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Immunohistochemical Expression of p63 and p16 in Different Grades of Oral Squamous Cell Carcinoma

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ABSTRACT

Background: Oral cancer constitutes the sixth most common cancer worldwide and third most common cancer in South-East Asia. The role of p63 and p16 expression in the prognosis of oral squamous cell carcinoma is still debatable. The present study aimed to evaluate the immunohistochemical expression of p63 and p16 and its association with histopathological grading of oral squamous cell carcinoma.

Methods: This cross-sectional study was conducted in the Department of Pathology, Chittagong Medical College over a period of 21 months. Specimens from 58 patients were fixed in 10% neutral buffered formalin and embedded in paraffin. Hematoxylin and Eosin-stained slides of each case was prepared from each paraffin block for proper evaluation of tumor grades. Two slides were prepared from representative tumor blocks of each specimen and stained with primary antibodies against p63 and p16 (mouse monoclonal Ab-1; DAKO Denmark). Association between histological grading with p63 and p16 expression was assessed by Fisher's exact test.

Results: The mean age of the patients with OSCC was 56.98 ± 12.96years. Among the 58 patients, majority (63.9%) was grade I tumor with low p63 expression. High p63 expression was observed in 100% cases of grade II and 50% cases of grade III tumor. Significant association was observed between p63 expression with grading of OSCC. Regarding p16 expression, 20 (34.5%) cases were positive and 38 (65.5%) cases were negative. There was no statistically significant association between p16 expression and grading of OSCC. Significant association was found between p63 expression and p16 positivity in OSCC.

Conclusion: p63 overexpression is significantly associated with higher grade of OSCC. Although no significant association was found between p16 expression and tumor grades but higher percentages of immunostaining were observed with poorly differentiated OSCC. Positive p16 expression significantly correlated with the high expression of p63 in OSCC. A combination of these markers could be used as a prognostic purpose and potential targeted therapy for OSCC.

Key words: Oral Squamous Cell Carcinoma, p63, p16.

I. INTRODUCTION

Oral cancer is a subgroup of head and neck cancers; it is the sixth most frequent type of cancer in the general population, with variable incidence rates across countries[1]. More than 90% of all oral cancers are squamous cell carcinoma[2].Cancers of the oral cavity and hypopharynx are highly prevalent in Asian countries. One-third of global cases and one-half of oral cancer-related deaths are reported from Southeast Asia. In certain countries, such as Srilanka, India, Pakistan, and Bangladesh, oral cancer is the most common cancer[3]. More than 7000 people are newly diagnosed in Bangladesh each year, and among them, 6.6% of people died due to their lifestyle and other factors[4].Oral cancer is related to deleterious oral habits such as tobacco chewing, betel quid chewing, tobacco smoking, reverse smoking, as well as other factors such as alcohol consumption, low socioeconomic status, poor hygiene, poor diet, viral infections, chronic

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irritation from ill-fitting dentures, rough, or fractured teeth[3].Various staining patterns were observed for p63 expression in OSCCs. It was observed that the pattern of staining differs between the grading of neoplasms**.**Studied showed that; cases with diffuse p63 expression were more aggressive and poorly differentiated & related to a poorer prognosis. P63 expression may be useful to identify cases of oral squamous cell carcinoma with more aggressive & invasive phenotype providing novel diagnostic &prognostic information on individual patient survival with oral cancers[5].Overexpression of p63 has been found in several cancers, including lung, skin, cervix, and head and neck cancers, associated with aggressiveness and poor prognosis[6].P16 is a cyclin-dependent kinase inhibitor (CDKI) encoded by the CDKN2A locus; p16 is a cellular protein which is involved in cell cycle regulation and arrests the cell cycle in G1 stage[7]. The p16 expression in normal oral mucosa was detected in the basal layer of epithelium and less in suprabasal layer. But in squamous cell carcinoma, p16 expression were detected more intense in basal layer, suprabasal layer of epithelium and connective tissue. It has been suggested that p16 expression is higher in well-differentiated tumors and lower in poorly differentiated tumors.[8]

In HNSCC, the p16 tumor suppressor gene is inactivated primarily by various genetic and nongenetic (eg, promoter hypermethylation) modifications such that the expression of its protein product is lost, diminished, or limited to tumor nuclei. In contrast, integration of high-risk HPV into the host genome is associated with upregulation of the p16 gene product. HPV integration results in loss of the regulatory viral E2 gene promoter, causing increased transcription of E6 and E7. Binding of the E7 oncoprotein to the Rb protein leads to Rb protein degradation and presumably to the compensatory overexpression of both cytoplasmic and nuclear p16 protein in HPV-infected tumor cells.

