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RESEARCH ARTICLE

EXPRESSION OF p16 AND Ki-67 IN ORAL SQUAMOUS CELL CARCINOMA

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Abstract

Introduction: Oral Squamous Cell Carcinomais a major health challenge with high recurrence and poor prognosis.Asper Globocan 2022, lip and oral cancer ranked 16th in terms of incidence with 389,485(2%)newcases. p16 helps identify HPV infection and induces apoptosis, while Ki-67, a key cell cycle regulator, is crucial for assessing cell proliferation, tumor development and prognosis.

Aim: The study aims to evaluate the immunohistochemical expression of p16 and Ki-67 in oral squamous cell carcinoma and correlation with various clinicopathological parameters.

Material and Methods: This descriptive analysis was conducted at Muzaffarnagar Medical College & Hospital, involving 50 histologically confirmed oral squamous cell carcinoma cases. The study involved p16 and Ki-67 expression through immunohistochemistry on histologically diagnosed OSCC cases and scoring was done.

Results: The study found that most OSCC patients belong to fifth and sixth decades of life, with buccal mucosa being the most affected site. Tobacco use was prevalent among the patients. Most cases were moderately differentiated. p16 and Ki-67 expression werehigher in poorly differentiated cases. Positive p16 expression was observed in 38% of the cases but showed no significant correlation with age, gender, or histological grade. However, significant associations were found between age, histological grade, and Ki-67 expression.

Conclusion: This study concluded that p16 expression was positive in 38% of OSCC cases, with higher rates in poorly differentiated tumors, but no significant links to age, gender, or histological grade. Ki-67 levels, higher in poorly differentiated cases, were significantly associated with age, indicating more aggressive disease in older ones.

Introduction:-

Oral squamous cell carcinoma (OSCC) is a common type of oral cancer, accounting for more than 90% of all oral malignancies. The primary risk factor for OSCC is tobacco use, either smoking, chewing or both.[1]According to Globocan 2022, lip and oral cancer ranked 16thand 15thin terms of incidence and mortality respectively worldwide

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with389,485(2%)newcasesand88,230(1.9%) deaths.[2] OSCC is more common in patients with oral potentially malignant diseases (OPMDs) and can be detected through preclinical phase indicators such as leukoplakia and lichen planus.[3]Oral squamous cell carcinoma (OSCC) is a multifactorial disease- tobacco use, alcohol use, viral infections as HPV, dietary deficiencies vitaminA,C, andE, consumption of spicy food, sharp teeth, and genetic factors.[4]

Multiple IHC markers are used to predict the likelihood of recurrence and assist in treatmentplanning.HPV infection is one among various risk factors of OSCC, and p16 expression is used as a surrogate marker for detecting HPV. p16 proteins are involved in regulating the cell cycle, facilitating the elimination of cancerous cells, and preventing their proliferation by maintaining the retinoblastoma protein in a hypophosphorylated state, and its activation leads to cellular aging.[5] HPV E6 and E7oncoproteins activate tumor suppressor proteins- p53 and pRB, causing genomic instability and malignant transformation.[6]

Ki-67, a proliferation marker protein, acts from the G phase to the M phase of the cell cycle. It is a large non-histone protein present in the nucleus and nucleolar region, which is seen in cells undergoing proliferation.[7] The Ki-67 protein has been examined in precursor lesions of oral cavity and oral squamous cell carcinoma, and proliferating cells increased according to the grade of dysplasia and is correlated clinically and prognosis of OSCC.[8]

Aim:-

To evaluate the immunohistochemical expression of p16 and Ki-67 in histologically diagnosed oral squamous cell carcinoma.

Materialand Methods:-

The study was performed in the Department of Pathology, Muzaffarnagar Medical College, Muzaffarnagar.

The study design was a hospital record-based descriptive study, and the study population included all specimens of oral lesions diagnosed as squamous cell carcinoma on histopathology in the Department of Pathology at Muzaffarnagar Medical College. The study duration was 18 months, and fifty cases of OSCC were included.

The specimens were fixed in 10% buffered formalin for overnight and then grossed, processed, paraffin embedded, and stained with Hematoxylin and Eosin and histopathological examination was done. Tumors of OSCCweregradedbasedonmorphologicalparameters as Well Differentiated (WD), Moderately Differentiated (MD), and Poorly Differentiated (PD) OSCC. Immunohistochemistry for p16 and Ki-67 was done.

