (0.0)	1 (0.8)	1 (0.8)	
N3	1 (0.8)	6 (4.5)	7 (5.3)	
N3b	0 (0.0)	2 (1.5)	2 (1.5)	
Total	11 (8.3)	122 (91.7)	133 (100.0)	

Likewise among Clinical Node stage N0, N1, N2, N3 cases 1.5%, 2.3%, 3.8% and 0.8% of the cases were positive for p16 marker respectively in our study. The association between p16 and the clinical nodal stage was also found to be insignificant statistically (p value=0.491).

Table 26: Association Between Histopathological Finding And P16 Expression Among Cases

Histopathological findings	p16 Positive	p16 Negative	Total	P value
Well differentiated SCC	4 (3.0)	37 (27.8)	41 (30.8)	0.0437*
Moderately differentiated SCC	6 (4.5)	81 (60.9)	87 (65.4)	
Poorly differentiated SCC	1 (0.8)	4 (3.0)	5 (3.8)	
Total	11 (8.3)	122 (91.7)	133 (100)	

*Significant

Among 30.8% of the cases with well-differentiated SCC 3% of the cases were p16 positive while among 65.4% of the cases with Moderately differentiated SCC 4.5% of the cases were p16 positive and among 3.8% of the cases with poorly differentiated SCC 0.8% of them had p16 expression positivity and the association was found to be statistically significant (p=0.0437).

Table 27: Lymphovascular Invasionvs P16 Marker Among The Participants

Lymphovascular invasion	p16 Positive	p16 Negative	Total	P value
Yes	2 (1.5)	25 (18.8)	27 (20.3)	0.855
No	9 (6.8)	97 (72.9)	106 (79.7)	
Total	11 (8.3)	122 (91.7)	133 (100.0)	

Lympho vascular invasion was found to be present in 20.3% of

the participants among whom 1.5% were positive for p16 marker, but there was no significant difference recorded for p16 expression and Lympho vascular invasion (p value =0.855).

Table 28: Perineural Invasion Vs p16 Expression Among The Study Subjects

Perineural invasion	p16 Positive	p16 Negative	Total	P value
Yes	4 (3.0)	35 (26.3)	39 (29.3)	0.592
No	7 (5.3)	87 (65.4)	94 (70.7)	
Total	11 (8.3)	122 (91.7)	133 (100.0)	

The difference between perineural invasion and the p16 expression was statistically insignificant and the p value was noted to be 0.592.

Table 29: Treatment Modality Vs p16 Expression Among Cases With Oral SCC

Treatment	p16 Positive	p16 Negative	Total	P value
Surgical	6 (4.5)	67 (50.4)	73 (54.9)	0.981
CTRT	5 (3.8)	55 (41.4)	60 (45.1)	
Total	11 (8.3)	122 (91.7)	133 (100.0)	

P16 marker was positive for 4.5% of the patients who underwent surgical treatment and 3.8% of the cases who underwent CTRT, but the difference was insignificant with p value 0.981.

Table 30: Smoking vs p16 Marker Among The Participants

Smoking	p16 Positive	p16 Negative	Total	P value
Yes	2 (1.5)	42 (31.6)	44 (33.1)	0.273
No	9 (6.8)	80 (60.2)	89 (66.9)	
Total	11 (8.3)	122 (91.7)	133 (100.0)	

In this current study among 33.1% of the smokers 1.5% cases was p16 marker positive while among 66.9% of the cases 6.8% were p16 marker positive. The difference in smokers and non-smokers for p16 marker was not statistically significant (p value =0.273).

Table 31: Tobacco Consumption Vs p16 Marker Among The Participants

Tobacco chewing	p16 Positive	p16 Negative	Total	P value
Yes	9 (6.8)	99 (74.4)	108 (81.2)	0.957
No	2 (1.5)	23 (17.3)	25 (18.8)	
Total	11 (8.3)	122 (91.7)	133 (100.0)	

Tobacco consumption was recorded among 81.2% of the cases in our study among whom 6.8% of the cases had p16 marker positive whereas among 18.8% of the cases who were non tobacco users 1.5% was p16 marker positive. The difference was statistically insignificant for p 16 expression among tobacco chewers and non-tobacco users (p value =0.957).

