

“ASSOCIATION OF p16 EXPRESSION IN ORAL AND OROPHARYNGEAL SQUAMOUS CELL CARCINOMA”

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ABSTRACT

➤ Introduction:

The recent trend shows that there is an epidemiological shift in head and neck squamous cell carcinoma (HNSCC) attributable to HPV infection. HPV positive squamous cell carcinoma(SCC) has unique pathogenesis, risk factors, clinicopathological characteristics and outcome. There is a large variation in the published prevalence of the HPV related HNSCC in India ranging from 7 -78.7%.

➤ Aim:

“To study the association of p16 expression in oral and oropharyngeal squamous cell carcinoma.”

➤ Material and methods:

86 patients of oral and oropharyngeal squamous cell carcinoma were included in the study. H&E sections and immunohistochemistry was done using monoclonal mouse p16 antibody.

➤ Result:

Out of total 86 cases, 6 cases showed positivity for p16(7%). Out of which maximum patients were in age group of 31-40 years(50%) and all of them were males(100%). p16 positivity was found more in oropharyngeal region(67%) and it was Moderately differentiated SCC with maximum cases showing focal intensity for p16.

➤ Conclusion:

In our study, p16 positivity was found more commonly in younger age and at oropharyngeal region. Its prognosis is better than SCC of oral cavity.

Keywords: HNSCC-Head and neck squamous cell carcinoma, SCC-squamous cell carcinoma, HPV-Human papilloma virus, p16.

Introduction:

Squamous cell carcinoma constitutes the most common type of cancer of oral cavity and oropharynx. It is considered to be amongst the leading malignancies worldwide, with an overall incidence of 16.1 adults per 100,000, and along with it there is marked geographic variation in its distribution. Oral cancer is among the top three types of cancers in India and 90-95% of the oral cancer is squamous cell carcinoma.¹

There are various etiologies of oral squamous cell carcinoma. There is a strong association between oral cancer and risk factors like cigarette smoking, alcohol consumption, tobacco chewing, betel nut chewing with and without tobacco. Recent studies have shown that there is

an association of Human Papilloma virus gene (HPV) with premalignant as well as malignant oral lesions².

Protein p16 is a cellular protein involved in cell cycle regulation. It is a surrogate marker identifying an oncogenic human papillomavirus (HPV) infection an inhibitor of cell cycle hence any deletion, mutation or hypermethylation of its gene results in loss or reduced expression of this protein. Other mechanism is that pRB gene is directly inactivated by E7 oncogene released by high-risk HPV³.

It has been observed in recent studies that the oral carcinoma caused by Human papilloma virus has better prognosis and a long term survival rate than those caused by tobacco and alcohol^{4,5}.

In this present study, we will correlate p16 expression in different histological grades of squamous cell carcinoma.

Aims & Objectives:

Aim:

“To study the association of p16 expression in oral and oropharyngeal squamous cell carcinoma.”

Objectives:

- To detect squamous cell carcinoma in oral cavity and oropharynx.
- Morphological changes and histological grading of squamous cell carcinoma.
- To analyse and compare p16 immunoeexpression in patients of oral and oropharyngeal squamous cell carcinoma.

Material and method:

Place of Study:

The present study named “Association of p16 expression in Oral and oropharyngeal squamous cell carcinoma” was carried out in department of Pathology on both the outpatients and inpatients, in tertiary care hospital after clearance from ethical committee.

Study Type:-

Descriptive study.

Study Period:-

The proposed study was done over a period of 18 months from November 2018 to April 2020. Study was conducted over a period of total 18 months.

Sample size:-

Total 86 cases which were diagnosed as squamous cell carcinoma of oral cavity and oropharyngeal in histopathology section.

Inclusion Criteria :-

- Both male and female having histopathological diagnosis of squamous cell carcinoma.
- Age-18-60 years of age.
- Currently not taking any medications. (e.g. chemotherapy)

Exclusion Criteria :-

- Patients with metastatic lesions.
- Patients previously or currently treated with chemotherapy and radiotherapy or both.
- Other malignancies or non-squamous lesions of the oral cavity and oropharynx.

Methods:-

- Sample of tissue or biopsy was received in Pathology department in 10% formalin with proper labelling like full name,age,sex,registration number along with completely filled histopathological form having proper clinical history and probable diagnosis.
- Gross examination of specimen was done including various parameters like shape,size,external surface of tissue.Thereafter,relevant tissue was taken and processed in tissue processor(STP 120-3)in our department.
- After processing of tissue embedding was done,blocks were prepared , tissue sections were taken and stained with hematoxyline and eosin stain.
- Microscopic examination was done and morphological classification was given.
- All 86 cases were subjected to immunohistochemical staining with Monoclonal Mouse Antihuman p16 of Diagnostic BioSystems, LOT NO:N226-QD and ready to use.
- The technique used was based on PAP (peroxidase anti-peroxidase) method.