The expression of p16 and Ki-67 was scored based on the percentage of positive cells observed in 10 high-power fields. Malignant cells were scored for p16 cytoplasmic andnuclear staining, positive cells score of 1+(1%to25%), 2+ (26%to 50%), 3+(51% to 75%) and4+ (>75%).Scoreof0 for p16wastakenasnegative.[9]MalignantcellsscoredforKi-67nuclearstaining,positivecells werescore as 1+ (1%to 25%),2+ (26%to50%),3+(51%to75%)and4+ (>75%).[10]Scoreof1+ and 2+ for Ki-67wastakenasnegative.

The observations and results of the study were expressed in the form of tables and figures and were analyzed using statistical techniques.

Theappropriatestatisticaltestwas appliedusingMedCalcsoftware version 22.020and datawascollectedandallstatistical calculationwere performedusingMS-Excel2010. A p-value of less than .05 was deemed statistically significant.[11]

Result:-

The study included 50 patients aged 30 to 80 years, withmean age was 48.39 ± 13.13 years, over half being in their fifth decade (56%).Most of the cases were predominantly male with male-to-female ratio of 5.2:1. In the fifth decade, OSCC was more prevalent in both males as well as females. Common sites of OSCC were buccal mucosa (36.0%), tongue (28.0%), and gingiva (16.0%).**[Figure 1,Table 1]**

Out of total 50 cases, 72% were tobacco users and 28.0% were non-tobacco users. We classified the tobacco users into three distinct groups: Group I: Only tobacco chewers; Group II: Only smokers; and Group III: Both chewers

and smokers. Most of the patients were in Group III 52.8%, 30.5% were in Group II, and the rest 16.7% were in Group I.**[Figure 2]**

Out of 50 cases, 26 specimens were excisional biopsy and 24 were incisional biopsies. Grossly, most biopsies showed ulcero-proliferative growth 54%, followed by proliferative growth in 28% and ulcerative growth in 18%. We graded all the cases of OSCC histologically. 54% were moderately differentiated (MD), followed by 32% well-differentiated (WD), and the remaining 14% cases were poorly differentiated (PD) OSCC. **[Table 1]**

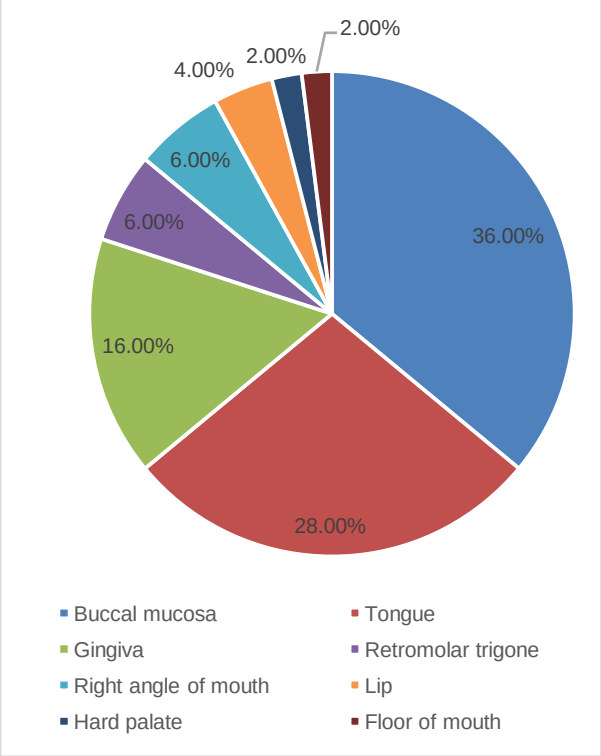

Figure 1:- Pie chart showing site wise distribution.

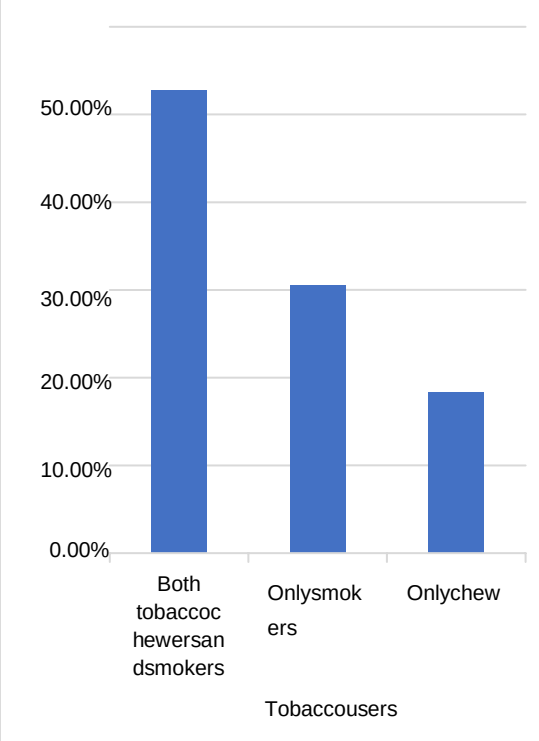

Figure 2:- Bar chart showing distribution of tobacco users.