Table 32: Alcohol Consumption vs p16 Marker Among The Patients With Oral SCC

Alcoholconsumption	p16 Positive	p16 Negative	Total	P value
Yes	0 (0.0)	12 (9.0)	12 (9.0)	0.275
No	11 (8.3)	110 (82.7)	121 (91.0)	
Total	11 (8.3)	122 (91.7)	133 (100.0)	

In this study among 9% of the alcohol consumers no case was found to be positive for p16 marker, but the difference between alcohol consumers and non- alcoholics was not proved to be statistically significant with p value of 0.275.

Table 33: Difference in mean age among the study participants

Variable	p16 Positive	p16 Negative	P value
Mean Age (in years)	47.55±13.4	51.34±14.4	0.404

The mean age among p16 positive cases was 47.55±13.4 years and the mean age of p16 negative cases was 51.34±14.4 years. The difference between mean age among p16 positive and negative cases was not significant (p value=0.404).

Table 34: Difference In Mean Depth Of Invasion Among The Study Participants

Variable	p16 Positive	p16 Negative	P value
Mean depth of invasion (mm)	7.00±1.0	7.25±5.9	0.919

The mean depth of invasion among p16 positive cases was 7.00±1.0 mm whereas the mean depth of invasion among p16 negative cases was7.25±5.9 mm. The p value was found to be 0.919 and the difference in mean depth of invasion between p 16 positive and negative cases were statistically insignificant.