II. OBJECTIVES

General objective:

1. To observe the expression of p63 and p16 with tumor grades of the oral squamous cell carcinoma.

Specific Objectives:

1. To see the histopathological grading of oral squamous cell carcinoma.
2. To observe the immunohistochemical expression of p63 and p16 in oral squamous cell carcinoma.
3. To observe the pattern of p16 expression in oral squamous cell carcinoma.
4. To evaluate the association between p63 and p16 expression with tumor grades of the oral squamous cell carcinoma.

III. METHODOLOGY

It was a cross sectional observational study that was started after getting permission from the Institutional Review Board. Then, after taking informed written consent from the patient attending the Department of Pathology, Chittagong Medical College.Incisional biopsy was taken from the most representative site of the oral lesion by a surgeon with all aseptic conditions. Biopsy specimen was preserved in 10% neutral buffered formalin solution. Gross examination of specimen was done and biopsied tissue was processed, embedded in paraffin wax and 3-4µm (micrometer) thick sections were made. After dewaxing the section, one section for hematoxylin and eosin stain and two section for p63 and p16 immunostaining were prepared from each case and examined under light microscope.

Immunohistochemical analysis:

Immunostaining for p63 and p16 were done at Care investigation, Chattogram, and BSMMU, Dhaka. Both p63 and p16 immune staining were done on all these 58 cases. For immunohistochemistry staining, 4-micrometer thick tissue sections were taken on Poly –L lysine coated slide from the paraffin blocks of the tumor.

Primary Antibody:

For p63: Monoclonal mouse anti-Human p63 antigen (Clone 4A4, Dako, Denmark).

For p16: Monoclonal mouse anti-Human p16 antigen (E6H4 Clone, Dako, Denmark) was used as primary antibody.

Secondary Antibody:

Envision (ready to use DEKO) was used for both p63 and p16 as secondary antibody.

Positive control:

Uterine cervical tissue was used as positive control for both p63 and p16 immunostaining.

Assessment of p63 Immunohistochemical Staining:

Only nuclear staining of epithelial cells was observed. To evaluate the p63 expression, a mean percentage of positive tumor cells was determined from the percentage of positive nuclei derived from the analysis of 100 cells in 10 random areas at x 400 magnification. Immunolabeled tumor cells were scored according to the percentage of the total number of cells and intensity of staining, as follows: the intensity of

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staining [0 (absent), 1 (weak), 2 (moderate) and 3 (strong)] and percentage of stained tumor cells [0 (negative, or <10%), 1 (10%–24%), 2 (25%–49%), 3 (50%–74%) and 4 (≥75%)]. Then, we performed an algorithm using intensity and percentage of positive cells, resulting in a final score from 0 to 7 [(0–1) negative; (2–3) 1 + (4–5) 2 + (6–7) 3 +]. For data analysis, score 0/1 + was considered as low expression and scores 2 + / 3 + as high expression of the marker.[9]

Assessment of p16 Immunohistochemical Staining:

In the newly published CAP guidelines for HPV testing in head and neck carcinomas in routine clinical practice, the recommendation for workup of patient specimens from oropharyngeal primary tumors is to perform p16 IHC testing, with mild staining in <70% of tumor cells with cytoplasmic and nuclear staining classified as HPV-negative and moderate or strong diffuse nuclear and cytoplasmic staining in ≥70% of tumor cells classified as HPV-positive.[10]

Statistical analysis:

The statistical analysis was conducted using SPSS (statistical package for the social science) version 26 statistical software. The findings of the study were presented by frequency, percentage in tables. Means and standard deviations for continuous variables and frequency distributions for categorical variables were used to describe the characteristics of the total sample. Associations of categorical data were assessed using the Chi-square test and Fisher's exact test. P<0.05 was considered significant, and all tests were two-sided.