Table 1:-Clinicopathological characteristics of 50 patients of histologically diagnosed OSCC.

Variables		**No & Percentage (%)**
Age		
≤50 years		36 (72%)
>50years		14 (28%)
Gender		
Male		42 (84%)
Female		08 (16%)
Site of the tumor		
Buccalmucosa	Leftbuccalmucosa (13)	18 (36%)
	Rightbuccalmucosa (5)	
Tongue	Left lat. Tongue (8)	14 (28%)
	Rightlat. Tongue (6)	
Gingiva	Leftuppergingiva (2)	8 (16%)
	Leftlowergingiva (3)	
	Rightlowergingiva (3)	
Retromolar trigone	Rightretromolar (2)	3 (6%)
	Leftretromolar (1)	

Right angle of mouth		3 (6%)
Lip	Upperlip (1)	2 (4%)
	Lowerlip (1)	
HardPalate		1 (2%)
Floorof mouth		1 (2%)
Histological grade		
Well-differentiated		16 (32%)
Moderately-differentiated		27 (54%)
Poorly-differentiated		07 (14%)
Total no. of cases		**50 (100%)**

Table 2:- Immunohistochemical expressionof p16 with clinical parameters.

Clinical &Histologicalparameter	**Number of cases positive forp16expressionand percentage (%)**				**Numberofcasesnegative for p16expression and percentage (%)**	**Total no.ofcases and percentage (%)**	**p-value**
	4+ score	**3+ Score**	**2+ score**	**1+ score**	**0+score**		
AGE							
≤50 years	2 (5.6)	3 (8.3)	3 (8.3)	4 (11.2)	24 (66.6)	**36**	**0.6801**
>50years	1 (7.2)	1 (7.2)	2 (14.2)	3 (21.4)	7 (50.0)	**14**	
GENDER							
Male	2 (4.8)	4 (9.5)	4 (9.5)	7 (16.6)	25 (59.6)	**42**	**0.6547**
Female	1 (12.5)	0	1 (12.5)	0	6 (75.0)	**8**	

The study examined the correlation of p16 and Ki-67 expression with clinical parameters (age and gender) and histologic grades.

The study found that p16 expression was positive in 38% of cases. Age was categorized into two age groups: ≤ 50 years and > 50 years. P16 was positive among (33.4%) of patients ≤ 50 years, while it was positive in half of patients with> 50 years [No significant statistical association]. Also, there was no significant statistical association between the gender of the patients in relation to P16 expression, which was positive among (40.4%) of the males compared to (25.0%) among females.**[Table 2]**

Table 3:-Correlation of Ki-67 expression with clinical parameters.

Clinical &Histologicalparameter	**Number of cases with Ki-67expression and percentage (%)**				**Total no.ofcases and percentage (%)**	**p-value**
	4+ Score	**3+ Score**	**2+ score**	**1+ Score**		
AGE						
≤50 years	1(2.9)	11 (30.5)	16 (44.4)	8 (22.2)	**36**	**0.0434**
>50years	3 (21.5)	6 (42.8)	5 (35.7)	0	**14**	
GENDER						
Male	3 (7.4)	16 (38.0)	17 (40.4)	6 (14.2)	**42**	**0.5399**
Female	1 (12.5)	1 (12.5%)	4 (50.0)	2 (25.0)	**8**	

Regarding the association of Ki 67 expression with the cases of OSCC, all of them had a positive expression of Ki 67. In patients > 50 years old, a high score of 3 was observed in 6 (42.8%) cases, followed by scores of 2 in 5 (35.7%) cases and rest 3 (21.5%) score 4+ for Ki-67 expression. In patients ≤ 50 years, a score of 2 was expressed in 16 (44.4%) cases, followed by a score of 3 in 11 (30.5%), score 1 was expressed in 8 (22.2%) and only 1 (2.9%) case expressed score 4. The study found a statistical association between Ki-67 expression and the age of patient with OSCC.

There was no significant association between the patient's gender and Ki-67 expression.**[Table 3]**

Table 4:-Correlation of p16 and Ki-67 score with histological grades in OSCC.