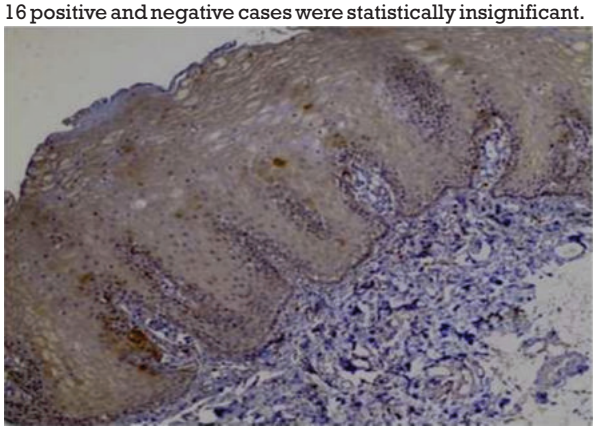


Figure 1: IHC – p16 Expression With Severe Dysplasia

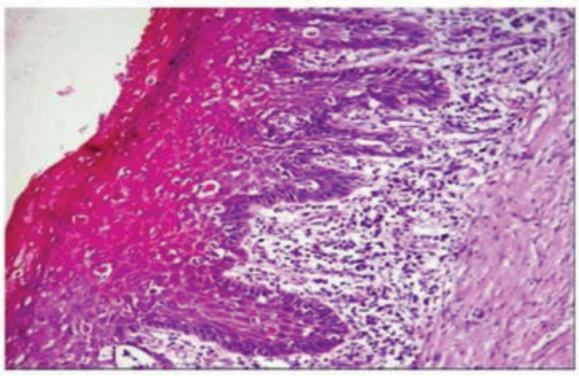


Figure 2: H&E – p16 Expression With Severe Dysplasia

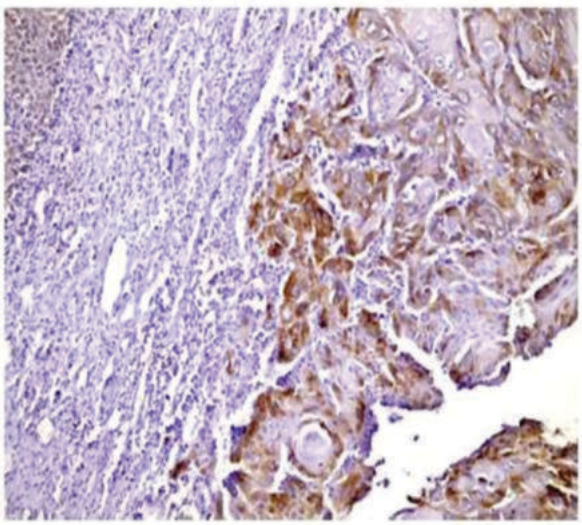


Figure 3: IHC – p16 expression of well differentiated oral SCC

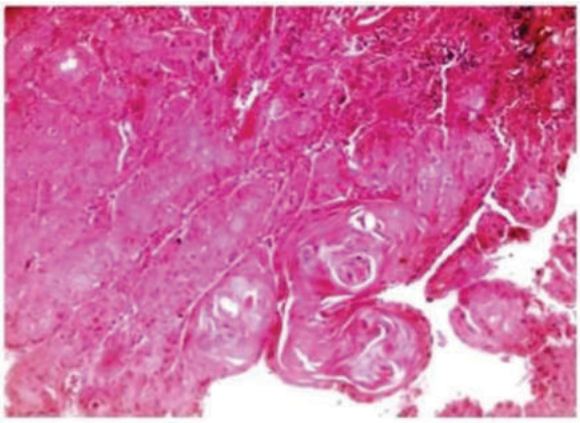


Figure 4: H&E – p16 Expression Of Well Differentiated Oral SCC

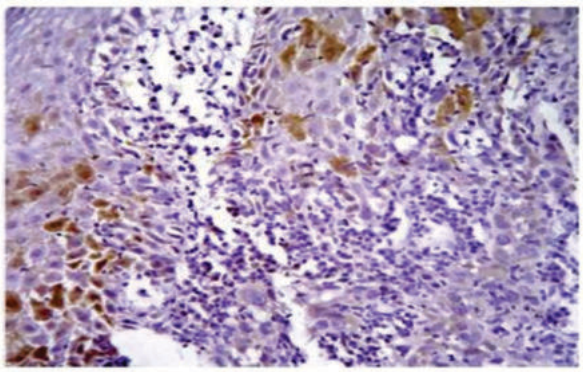


Figure 5: IHC – p16 Expression Of Moderately Differentiated Oral SCC

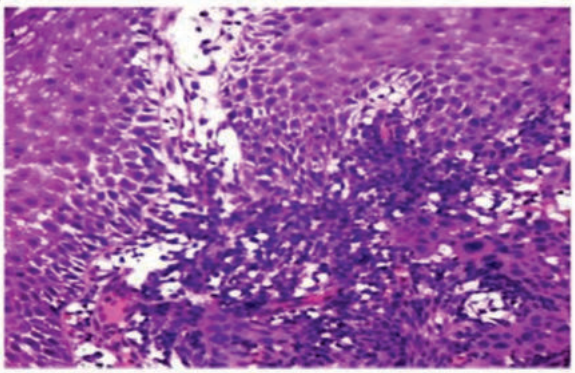


Figure 6: H&E – p16 Expression Of Moderately Differentiated Oral SCC

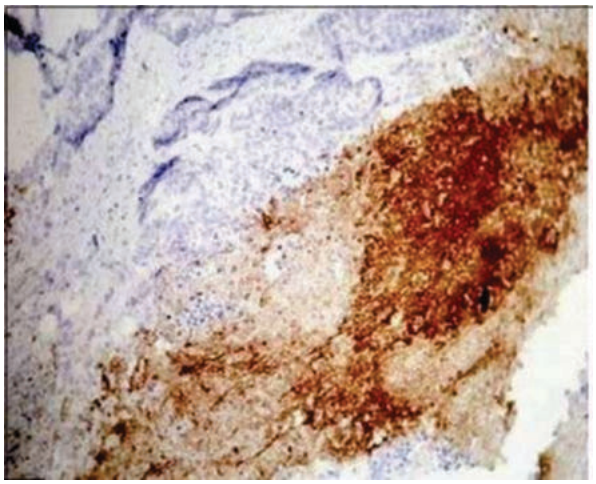


Figure 7: IHC – p16 Expression Of Poorly Differentiated Oral SCC

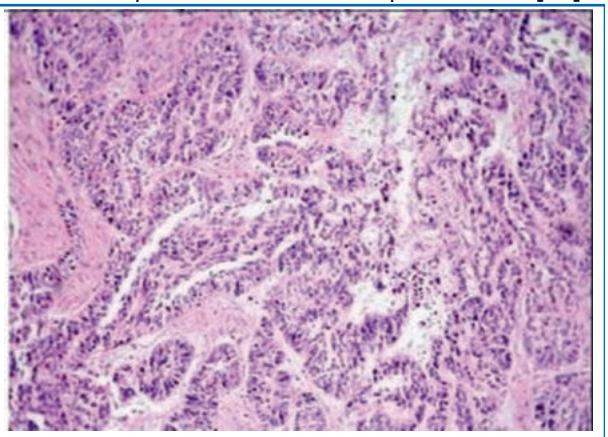


Figure 8: H&E – p16 Expression Of Poorly Differentiated Oral SCC

DISCUSSION:

In this study to evaluate the p16 expression in oral squamous cell carcinoma there were 5.3% of the participants below 30 years of age, 18.8% of the participants between 31-40 years of age and 30.8% of the cases were in the age group of 41-50 years. In the age range of 51-60 years, 61-70 years and above 70 years of age the proportion of study participants were 21.1%, 15.8% and 8.3% respectively. Among the total 133 cases maximum of 72.2% of the cases were males and 27.8% of the cases were females in this study.

The most common side of oral pathology was found to be on right side (44.4%) while on left side the pathology was seen among 42.1% of the cases. Bilateral or generalized lesion was noted among 13.5% patients. Regarding the site of oral lesion the most common site was Buccal mucosa (45.1%) followed by Tongue (42.1%), Lower alveolous (7.5%) and Retromolar trigone (2.3%). However Gingivobuccal sulcus, hard palate and Floor of mouth were the site of lesion for 1.5%, 0.8% and 0.8% of the cases with oral squamous cell carcinoma. In this current study recurrence of carcinoma was recorded among 8.3% of the cases after treatment.

There were 40.6%, 27.1%, 19.5%. 7.5% and 5.3% of the cases in the clinical tumor stage T 4a, T 2, T 3, T 4b and T1 respectively in this current study. Likewise the Clinical Node stage among the study subjects was noted to be N0, N1, N2, N2b, N3 and N3b among 27.8, 42.1, 22.6, 0.8, 5.3 and 1.5% respectively. Among all the cases in this present study 0.8% of the patients were in M1 clinical metastatic stage while the rest 99.2% of the patients were in M0 clinical metastatic stage.

Based on the Histo-pathological findings 65.4% of the cases had moderately differentiated SCC which was found to be predominant in our study, 30.8% of the cases had well differentiated SCC and 3.8% of the patients were found with poorly differentiated SCC. In this present study Lympho vascular invasion was noted among 20.3% of the subjects with oral SCC. Likewise perineural invasion was seen among 29.3% of the subjects with oral SCC while 70.7% of the cases had no perineural invasion.

On assessing the Pathological Tumor stage 45.1% of the cases were found to be non-surgical cases, 24.8% of the cases were in T2 stage, 13.5% of the cases were in T3 stage while 9% and 7.5% of the cases were under T1 and T4a pathological tumor stage respectively. In this present study 45.1%, 25.6%, 15.0%, 2.3%, 3.8%, 3.0%, 0.8% and 4.5% of the cases had non-surgical, N0, N1, N2, N2a, N2b, N2c and N3b nodal pathological stage respectively.

Smoking habit was recorded among 33.1% of the total participants in our study. In this study 81.2% of the patients with oral SCC were found to be tobacco chewers while 18.8%

of the cases were non tobacco users. In this current study 9% of the cases gave history of alcohol consumption.

On assessing the p16 surrogate marker 8.3% of the study subjects were found to be positive for p16 while 91.7% of the cases were negative. In this study p16 marker was found to be positive among 0.8% of the cases below 30 years of age, 2.3% of the cases between 31-40 years of age and 2.3% of the cases between 41-50 years of age. In the age range of 51-60 years, 61-70 years there were 0.8% and 2.3% cases with p16 positive respectively, but there was no significant statistical association noted between age group and p16 marker.

Among 27.8% of the female participants 2.3% were found with p16 positive marker while among 72.2% of the male participants 6% of them had positive p16 marker. The association for sex of the patient and p 16 marker was insignificant. Based on the side of pathology 5.3%, 1.5% and another 1.5% of the cases with left, right and bilateral or generalized lesion were found to have positive p16 marker but there was no statistical significant association between side of pathology and p 16 expression in our study.

P16 marker was found to be positive among 3% of the cases with buccal mucosa pathology, 3.8% of the cases with pathology in the tongue and 1.5% of the cases with lesion in the lower alveolous. There was no significant association found for site of pathology vs p16 expression. In this present study 8.3% of the cases were positive for p16 marker among 91.7% cases without recurrence of oral SCC, but the difference was not statistically significant.