IV. RESULTS

To evaluate the immunohistochemical expression of p63 and p16 protein in OSCC. Total 58 cases were included in this study. All the specimens were obtained by excisional biopsy. Among the 58 patients, majority (n=18,31.0%) were from 51-60 years age group and 12 (20.7%) were from 41-50years age group. The mean age of the patients was 56.41 (± 13.03) years where minimum age was 30 years and maximum age was 80 years.

Figure 1 shows that 25 (43.1%) patients had low p63 expression while 33 (56.9%) had high p63 expression.

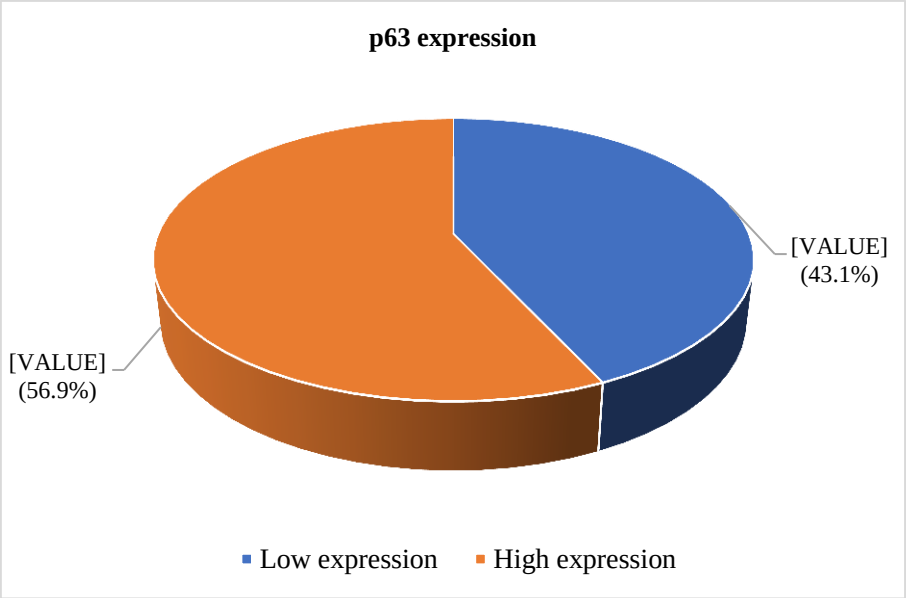

Figure 1: Distribution of patients by p63 expression (n=58)

Figure 2 shows that 38 (65.5%) patients had negative p16 expression while 20 (34.5%) had positive p16 expression.

International Journal Dental and Medical Sciences Research
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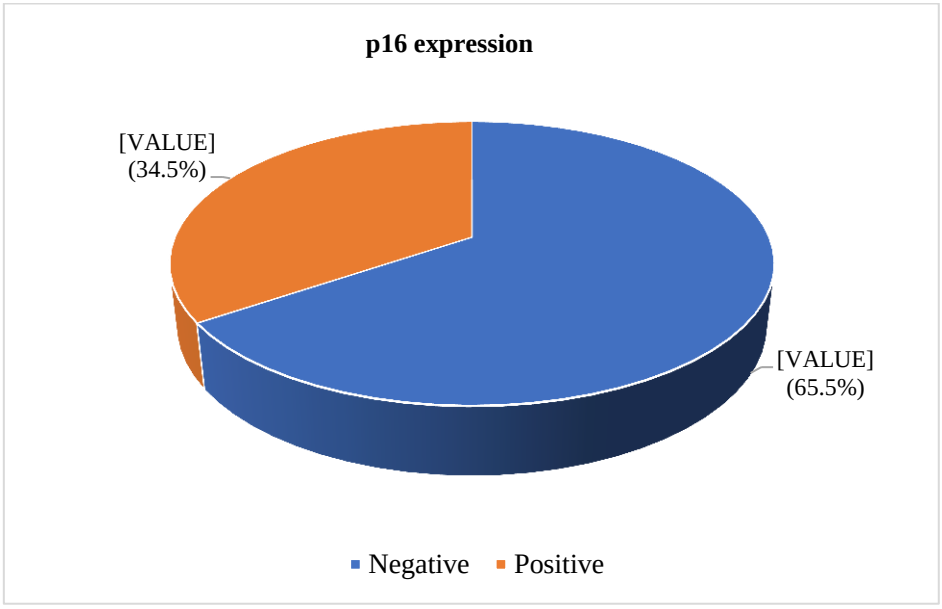