Grade	p16 score					Ki-67 Score				
	0	**1+**	**2+**	**3+**	**4+**	**1+**	**2+**	**3+**	**4+**	**Mean % (Labelling index of Ki-67)**
Well-Differentiated (16)	12 (75.0%)	3 (18.8%)	1 (6.2%)	0	0	5 (31.3%)	11 (68.7%)	0	0	26.87 ± 9.90
Moderately-Differentiated (27)	16 (59.2%)	4 (14.8%)	3 (11.2%)	3 (11.2%)	1 (3.6%)	3 (11.1%)	10 (37.1%)	12 (44.4%)	2 (7.4%)	49.07 ± 17.2
Poorly-Differentiated (7)	3 (48.8%)	0	1 (14.3%)	1 (14.3%)	2 (28.6%)	0	0	5 (71.4%)	2 (28.6%)	76.40 ± 8.01
p-value	**0.5657 (statistically not significant)**					**0.0006 (statistically significant)**				

The study observed that p16 expression varied with histological grade: 25% of well-differentiated cases exhibited p16, with 18.8% scoring 1+ and 6.2% scoring 2+. In moderately differentiated cases, 40.8% showed p16 expression, with 14.8% cases scoring 1+ and 11.2% scoring 2+ and 3+ each and rest 3.6% of cases expressed score 4+. Among poorly differentiated cases, 57.2% were positive for p16, with 28.6% achieving a score of 4 and 1 (14.3%) case of score 3+ and 2+. High p16 scores of 4+ were notably more common in poorly differentiated carcinomas, while well-differentiated cases did not show scores of 4+ or 3+. Overall, the findings suggest an increase in p16 expression with higher histological grades, though there was no statistically significant link between histological grade and p16 expression.**[Table 4]**

Ki-67 was expressed in all 50 cases, out of 16 cases of well-differentiated OSCC, 11 cases (68.7%) had a Ki-67 score of 2+, while 5 cases (31.3%) had a score of 1+. Among 27 cases of moderately differentiated OSCC, 12 cases (44.4%) had a score of 3+, 10 cases (37.1%) had a score of 2+, 3 cases (11.1%) had a score of 1+, and 2 cases (7.4%) had a score of 4+. For the 7 cases of poorly differentiated OSCC, 5 cases (71.4%) had a score of 3+ and 2 cases (28.6%) had a score of 4+. Ki-67 scores of 3+ and 4+ were notably higher in poorly differentiated OSCC compared to moderately differentiated cases. The mean Ki-67 labelling index (LI) was 26.87 ± 9.90% in well-differentiated carcinoma, increased to 49.07 ± 17.2% in moderately differentiated carcinoma, and further rose to 76.40 ± 8.01% in poorly differentiated carcinoma. The mean percentage of Ki-67 was significantly higher in poorly differentiated cases. There was a significant statistical correlation between Ki-67 expression and histological grade, indicating that Ki-67 expression increases with higher histopathological grades.**[Table 4]**

Discussion:-

Oral squamous cell carcinomas (OSCC) present as significant health challenge due to their unpredictable behaviour and varying prognosis. Oral squamous cell carcinoma (OSCC) is a multifactorial disease, influencing its development and progression.[5] The study included 50 cases of oral squamous cell carcinoma (OSCC) and compared the expression of p16 and Ki-67 with clinical parameters (Ageandgender)andhistologic grade.

In the present study, patient included were in age group 30-80 years of age. Most patients were in their fifth decade of life (56%), with fewer in their fourth (12%), sixth (16%), and seventh decades (10%). These findings are concordant with those of Yasin M et al[12] (mean age 48 years), Saadia Akram et al[13] (mean age 47.8 years), and Sahaf R et al[14] (mean age 51.8 years). However, Yu YH et al[15] reported a mean age in the seventh decade, highlighting how sample size and geographic differences can impact age-related findings in OSCC studies.

In the current study, OSCC was more common in males (84%) than females (16%), aligning with previous research by Omer SM et al[5], Ghai et al[16], Pires FP et al [17], Singh MP et al [18], and Chandrakanta et al[19] In contrast, Jing Y et al[7] reported a female preponderance (53.2%) in their study of 298 OSCC cases, highlighting how differences in sample size can influence findings.

In this study, 72% of the 50 OSCC cases were tobacco users, while 28% were non-tobacco users. Among the tobacco users, 52.7% were both smokers and tobacco chewers, 30.5% were only smokers, and 16.8% were only tobacco chewers. These findings are consistent with research by Feghali et al[20], Sajith Edirisinghe et al[21], and Chandrakanta et al[19].

