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RESEARCH ARTICLE

STUDY OF ORAL SQUAMOUS CELL CARCINOMA IN CORRELATION WITH P16 IHC MARKER

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Abstract

Introduction: Oral squamous cell carcinoma consistently ranks as one of the top seven cancers world wide. P16 expression is now being used as surrogate marker of HPV infection in squamous cell carcinoma.

Aims: To study and correlate oral squamous cell carcinoma in indianpopulation with age, sex, habit of tobacco, anatomical site, histological grade and scoring of p16 positivity on IHC.

Material and methods:100 cases oforal squamous cell carcinomawere correlated withp16 IHC marker positivity with age, sex,habit of tobacco, anatomical site and grading of squamous cell carcinoma and scoring of p16 positivity on IHCbased on system by **M. Oliver et, al.**

Result: Out of 8 p16 expression positive cases: 3 cases were< 50 years of age while 5 cases observed in > 50 years of age. M:F ratio was 7:1.2 cases were tobacco chewers and 1 case was smoker, numbers of welldifferentiated, moderately and poorly differentiated cases were 2,4 and 2 respectively, Out of total 100 cases of oral squamous cell carcinoma 92cases with 0 score of p16 immunostaining,3 cases with 1+,2 cases with 2+ and 3cases with 3+ scores observed.

Introduction:-

Squmous cell carcinoma represents almost 95% of the head and neck cancers.[(1)]There are 4000,000 new cases each year and most of these cases have been reported from asiancountries like India, Pakistan, Shri-lanka, Bangldesh.[(2)]In India, oral cancer represent more than 50% of all malignancies.[(1)] It ranks first among male and the third among female population in India.[(3)] The most significant etiologic agent associated with the development of oral SCC is tobacco and alcohol consumption[(4)]The buccal mucosa is the most common site for OSCC, followed by anterior 2/3rd of tongue, lower gum, lip, hard palate, floor of mouth & upper gum.[(5)]Transcriptionally active HPV type 16 accounts for nearly all of the HPV-driven oral cavity squamous cell carcinomas with a minority caused by HPV types 18, 31, 33, and 35.[(6)]Tumor suppressor p16INK4A (p16), is one of the INK4 class member of CDK inhibitors. Mechanisms of inactivation include homozygous gene deletion, gene mutation and hypermethylation of upstream CPG regions.[(7)]P16 expression is being used as surrogate marker of HPV infection in squamous cell carcinoma. This may help in providing important prognostic information and future therapies aimed at targeting this pathway of HPV tumor genesis.[(7)]

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Aims:-

To study and correlate oral squamous cell carcinoma in Indianpopulation with age, Sex, habit of tobacco,anatomical site, histological grade and scoring of p16 positivity on IHC.

Material and Methods:-

In the present study, 100 cases oforal squamous cell carcinomawere correlated withp16 immunostainig.We had carried out immunohistochemistry for p16 marker by manual method on formalin fixed paraffine embedded tissue sections. The immunostaining for p16 was scored usingsystem by **M. Oliver et, al.** Further, the results obtained were analysed for various parameters like - age, sex,habit of tobacco, anatomical site and grading of Squamous cell carcinomaand their association with p16 immunostaing using chi-square test.A value of $P < 0.05$ was taken as significant.

Table I:- M. Oliver, et al.[8]system for staining.

Score	Staining
0	no staining
1+	moderate to strong staining in <25% of cells or weak staining to any extent
2+	moderate to strong staining in 25–50% of cells
3+	moderate to strong staining in >50% of cells

Inclusion criteria:-

All patients with oral squamous cell carcinoma undergoing biopsy at civil hospital,Ahmedabad.

Exclusion criteria:-

Inadequate biopsy material

Observations and Results:-

Table II:- Summary of p16 Positive cases.