Figure 2: Distribution of patients by p16 expression (n=58)

Table 1: Association between p63 expression and socio-demographic variables and personal habit (n=58)

Characteristics	p63 expression		
	Low expression	High expression	p value
Age groups (in years)			
Up to 50	6 (27.3%)	16 (72.7%)	0.057
>50	19 (52.8%)	17 (47.2%)	
Sex			
Male	14 (46.7%)	16 (53.3%)	0.571
Female	11 (39.3%)	17 (60.7%)	
Socio-economic class			
Lower	17 (50.0%)	17 (50.0%)	0.207
Middle	8 (33.3%)	16 (66.7%)	
Smoking history			
Non-smoker	11 (36.7%)	19 (63.3%)	0.306
Smoker	14 (50.0%)	14 (50.0%)	
Betel quid chewing habit			
No	5 (35.7%)	9 (64.3%)	0.522
Yes	20 (45.5%)	24 (54.5%)	

*Fisher's exact test

Table 1 shows that there was no significant association between p63 expression and socio-demographic variables and personal habit of the patients as P>0.05 (obtained by Chi-square test).

Table 2: Association between p63 expression and grading of tumor (n=58)

Grading	p63 expression		p value
	Low expression	High expression	
Grade I	23 (63.9%)	13 (36.1%)	<0.001
Grade II	0 (0.0%)	18 (100.0%)	
Grade III	2 (50.0%)	2 (50.0%)	

Table 2 shows that one-third of the patients (n=13,36.1 %) with grade I tumor had high p63 expression while grade II and grade III carcinoma were mostly high P63 expression, 18 (100 %) & 2 (50%), respectively. Fisher's Exact test showed that there was statistically significant association between p63 expression and grading of the tumor as P<0.001.

International Journal Dental and Medical Sciences Research
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Table 3: Association between p16 expression and socio-demographic variables and personal habit (n=58)

Characteristics	p16 expression		p value
	Negative	Positive	
Age groups (in years)			
Up to 50	15 (68.2%)	7 (31.8%)	0.739
>50	23 (63.9%)	13 (36.1%)	
Sex			
Male	20 (66.7%)	10 (33.3%)	0.849
Female	18 (64.3%)	10 (35.7%)	
Socio-economic class			
Lower	21 (61.8%)	13 (38.2%)	0.474
Middle	17 (70.8%)	7 (29.2%)	
Smoking history			
Non-smoker	19 (63.3%)	11 (36.7%)	0.717
Smoker	19 (67.9%)	9 (32.1%)	
Betel quid chewing habit			
No	9 (64.3%)	5 (35.7%)	1.000*
Yes	29 (65.9%)	15 (34.1%)	

*Fisher's Exact value

Table 3 shows that there was no significant association between p16 expression and socio-demographic variables and personal habit of the patients as P>0.05 (obtained by Chi-square test).

Table 4: Association between p16 expression and grading of tumor (n=58)

Grading	p16 expression		p value
	Negative	Positive	
Grade I	25 (69.4%)	11 (30.6%)	
Grade II	12 (66.7%)	6 (33.3%)	0.221
Grade III	1 (25.0%)	3 (75.0%)	

Table 4 shows that there was no significant association between p16 expression and grading of tumor as P>0.05 (obtained by Fisher's Exact test).