Interpretation for p16 :-

- Immunostained sections were reviewed and a strong cytoplasmic staining was considered as positive reaction.Distribution of p16 positivity is interpreted as follows:

p16 expression:-

0- Negative (<1% cells positive)

Positive:-

Score 1- Sporadic (<5% cells positive)

Score 2- Focal (<25% cells positive)

Score 3- Diffuse (>25% cells positive)

RESULT:

Table 1: Relation of p16 expression in cases of SCC.

Result	No. of cases	Percentage(%)
Positive	6	7%
Negative	80	93%
Total no of cases	86	100

In the present study, positive p16 staining were found in 6 cases(7%) and were negative in 80 cases(93%).

Table 2: Relation of p16 expression amongst various age groups.

Age group (years)	No of cases			
	p16 positive	Percentage (%)	p16 negative	Percentage (%)
21-30	1	17	9	11.3
31-40	3	50	22	27.5
41-50	0	0	30	37.5
51-60	2	33	19	23.7
Total cases	06	100	80	100

In the present study, out of total 6 positive p16 patients, maximum patients were in age group of 31-40 years-3 patients(50%),2 patients were in age group of 51-60 years(33%) and one patients was in age group of 21-30 years(17%).No patients were found in age group of 41-50 years.

p16 negative patients occur maximum in age group of 41-50 years-30 patients (37.5%), followed by 22 patients in age group of 31-40 years (27.5%).

Gender	No of cases			
	p16 positive	Percentage(%)	p16 negative	Percentage(%)
Total no of males	06	100	55	69
Total no of females	00	00	25	31
Total cases	06	100	80	100

Table 3: Relation of p16 expression with gender.

In present study, total 6 positive p16 patients were found, and all of them were males(100%). Among p16 negative patients,55 were males (69%) and 25 were females(31%).

Table 4: Site distribution amongst p16 positive SCC cases.

Site	p16 positive		p16 negative	
	No of cases	Percentage(%)	No of cases	Percentage(%)
Tongue	0	0	26	32.5
Buccal mucosa	1	16	19	23.75
Base of tongue	4	67	7	8.75
Floor of mouth	0	0	10	12.5
Hard palate	0	0	7	8.75
Tonsil	1	17	3	3.75
Soft palate	0	0	3	3.75
Gingivobuccal sulcus	0	0	3	3.75
Lip	0	0	1	1.25
Cheek	0	0	1	1.25
Total no of cases	6	100	80	100

In present study, the most common site for p16 positive SCC cases was Base of tongue-4 cases(67%),followed by tonsil and buccal mucosa-1 case each(17% and 16% respectively).

In our study,total 6 cases were p16 positive , out of which 5 patients had no history of tobacco/betel nut chewing.So other risk factors of p16 were responsible for cancer, but the patients were not comfortable in sharing such details,hence we were not able to elicitate it.

1 case showed positivity for p16 and also had habit of tobacco/betel nut chewing.

Table 5: Relation of HPE diagnosis according to its site and p16 expression.