Cases	Age/Sex	Habits	Site	Histological Grade	p16 IHC
Case 1	59/M	Tobacco Chewing	Tongue middle 1/3rd	Moderately differentiated	3+ Positive
Case 2	30/M	None	Left Buccal Mucosa	Well differentiated	3+ Positive
Case 3	52/M	None	Left Lateral Border Of Tongue	Moderately differentiated	3+ Positive
Case 4	65/M	Smoking	Right Buccal Mucosa	Poorly differentiated	2+ Positive
Case 5	52/M	Tobacco Chewing	Left Lateral Border Of Tongue	Moderately differentiated	2+ Positive
Case 6	60/F	None	Left Buccal Mucosa	Poorly differentiated	1+ Positive
Case 7	35/M	None	Right Buccal Mucosa	Well differentiated	1+ Positive
Case 8	40/M	None	Left Lateral Border Of Tongue	Moderately differentiated	1+ Positive

P16 Expression:

Out of 100 cases, 92 (92%) cases 0, 3 (3%) cases with 1+ positive, 2+ positive in 2 (2%) cases while 3+ positive in 3 (3%) cases.

Table III:- Correlation of p16 expression with clinical parameters and histologic grade.

Clinical & Histologic parameter	**3+ Score (No. of cases)**	**2 + Score (No. of cases)**	**1+ Score (No. of cases)**	**0 Score (No. of cases)**	**Total No. of Cases**	**P* value**
Age (Years)						
< 50 years	1	-	2	46	49	0.452
> 50 Years	2	2	1	46	51	
Total	3	2	3	92	100	
Sex						
Male	3	2	2	73	80	0.657
Female	-	-	1	19	20	
Total	3	2	3	92	100	
Habits						
Smoking	-	1	-	21	22	0.382
Tobacco chewing	1	1	-	30	32	
None	2	-	3	41	46	
Total	3	2	3	92	100	
Site						
Lower lip mucosa	-	-	-	2	2	0.964
Middle 1/3 of tongue	1	-	-	3	4	
Tip & Base of Tongue	-	-	-	2	2	
Right lateral border of tongue	-	-	-	20	20	
Left lateral border of tongue	1	1	1	25	28	
Right buccal mucosa	-	1	1	13	15	
Left buccal mucosa	1	-	1	11	13	
Hard palate	-	-	-	5	5	
Right gingivo-buccal sulcus	-	-	-	1	1	
Left gingivo-buccal sulcus	-	-	-	4	4	
Right Posterior Mandibular teeth	-	-	-	4	4	
Floor of the mouth	-	-	-	2	2	
Total	3	2	3	92	100	
Histological grade						
Well differentiated	1	-	1	10	12	0.393
Moderately differentiated	1	1	1	70	73	
Poorly differentiated	-	1	1	13	15	
Total	3	2	3	92	100	

Correlation of p16 expression with age, sex, habits , site of tumor and tumor grade (p value = 0.497, 0.580, 0.605, 0.689 and 0.296 respectively), were not found to be statistically significant.

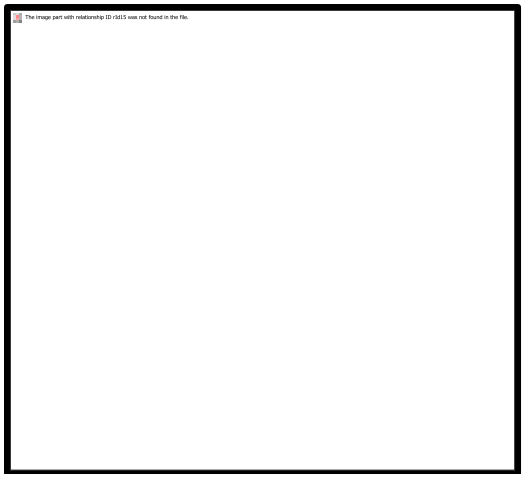

Figure I:- p16 Negative, Welldifferentiated oral squamous cell carcinoma(IHC,40x).

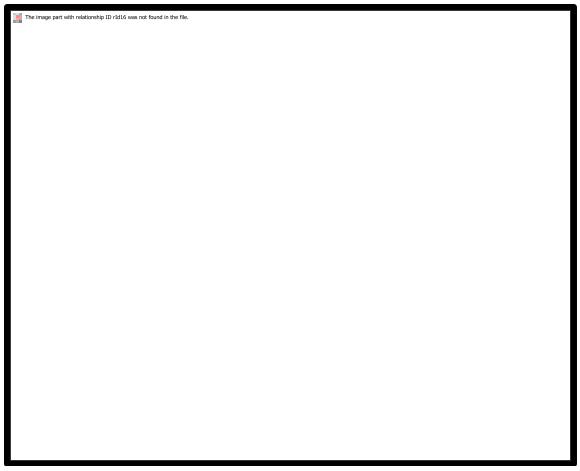

Figure II:- p16 1+ Score Moderately differentiated oral squamous cell carcinoma (IHC,20x).