In this study, the buccal mucosa was the most common site for OSCC, affecting 36% of cases, followed by the tongue (28%), gingiva (16%), retromolar trigone (6%), and angle of the mouth (6%). Other sites like the hard palate, floor of the mouth, and lip were less affected. Chewing tobacco and holding it against the buccal mucosa were significant risk factors. These results are consistent with findings from Lin NC et al[22], Singh MP et al[18], and AR Gadbail et al[23] In contrast, Chandrakanta et al[19] found the tongue to be the most common site (50%), with the buccal mucosa at 18.42%. Similarly, Moro JS et al[24] and Krishna Rao SV et al[25] reported high rates of tongue involvement (28% and 42%, respectively). The differences in findings may be due to variations in sample sizes and study durations.

In this study, most OSCC tumors were moderately differentiated (54%), followed by well differentiated (32%), and poorly differentiated (14%). These findings align with studies by Yasin Met al[12], Gadbail AR et al[23], and Omer SM et al[5], which also reported a predominance of MD-OSCC, with fewer cases of WD and PD.

In our study, 33.4% of patients aged ≤50 years and 50% of those aged >50 years were positive for p16 expression, with no significant relationship observed between p16 expression and age. This aligns with findings from Kalsariya P et al[26](62.5%) and Ralli et al[27] (52.5%). However, Agarwal VK et al[28]found that 16.67% of patients aged <40 years and 31.82% of ≥40 years were positive for p16, contrasting with Western literature, which suggests higher p16 expression in younger individuals.[28]

In our study, 40% of male patients and 25% of female patients showed positive p16 expression, with no statisticallysignificant correlation between p16 positivity and gender (p-value >0.05). These findings are consistent with Saxena P et al[29] which reported 39.1% p16 positivity in females and 78.7% in males, as well as Kalsariya P et al[26] and Ralli et al[27], who observed male predominance in p16 positivity (87.50% and 86.40%, respectively). In contrast, Omer SM et al[5]found a lower p16 positivity in males (7.7%) compared to females (25.0%).

In our study of 50 OSCC cases, p16 expression varied by histological grade: 25% of well-differentiated cases showed p16 expression, out of which 18.8% had a score of 1, and 6.2% had a score of 2. Among moderately differentiated cases, 40.8% had p16 expression, out of 14.8% scored 1, and the rest had scores of 2, 3, or 4. In poorly differentiated cases,57.2% were positive, with 28.6% scoring 4. Our findings, showing increased p16 positivity with higher histopathological grade, align with Agarwal VK et al[28], Patil S et al[30] and Ralli M et al[27].

However, they contrast with Kalsariya M et al[26] and Saxena P et al[29] who reported higher p16 expression in moderately differentiated cases. The differences may be due to sample size and inclusion of dysplastic lesions included in the studies.

In our study, Ki-67 expression was showing a high score of 3 in 42.8% of cases, score 2 in 35.7%, and score 4+ in 21.5%. No cases scored 1 in patients over 50 years old, while in those under 50, 44.4% had a score of 2, 30.5% had

a score of 3, 22.2% had a score of 1, and 2.9% had a score of 4+. These results align with Sharma G et al[31] and Jing et al[7], who found higher Ki-67 expression in older patients and lower expression in younger ones. However, our findings contrast with Alaa S. Saeed et al[32], who reported higher Ki-67 expression in younger patients compared to older ones.

In our study of 50 OSCC cases, all patients demonstrated Ki-67 expression, with no significant correlation between gender and Ki-67 levels. This finding is consistent with Omer SM et al[5] who also found no significant gender-based differences in Ki-67 expression (80.8% in males and 70.8% in females). Similarly, Alaa S. Saeed et al[32] and Sharma G et al[31] did not find a significant association between gender and Ki-67 expression in their studies.

In our study of 50 OSCC cases, Ki-67 scores of 3+ and 4+ were significantly higher in poorly differentiated tumors. The mean Ki-67 labeling index (LI) increased with histopathological grade: 76.40% ± 8.01% in poorly differentiated, 49.07% ± 17.20% in moderately differentiated, and 26.87% ± 9.90% in well-differentiated cases. This trend aligns with the findings of Chandrakanta et al[19], who reported increasing Ki-67 LI with tumor grade, and Dash KC et al[33], Agarwal et al[34], and AR Gadbail et al[23], who also found higher Ki-67 expression in more poorly differentiated OSCC. Takeem A et al[35] similarly observed that Ki-67 expression rose with tumor grade.[35] Our results indicate a significant association between histopathological grade and Ki-67 expression, with higher scores in poorly differentiated OSCC. Notably, none of the well-differentiated cases had Ki-67 scores of 3+ or 4+.