Table 5: Association between p63 expression and p16 expression (n=58)

p16	p63 expression		p value
	Low expression	High expression	
Negative	21 (55.3%)	17 (44.7%)	0.013
Positive	04 (20.0%)	16 (80.0%)	

Table 5 shows that among the 38 patients who had p16 negative expression, 17 (44.7%) had p63 high expression while 20 patients who had p16 positive expression, 16 (80.0%) had p63 high expression. There was significant association between p63 and p16 expression as P=0.013 (obtained by Fisher's exact test).

Table 6: Association between p16 pattern and p16 expression (n=58)

p16 pattern	p16 expression		
	Negative	Positive	p value
High cytoplasm	21 (100.0%)	0 (0.0%)	
High nucleus	0 (0.0%)	20 (100.0%)	<0.001
Both low	17 (100.0%)	0 (0.0%)	

Above table-6 shows that among the 17 patients who had high nucleus, 16 (94.1%) had positive p16 expression while 17 patients who had both low, all (100.0%) had p16 negative p16 expression. There was significant statistical difference between p16 pattern and p16 expression as P<0.001 (obtained by Fisher Exact test).

International Journal Dental and Medical Sciences Research
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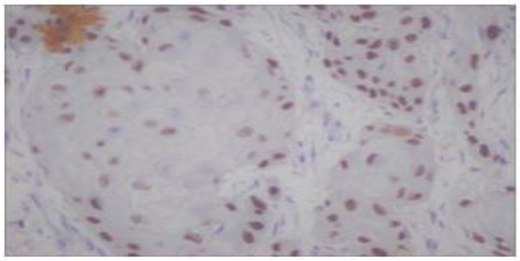

Figure 3: Oral squamous cell carcinoma, grade-I (20X) Low expression, p63 immunostaining

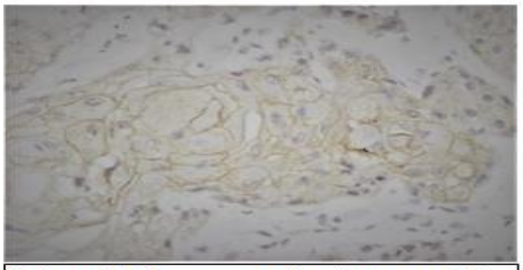

Figure 4: Oral squamous cell carcinoma, grade-I (20X) p16 negative (Both low staining)

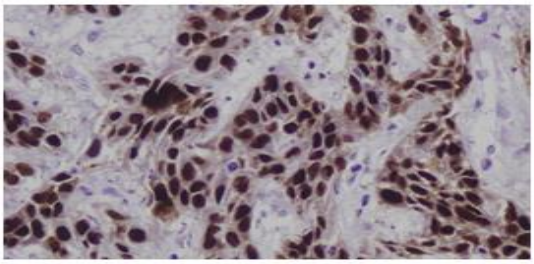

Figure 5: Oral squamous cell carcinoma, grade-II (20X) High expression, p63 immunostaining

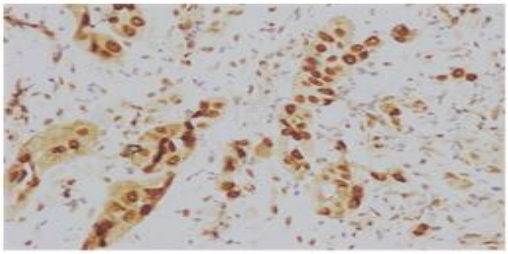

Figure 6: Oral squamous cell carcinoma, grade-II (20X) p16 positive (High nuclear staining)

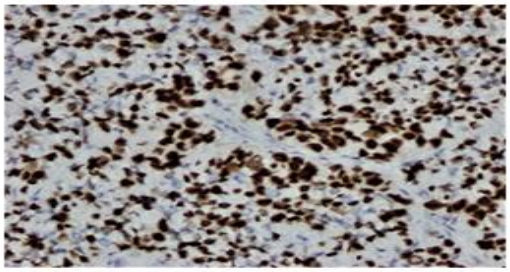

Figure 7: Oral squamous cell carcinoma, grade-III (20X), High expression, p63immunostaining