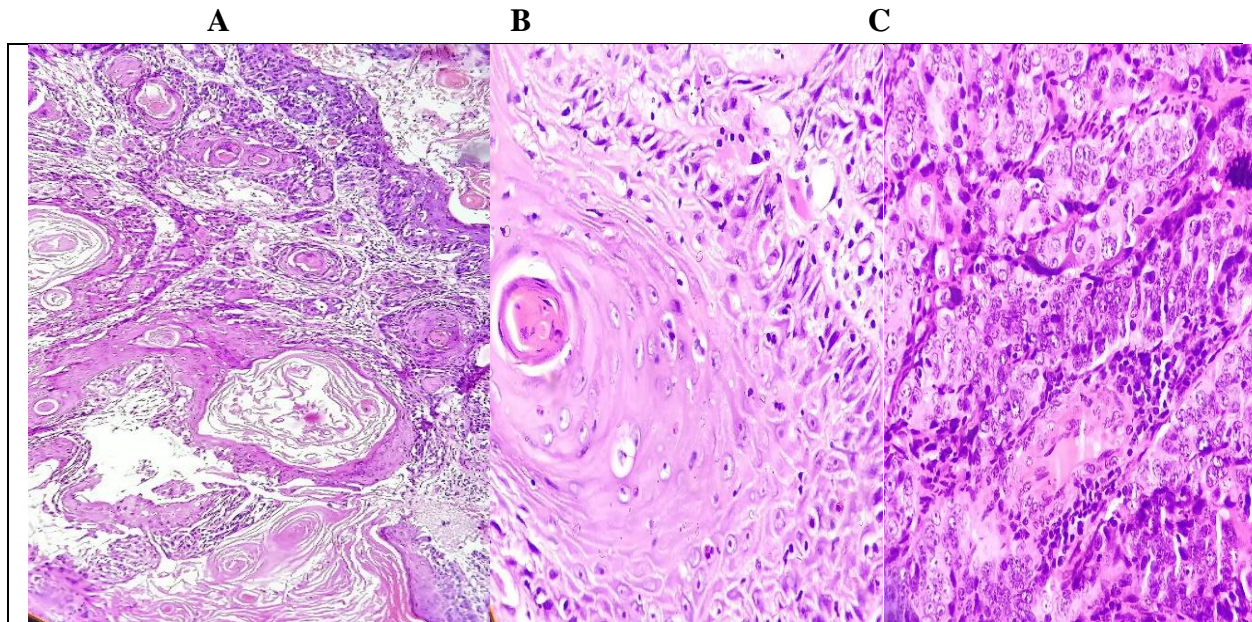
HPE diagnosis	Oral cavity(n=71)		Oropharynx(n=15)	
	p16 positive	p16 negative	p16 positive	p16 negative
Well differentiated	0	35	0	0
Moderately differentiated	1(Sporadic)	31	3(Focal and diffuse)	9
Poorly differentiated	0	4	2(Focal and diffuse)	1
Total cases	1	70	5	10

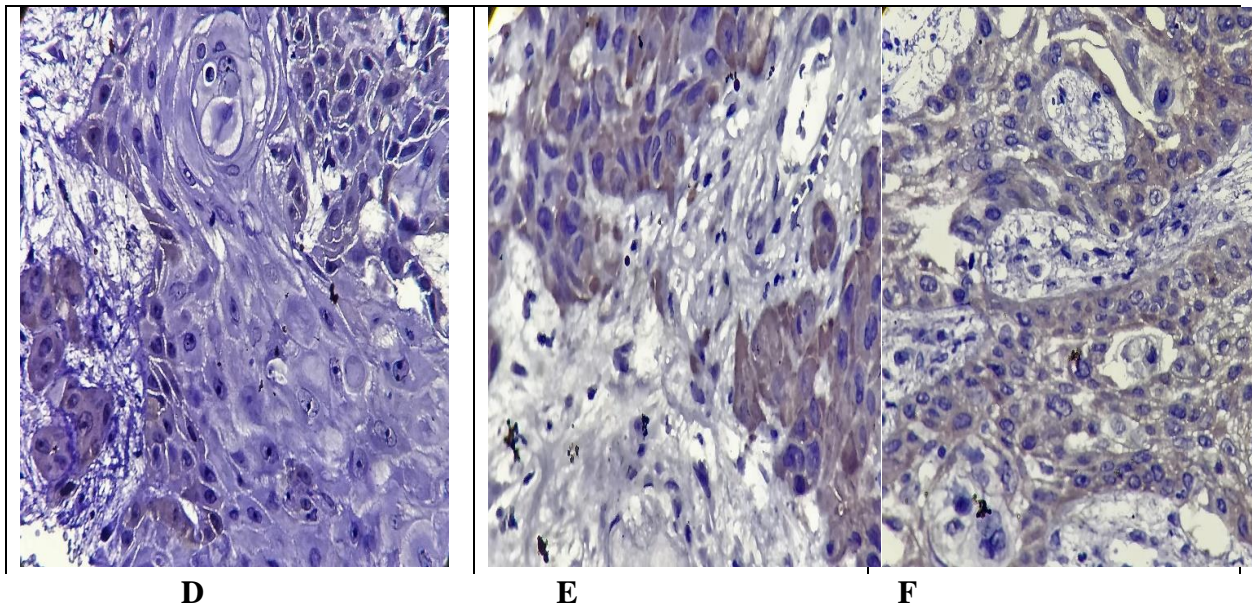
In our study, the most common site for p16 positive cases was Oropharynx-3 cases, and most common type was Moderately differentiated SCC, showing focal and diffuse positivity respectively.

Only one case showed p16 positivity in oral cavity.

In this study, the most common site for p16 negative cases were oral cavity-35 cases, and in that the most common diagnosis was Well differentiated SCC.