Conclusion: -

OSCC is the most common carcinoma among oral malignancies. p16 and Ki-67 are valuable markers for assessing the aggressiveness and progression of OSCC.p16 expression is the most important immunohistochemical marker to differentiate HPV positive OSCC and HPV negative OSCC for better management. HPV vaccines may also benefit for preventing OSCC. Ki-67 antigen is a proliferative marker used to determine the tumor behaviour, prognosis and deciding treatment modalities. Ki-67 protein increase with decreasing tissue differentiation of OSCC.

Limitations:-

The study's findings are based on a hospital-based sample and a limited number of cases, which may not fully represent the broader population. Further research is needed to validate these findings and explore the roles of p16 and Ki-67 in management of OSCC.

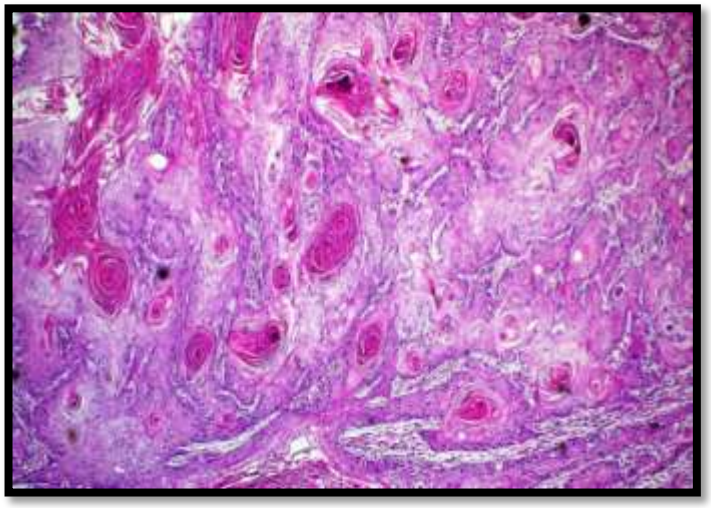

Image 1: Photomicrograph of tissue section shows Oral Squamous Cell Carcinoma [Well-Differentiated]. (H&E 100X)

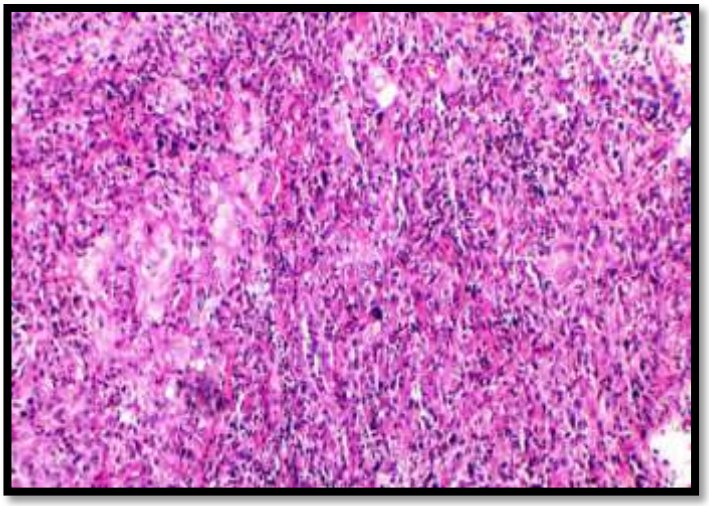

Image 2: Photomicrograph of tissue section shows Oral Squamous Cell Carcinoma[Moderately-Differentiated]. (H&E 100X)

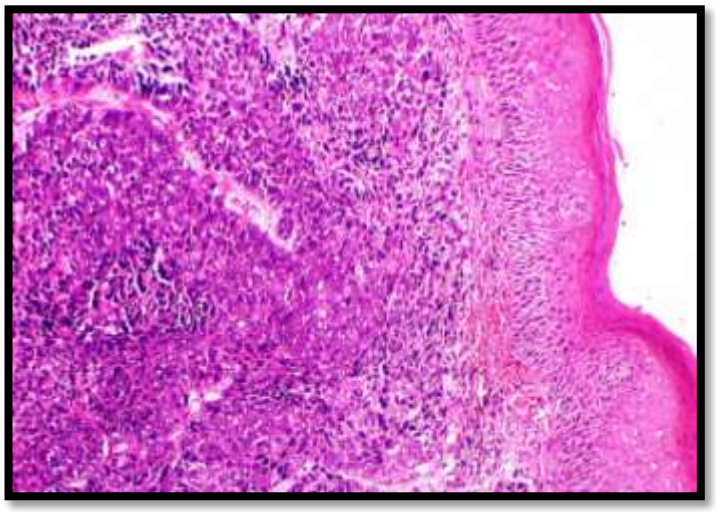

Image 3: Photomicrograph of tissue section shows Oral Squamous Cell Carcinoma[Poorly-Differentiated]. (H&E 100X)