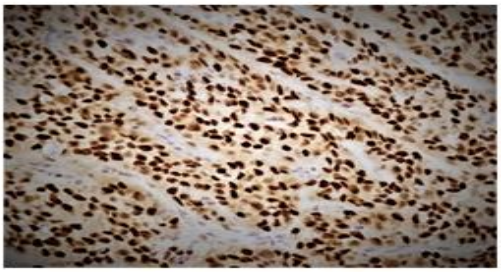

Figure 8: Oral squamous cell carcinoma, grade-III (20X), p16 positive (High nuclear staining)

V. DISCUSSION

Squamous cell carcinoma is the most common malignancy affecting the oral cavity. Most of the cases manifest with advanced lesions at the time of diagnosis. Histological grade of differentiation, invasion and staging determines the prognosis of the patient. Despite advances in therapeutic options for head and neck squamous cell carcinoma over the last decades, mortality and morbidity rates have been improved moderately, encouraging the search for new and better molecular markers that relate comprehensively with known alterations of tumor progression.[11] Molecular markers that could act as a therapeutic target could play an important role in effective treatment strategies of oral cancer[8].P63 is known to play an essential role in epithelial development and maintenance. Mutation of p63 rarely occurs in malignancies, but amplification appears to be responsible for overexpression of p63 protein in many SCCs from several sites, including the head and neck.[12]The protein p16 is a cellular protein involved in cell cycle regulation. In normal cells, p16 protein is expressed in very low levels and is almost undetectable by IHC. However, due to the transforming activity of the E7 oncogene, p16 is strongly expressed in tumor cells affected by HPV and may be easily detected by IHC. Hence, p16 positivity correlates strongly with HPV positivity.[13] The present study was carried out in the Department of pathology, Chittagong Medical College, Chattogram, to evaluate the expression of p63 and p16 in invasive squamous cell carcinoma of the oral cavity using immunohistochemistry. In the current study, 37.9% of cases were from buccal mucosa, 20.7% from the tongue, 18.9% from the palatine tonsil, 8.6% from the floor of the mouth and retromolar area, and 5.2 % from the lip

Similarly, Venkatesh et al., 2018 found most of the cases were from buccal mucosa (70.37%).[14]In this study, it was observed that the age of the patients varied from 30-80 years. Out of 58 patients, 31% of patients belonged to age 51-60 years, and mean ± SD age was 56.41 ± 13.3years.Similarly, in Brazil, Lauxen et al., 2014 found in their study that the age range of 51-60 years (35.4%).[15] In India, Venkatesh et al., 2018 showed the age distribution ranged between 50-59 years[14]. Some other investigators found a little higher age distribution. In Italy, Muzio et al., 2005 noticed most of the patients aged >65 years[5]. Similarly, in Portugal, Monteiro et al., 2016 found most patients were aged ≤62 years. These age differences may be due to different subcontinents and places of study[9].In the final scoring of p63 expression, 56.9% cases were found with high expression. In contrast¸ low expression of p63 was found among 43.1% of cases. No negative p63 expression was found in any of the cases. In the present study, it was observed that no statistically significant difference between p63 immunoreactivities and age, sex, betel nut, smoking and alcohol habit, location of tumors (P > 0.05), which is quite similar to some previous studies like Muzio et al and Monteiro et al.[5,9] In the present study, 20 (34.5%) cases were p16 positive, and 38 (65.5%) were p16 negative. In total 58 cases, 24 (41.1%) cases showed HC staining pattern, 17(29.3%) cases with HN and 17(29.3%) had LS staining pattern. In the present study, no significant difference observed between p16 expression with age, sex, betel nut, smoking, alcohol habit, site of the tumor, and also grading of tumors (P > 0.05) which is quite similar to some previous studies. Among four patients of poorly differentiated OSCC, majority of patients (75.0%) were showed positive p16 expression, which is similar to some previous studies like Ralli et al., Smith et al., and Muirhead et al.[16]In total 58 cases, 21 (35.6%) cases showed high cytoplasmic staining pattern, 20 (33.9%) cases with high nuclear and 17 (29.3%) had both low staining patterns. Human papillomavirus infection has been strongly associated with both cytoplasmic and nuclear p16 positivity. In this study, the p-value was significant between p63 expression and p16 expression in OSCC. Positive p16 expression significantly correlated with the high expression of p63 in OSCC. Shirendeb et al., found the correlation between p63 and HPV 16 expression in SCC, suggesting that HPV 16 presents a tropism for squamous epithelial cells, while p63 may positively contribute to the viral life cycle by blocking apoptosis through the ΔNp63 isoforms. In contrast, Sharada et al., and de Oliveira et al., found increased expression of p63 and decreased expression of p16 are associated with higher grades of OSCC which may be attributed to loss of function of the tumor suppressor genes, a vital factor responsible for tumor progression.[15,17] This dissimilarity might be due to geographic distribution, the difference in prevalence of various risk factors, different isoforms of the p63 gene, the different scoring system used by different workers for evaluation of the positivity of this marker, inconsistency in specimen handling¸ and technical procedures. However, in this present study, increased p63 expression was significantly associated with a higher grade of OSCC, whereas no significant relationship was detected between p16 expression with tumor grades. These results can advance our understanding of the initiating mechanisms, pathogenesis, and prognosis of OSCC and result in novel therapeutic targets in cancer treatment.