Among 5.3% of the cases with T1 clinical staging no cases was found to be positive for p16 whereas among 27.1% of the cases with T2 staging 2.3% of the cases had p16 positive expression among 19.5% cases with T3 staging 1.5% cases had positive p16 marker. With T4a and T4b stage 3.8% and 0.8% of the cases were positive for p16 marker respectively. The p value was found to be statistically insignificant which indicates there is no significant association for p16 expression based on the clinical tumor stage. Likewise among Clinical Node stage N0, N1, N2, N3 cases 1.5%, 2.3%, 3.8% and 0.8% of the cases were positive for p16 marker respectively in our study. The association between p16 and the clinical nodal stage was also found to be insignificant statistically.

Among 30.8% of the cases with well-differentiated SCC 3% of the cases were p16 positive while among 65.4% of the cases with Moderately differentiated SCC 4.5% of the cases were p16 positive and among 3.8% of the cases with poorly differentiated SCC 0.8% of them had p16 expression positivity. The association was found to be statistically significant for histopathological finding and p16 expression. Lympho vascular invasion was found to be present in 20.3% of the participants among whom 1.5% were positive for p16 marker, but there was no significant difference recorded for p16 expression and Lympho vascular invasion. The difference between perineural invasion and the p16 expression was statistically insignificant.

P16 marker was positive for 4.5% of the patients who underwent surgical treatment and 3.8% of the cases who underwent CTRT, but the difference was insignificant. In this current study among 33.1% of the smokers 1.5% cases was p16 marker positive while among 66.9% of the cases 6.8% were p16 marker positive. The difference in smokers and non-smokers for p16 marker was not statistically significant.

Tobacco consumption was recorded among 81.2% of the cases in our study among whom 6.8% of the cases had p16 marker positive whereas among 18.8% of the cases who were non tobacco users 1.5% was p16 marker positive. The difference was statistically insignificant for p 16 expression among tobacco chewers and non-tobacco users. In this study among 9% of the alcohol consumers no case was found to be positive for p16 marker, but the difference between alcohol consumers and non- alcoholics was not proved to be statistically significant.

The mean age among p16 positive cases was 47.55±13.4 years and the mean age of p16 negative cases was 51.34±14.4 years. The difference between mean age among p16 positive and negative cases was not significant. The mean depth of invasion among p16 positive cases was 7.00±1.0 mm whereas the mean depth of invasion among p16 negative cases was7.25±5.9 mm. The p value was found to be 0.919 and the difference in mean depth of invasion between p 16 positive and negative cases were statistically insignificant.

Findings of this study were comparable with the findings of the following studies. Yuen PW et al[54] included 225 cases with SCC of head and neck. They stated that in 48 percent of the tumours, p16 expression was determined to be low. When comparing tumours of the larynx to those of the pharynx and oral cavity, tumours of the larynx had a higher frequency of decreased p16 expression, in their study. There was a link between lower p16 expression and a higher T stage. There was no link between p16 expression and gender, age, grade of the tumour, recurrence, metastasis, or prognosis in this study. They came to the conclusion that p16 expression was frequently down regulated in head and neck SCC. When compared to tumours of the oral cavity and throat, laryngeal tumours demonstrated a much higher prevalence of weak p16 expression. Also it didn't have any bearing on nodal metastases or survival, in their study.

Buajeeb W et al[56] assessed the p16 expression in oral SCC and premalignant lesions. They noted that P16 expression was found in 18.8% of OSCC patients, 26.7 percent of oral leukoplakia without dysplasia cases, and none of oral leukoplakia with dysplasia and normal mucosa cases, in their study. Oral SCC, oral leukoplakia with and without dysplasia, and normal mucosa had no significant changes in p16 expression prevalence. In oral SCC and oral leukoplakia without dysplasia, the percentages of positive cells were 0.89 and 0.17, respectively. There was no discernible difference in the percentage of positive keratinocytes, in their study. Ohta S et al[57] stated that p16 expression changes were found in 61.4 percent of oral SCC patients. The p16 gene promoter region was found to be methylated in 63.6 percent of the samples. In 9.0 percent of the samples, p16 gene alterations were found. These findings suggest that methylation, gene mutation, and allelic deletions frequently impact the status of p16 genes in oral SCC.