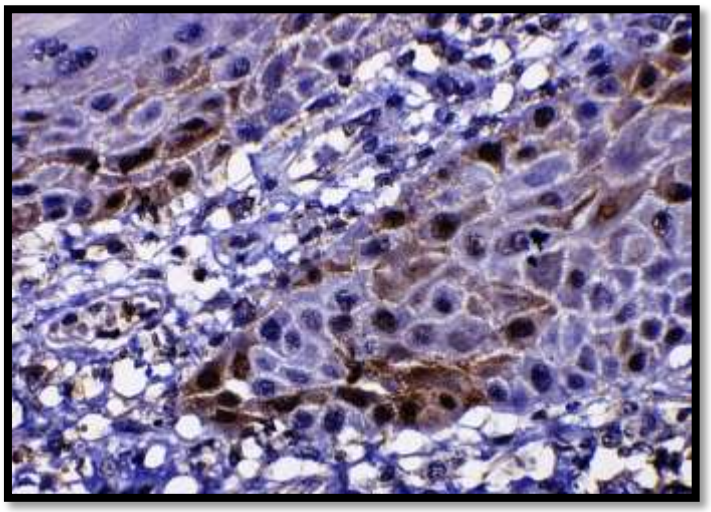

Image 4: Photomicrograph of Oral Squamous Cell Carcinoma[Well-Differentiated] showing immunopositivity for p16 score 1. (IHC 400x)

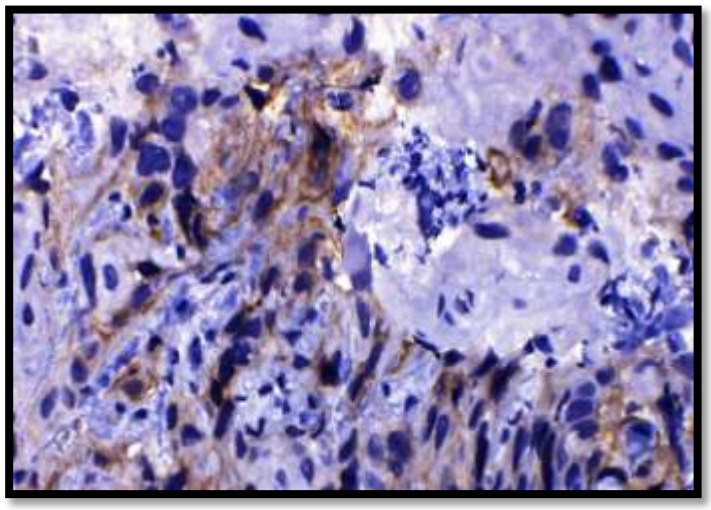

Image 5: Photomicrograph of Oral Squamous Cell Carcinoma[Moderately-Differentiated] showing immunopositivity for p16 score 3. (IHC 400x)

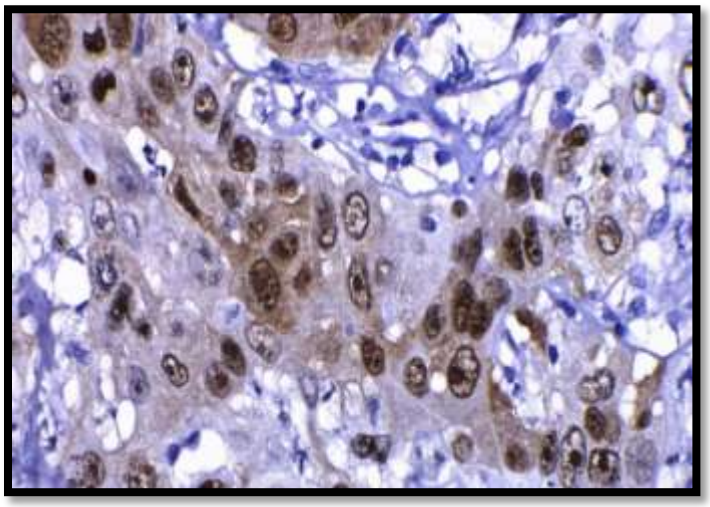

Image 6: Photomicrograph of Oral Squamous Cell Carcinoma[Poorly-Differentiated] showing immunopositivity for p16 score 4. (IHC 400x)