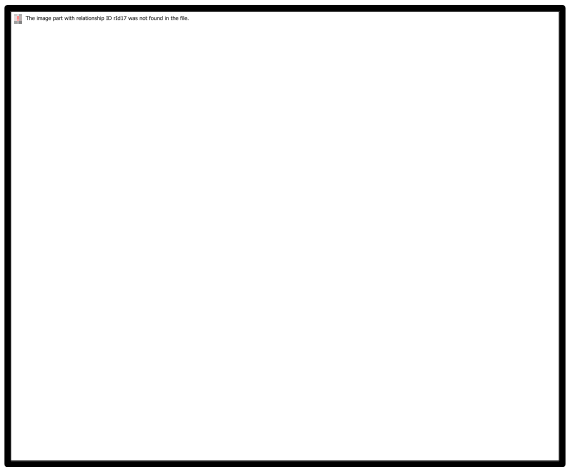

Figure III:- p16 2+Score Moderatelydifferentiated oral Squamous cell (IHC, 20x)

Figure IV:- p16 3+ Score, Welldifferentiatedoral squamous cell carcinoma(IHC,20x)

Discussion:-

In Present studytotal, 100 cases of the oral squamous cell carcinoma with p16 IHC marker expression were studied. Various clinical and histological parameters like, age, sex, habit of tobacco, grading of oral squamous cell carcinoma in Indianpopulation were correlated and compared with other similar studies.

Table IV:- Comparison Of p16 Staining Positivity with other study in correlation with Age.

Age	**p16 Staining Positivity (% of cases)**	
	In Ralli, et al.[7]Study	**In Present study**
< 50 years	47.5 %	37.5 %
> 50 years	52.5 %	62.5 %

In both the study,we observed that p16 positivity was more common in patients age >50 years and similar findings observed in study of Ralli, et al.[7]