In another study, Fischer CA et al[59] assessed the expression of p16 on prognosis of treated oral SCC cases. They reported that the presence of p16 was found to be associated with the location of oropharyngeal tumours. Oral SCC patients who had p16 positive tumours had a considerably greater overall survival rate than those who had p16 negative tumours, in their study. They also discovered that the health benefit of individuals with p16 positive oral SCC was unaffected by clinic pathological characteristics such cT and cN categorization or treatment mode. After radiation and surgery, patients with p16 positive oral SCC have a better prognosis, in their study. Duncan LD et al[51] investigated p16 expression in HPV positive oral SCC cases. A total of 81 cases were included with 44 men and mean age 63.9 years. They discovered that 55.6 percent of the cases had no staining, 27.2 percent had 1+ staining, and 8.6 percent had 2+ staining. In addition, 8.6% of patients exhibited 3+ staining, with all of them testing positive for HPV serotype 16 by PCR. Three of the seven HPV PCR-positive patients displayed keratinization that was consistent with an oral cavity site rather than the basaloid development seen in HPV oropharyngeal tumours.

However, Grobe A et al[63] examined the positivity of p16 and

HP virus in oral carcinomas. They reported that p16 expression was found positive in 74% of tumours, although it was not significantly connected with tumour characteristics, but it was strongly correlated with recurrence free longevity of the cases if the tumours were primarily nuclear. However, neither the level of p16 expression nor the presence of HPV had any impact on these two factors. They came to the conclusion that IHC expression of p16 alone is a limited diagnostic and prognostic tool for oral carcinomas. IHC examination of p16 could be used as a prognostic indicator for HPV infection, depends on its intracellular location. Patil S et al[65] assessed the p16 positivity in cases with head and neck SCC and reported that in 86.7 percent of the cases, p16 positive was found. P16 staining was positive in 70% of well differentiated oral SCC, 90% of moderately differentiated oral SCC, and 100% in poorly differentiated oral SCC out of 26 positive cases, in their study. In addition, single dispersed cell staining was seen in well differentiated oral SCC, patchy staining in moderately differentiated oral SCC, and a more diffuse staining pattern in poorly differentiated oral SCC.

Salehinejad J et al[67] conducted a study and reported that among the cases with oral SCC, if more than 70% of tumour cells had brown nuclear and cytoplasmic staining, it was considered positive for p16. All of the oral SCC and control group samples tested negative for p16, whereas 26.7 percent of oral leukoplakia samples tested positive. Their findings show that p16 expression in oral leukoplakia samples might not be employed as a useful marker for detecting malignancy progression.

Satgunaseelan L et al[70] conducted a study and reported that p16 expression was found to be positive in 17.2% of cases with Oropharyngeal SCC however it was not associated with keratinisation's extend. Also in their study they stated that the positive p16 expression was found to be markedly reduced with increasing pathological tumor(T) staging and also with thickness of tumour, in their study. They concluded that positive p16 expression was reported markedly with early stage oropharyngeal SCC and it was not a reliable predictor of survival.

In another study, Gonzalez JCC et al[71] assessed the expression of p16 and p53 in cases with oral epithelial dysplasia (OED) and oral SCC. They reported that the highest frequency of p16-positive dysplasia was found in 35.5 percent, while p53 is linked to moderate dysplasia. In 47 percent of patients, moderately differentiated OSCC showed a higher frequency of p16 and p53-positive instances, in their study. There was a significant link between p16 and p53-positive cells in the OED basal stratum and p16-positive and p53-positive cells in the OSCC perivascular zone.