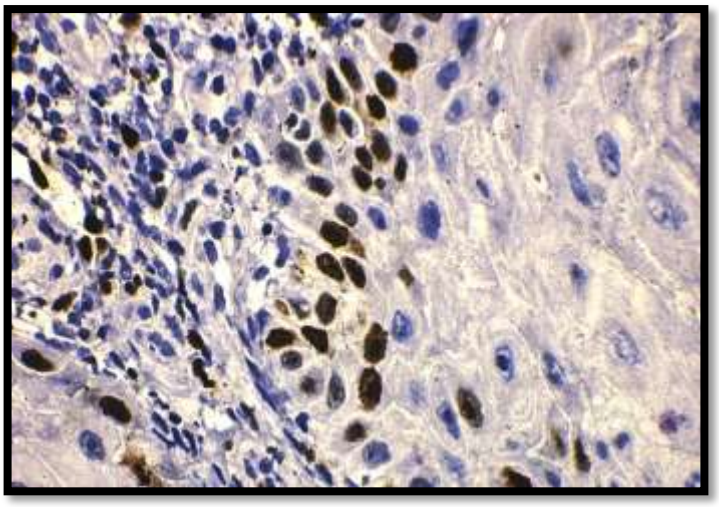

Image 7: Photomicrograph of Oral Squamous Cell Carcinoma[Well-Differentiated] showing immunopositivity for Ki-67 score 1. (IHC 400x)

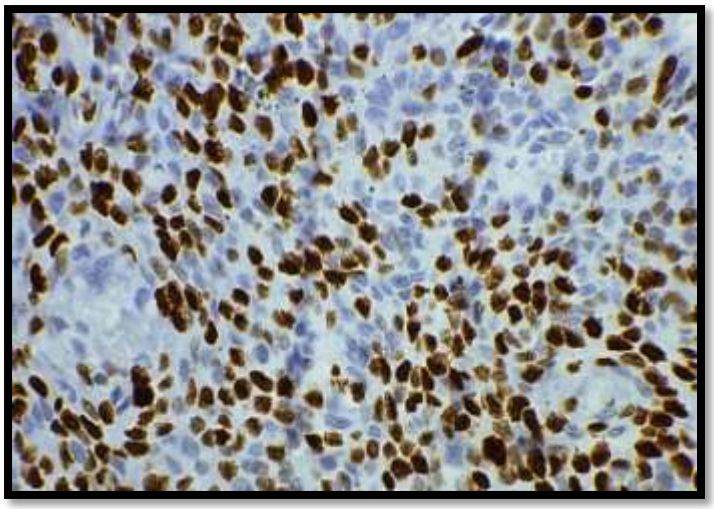

Image 8: Photomicrograph of Oral Squamous Cell Carcinoma[Moderately-Differentiated] showing immunopositivity for Ki-67 score 3. (IHC 400x)

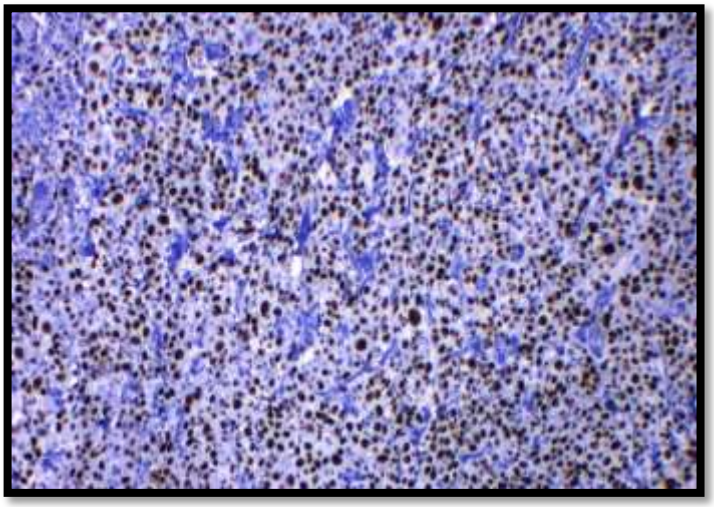

Image 9: Photomicrograph of Oral Squamous Cell Carcinoma[Poorly-Differentiated] showing immunopositivity for Ki-67 score 4. (IHC 100x)

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