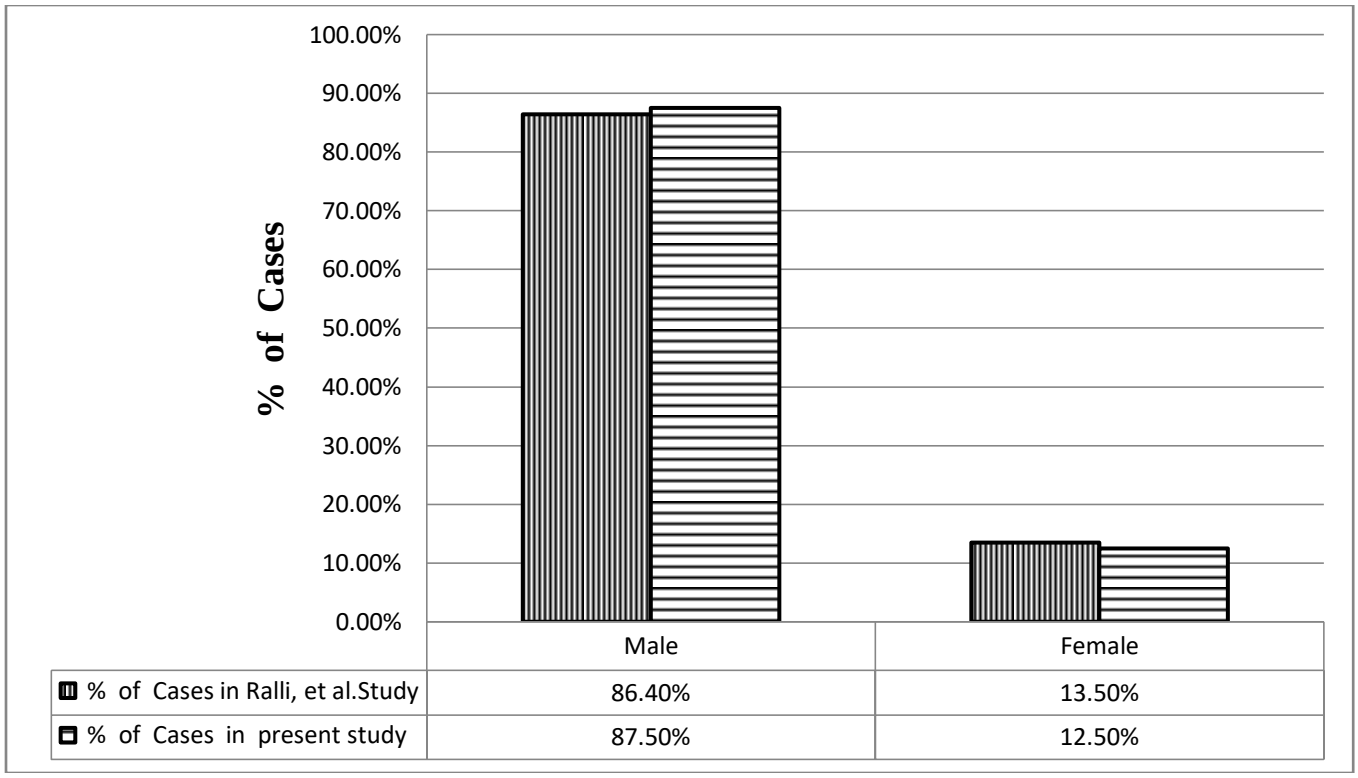

Figure V:- Comparison Of p16 Staining Positivity with other study in correlation with sex.

Above chart show that our observation for sex wise distribution of p16 IHC positivity in oral Squamous cell carcinoma were correlated with the study of Ralli,et al.[7]

Table V:- Comparison Of p16 Staining Positivity with other study in correlation with anatomical site.

Anatomical site	**p16 Staining Positivity (% of cases)**	
	In P. Prakash, et al.[9]Study	**In Present study**
Tongue	37.7 %	54%
Buccal mucosa	24.6 %	28 %
Cheek	18.8 %	0%
Alveolus	5.8 %	0%
Lip	2.9 %	2 %
Angle of mouth	2.9 %	4%
Soft palate	4.3 %	0%
Hard palate	1.45 %	5 %
Gingiva	1.45 %	5 %

In both the studies, tongue followed by buccal mucosa are comman anatomical sites.

HPV associated p16 positive oral squamous cell carcinoma were more frequent in oropharynx than oral cavity[10]. Though, in present study, HPV associated with squamous cell carcinoma was studied by p16 only in oral cavity; the incidence of p16 positive OSCC comparable with other studies. Limitation of this study were low sample volume and inclusion of only single anatomical site.

Table VI:- Comparison Of p16 Staining Positivity with other study in correlation with habits.

Habits	**p16 Staining Positivity (% of cases)**	
	In S. Gondha [11] Study	**In Present study**
Smoking	38 %	12.5 %
Tobacco chewing	18 %	25 %
None	44 %	62.5 %

Both the study show correlation of habit with oral squamous cell carcinoma and p16 positivity.

Table VII:- Comparison Of p16 Staining Positivity with other study in correlation with histological grade.

Histological Grade	p16 Staining Positivity (% of cases)	
	In P. saxena, et al[10] Study	In Present study
Well differentiated	29.5%	25 %
Moderately differentiated	56.8 %	50%
Poorly differentiated	13.7%	25%

Correlation of p-value with other studies

We had derived p value for our study and correlated with other studies for sex, site of tumor and habit. It wasconcordant with Murthy V et al.[12] and Babiker AY et al.[13]. For grade of the tumor p value was concordant with P. Saxena, et al[10],S.Gondha[11] andDr. Shalini G et al.[14]

In our study p16 positivity was low i.e. 8% cases. The low p16 positivity rate in the present study could probably be explained by the relatively small sample size.

Conclusion:-

p16 is a highly sensitive surrogate marker for the identification of HPV-driven tumors and this can be very well demonstrated by method of immunohistochemistry. P16 immunohistochemistry staining in oral Squamous cell carcinoma is scored and interpreted accordingly.

The p16 expression in Oral Squamous cell carcinomawith Age, Sex, Habits , Site of tumor and Tumor grade could be well correlated in the present study.

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