However, Parish PS et al[72] performed a systematic review and meta analysis and reported that there was a cases with p16-expressing malignancies have a much better prognosis. There is considerable evidence that patients with oropharyngeal SCC that expresses p16 have a better clinical result and prognosis. Ralli M et al[73] included 75 cases with oral SCC and they noted that P16 positivity was found in 78.7% of instances, whereas negativity was found in 21.3 percent. Nonsmokers and non-alcohol drinkers had greater levels of p16 expression, which was linked to paan chewing. There was no significant correlation with a history of abnormal sexual practices, but p16 expression was significantly found to correlate with multiple sexual partners, increasing histological grade, and lymph node metastasis in cases with multiple sex partners, increasing histological grade, and lymph node involvement. Satgunaseelan L et al[75] reported that among the cases with oral SCC, strong p16 expression was seen in 31.9 percent of patients, with 41 percent of non-metastatic tumours and 29.5 percent of metastatic tumours. p16 expression was found to be associated with size, depth of invasion, lymph node and vascular invasion, perineural invasion, keratinisation, node involvement, extranodal extension, and survival, but not with size, depth of invasion, lymph node invasion, perineural invasion, keratinisation, node involvement, extranodal extension, or survival. Positive p16 expression is found in nearly 32% of poorly differentiated head and neck SCCs. In areas where head and neck SCC is common, p16 positive neck nodal metastases should be considered a primary head and neck SCC. In head and neck SCC, p16 expression is just not linked to a better prognosis. Purwaningsih NMS et al[78] performed a study and in their study they included 32 cases with OPMDs, 46 cases with oropharyngeal SCC and 9 cases with normal oral mucosa. They reported that p16 were found to be positive in 96.7% of oropharyngeal SCC, in 84.4% of cases with oral potentially malignant disorders and none had positive p16 expression in cases with normal oral mucosa. Also they stated that the there was a markedly higher p16 positivity in oropharyngeal SCC when compared with the cases with normal oral mucosa. They concluded that p16 expressions were increased with the increasing degree of malignancy.

Also, Li P et al[87]stated that in 12.6 percent of those with SCC, p16 was positive. In patients with p16 negativity, the 5-year disease free overall survival were 52 percent, 39 percent, and 21 percent in cases with 0, 1–2, and 3–4 positive lymph nodes, respectively; in patients with 5 positive lymph nodes, all patients developed recurrence within two years after surgery, the variation was considerable; the 5-year disease specific survival rates were 60, 38, and 18 percent in patients with 0, 1–2, and 3–4 positive lymph nodes, respectively; in patients with 5 positive lymph nodes. The 3-year disease free overall survival in p16 positivity patients were 41 percent and 17 percent in patients with 0–2 and 3 positive lymph nodes, respectively, with a significant difference; the 3-year disease specific overall survival were 84 and 46 percent in patients with 0–2 and 3 positive lymph nodes, respectively, with a significant difference, in their study. Tokuzen N et al[88] in their study they reported that HPV mRNA expression was only discovered in 1% of patients, but positive p16expression was found in 10percent of cases, along with an HPV positive case. There were no strong relationship between p16 expression levels and clinic pathological characteristic variables. These findings revealed that oral SCC was less likely to be caused by HPV 16 infection, and that p16 expression was not a good diagnostic for HPV infection in oral SCC.

In an Indian study, Singh V et al[80] reported that in 19.4 percent of HPV positive patients and 12.9 percent of HPV negative cases, p16 was found. In all ten p16 positive instances, risk factors such as oral cigarette use and alcohol intake were present. HPV and p16 status had no effect on patient survival. Thambiah LJ et al[81] assessed the expression of p16 and p27 in oral SCC cases. They found that the both p16and P27 revealed lower intensity in oral SCC and high-grade potentially malignant diseases. They came to the conclusion that p16 and p27 can be employed as a predictive biomarker for epithelial dysplasia carcinogenesis.

Sudhakaran A et al[84] stated that from normal to oral submucosal fibrosis (OSF) to oral SCC with and without OSF, the percentage of p16 positive cells increased. In addition, a statistically significant change from nuclear to cytoplasmic expression from normal to OSCC was observed. Except for age and habits, no statistical significance was seen with any clinic pathologic factors. Wang H et al[85] in their study they reported that for identifying HPV infection in SCC in the head and neck region, IHC-p16 shows a good sensitivity and specificity, in their study. Brcic I et al[86] reported that Hematoxylin and eosin staining can be used to consistently measure tumour infiltrating lymphocyte density, with high concordance between small biopsy, resection specimen, and LN metastasis. Although the evaluation of p16 expression concordance is quite good, some cases may be misdiagnosed based on a tiny biopsy or lymph node metastases.

CONCLUSION:

In our study p16 marker was positive among 8.3% of cases with oral SCC. In our study age, gender, side and site of pathology, histopathological grade, recurrent disease, Clinical TNM staging, lymphovascular invasion, perineural invasion, treatment modalities, and addiction behaviors were not significantly associated with the positive expression of p16.

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