PHOTOS:





- A) Well differentiated SCC
 C) Poorly differentiated SCC
 E) SCORE 2

- B) Moderately differentiated SCC
 D) SCORE 1
 F) SCORE 3

DISCUSSION:

Squamous cell carcinoma of oral cavity and oropharynx is one of the most common cancer of head and neck. Now there is growing evidence to support that oral and oropharyngeal squamous cell carcinoma are two distinct diseases with different etiologies.

Tobacco and alcohol are the main etiological factors responsible for SCC. Recent studies have shown change in trend in oropharyngeal cancer, caused by HPV, occurs particularly in younger age group, and in absence of risk factors like tobacco and alcohol.

HPV positive SCC has different molecular biology, different risk factors like – history of multiple sexual partners, oral sex, higher socio-economic status, they also have different clinical presentation with different management of the tumour. The prognosis is better in HPV positive SCC than those associated with tobacco, alcohol and other risk factors.

Moreover in our country, the prevalence of HPV has not been extensively studied. We have tried to find any association of HPV as an etiological agent in our study for causing oral and oropharyngeal SCC.

In our study, maximum positive p16 cases were found in age group of 31-40 years in 50% of cases and in study conducted by Uppala et al⁶, maximum cases were found in age group of 40-49 years in 50% of cases. So in both the studies maximum patients were found in age less than 50 years. This showed young age group involvement.

However, in study conducted by Deng et al⁷, showed that maximum number of p16 positive cases occurred in age of more than 50 years-77% of cases. According to study conducted by Kalavathy Jayapal Elango et al⁸, out of total cases, 28% of patients belonging in age group of less than 45 years showed p16 positivity and 72% of patients of age more than 45 years showed p16 positivity.

In our study, maximum number of patients showing p16 positivity were males (100%), which was similar to study carried out by Murthy et al⁹-79.4% and Gul Kanyilmaz et al¹⁰ showing

82% positive cases amongst males. However, study carried out by Deng et al⁷ and Uppala et al⁶ showed maximum p16 positivity amongst females-81.1% and 66.67% respectively.

In our study, the most common site for positive p16 was Base of tongue in 67% cases - part of oropharynx, which is similar to study conducted by Murthy et al⁹ where the most common site was oropharynx in 58.8% cases.(But in its study tonsil was the most common site in oropharynx).Uppala et al⁶ also showed that the most common site to be base of tongue in 50% cases. Similarly, Deng et al⁷ also showed the most common site to be oropharynx in 66.7% cases.

In our study, the most common site for p16 positive cases was Oropharynx, and most common type was Moderately differentiated SCC. And in oropharynx, the most common site was base of tongue. The propensity for HPV to infect the base of tongue and tonsil is might be due to easy accessibility of the virus to the basal and proliferating squamous cells at this sites, possibly as a result of epithelial disruption¹¹.

In this study, the most common site for squamous cell carcinoma was tongue in oral cavity, and in that the most common type was Well differentiated SCC . This was due to the high consumption of tobacco/smoking and alcohol as most of the cases were p16 negative.

In our study, the most common HPE diagnosis in p16 positive cases was Moderately differentiated SCC in 67% cases, similar to the results shown by Deng et al⁷ showing 40% positive cases. However, study conducted by S.Patil et al¹² showed that maximum cases had showed positivity in Poorly differentiated SCC in 38.4% cases. And study conducted by Uppala et al⁶ showed equal incidence amongst all the three diagnosis.

In our study, among the positive p16 SCC cases, most (3.5% cases) were having focal pattern of p16 expression, followed by diffuse (2.3% cases) and sporadic (1.2% cases) pattern of expression. According to Pradyot Prakash et al¹³, diffuse pattern was the most common (31.9% cases), followed by sporadic (24.6% cases) and focal (14.5% cases) pattern of expression.

CONCLUSION:

In this study prevalence of p16 positivity is low (7%) as compared to west and other risk factors are high for aetiology of malignancy.Overall p16 positivity was seen only in 7% of cases and it was more common in oropharyngeal region, this may be due to less number of cases in our study, if the sample size was increased than more number of cases could be found in association with HPV.

p16 may be used as prognostic markers in squamous cell carcinoma for identifying the biological behaviour and outcome of HPV related SCC and could thus take into account for planning various therapeutic strategies. Further such type of studies are required which will be helpful to avail more data for the use and implementation of HPV vaccine in future. Thus, this study will be helpful both to the patients as well as to clinicians for management of squamous cell carcinoma in oral cavity and oropharynx.

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