VI. CONCLUSIONS

This study observed that¸ p63 overexpression was significantly associated with a higher grade of OSCC. Although no significant association was found between p16 expression and tumor grades but higher percentages of immunostaining were observed with poorly differentiated OSCC. Positive p16 expression significantly correlated with the high expression of p63 in OSCC. The combination of these markers could be used as important prognostic biomarkers in OSCC and might be evaluated as future targets for molecular therapies.

REFERENCE:

[1]. Laimer, K., Spizzo, G., Gastl, G. et al. (2007) 'High EGFR expression predicts poor prognosis in patients with squamous cell carcinoma of the oral cavity and oropharynx: a TMA-based immunohistochemicalanalysis'. Oral Oncol, Vol.43, pp.193–198.

[2]. Attar, E., Dey, S., Hablas, A. et al. (2010) 'Head and Neck Cancer in a Developing Country: A Population-Based Perspec-tiveAcross 8 Years'. Oral Oncology, Vol. 46 (8), pp. 591-596.

[3]. Tandon, A., Bordoloi, B., Jaiswal, R. et al. (2018)' Demographic and clinicopathological profile of oral squamous cell carcinoma patients of North India: A retrospective institutional study'. SRM J Res Dent Sci , Vol. 9, pp. 114-8.

[4]. Sultana, N., and Malik, M., (2014) 'The Overview of Oral Cancer and Risk Factors

in Bangladesh'. International Journal of Dental Sciences and Research, Vol. 2, No.5,pp. 8-10.

[5]. Muzio, L.Lo.,Santarelli, A., Caltabiano, R. et al. (2005) 'P63 overexpression associates with poor prognosis in head and neck squamous cell carcinoma'. Human Pathology, Vol. 36, pp. 187– 194.

[6]. Nylander, K., Vojtesek, B., Nenutil, R. et al. (2002)ʻ Differential expression of p63 isoforms in normal tissues and neoplastic cells'. J Pathol, Vol. 198, pp. 417–427.

[7]. Wang, L., Xin Wang, X., Li,Y. et al. (2017) ʻ Plasma lipid profiling and diagnostic biomarkers for oral squamous cell carcinoma'.Oncotarget, Vol. 8, pp. 92324-92332.

[8]. Chen, C.J., Hsu, L.S., Lin, S.H. et al.(2012). ʻ Loss of nuclear expression of Krüppel-like factor 4 is associated with poor prognosis in patients with oral cancer'. Human Pathology, Vol 43, pp. 1119–1125.

[9]. Monteiro, L.S., Delgado, M.L., Ricardo, S. et al. (2016) 'Prognostic significance of CD44v6, p63, podoplanin and MMP-9 in oral squamous cell carcinomas'. Oral Diseases, Vol. 22, pp. 303–312.

[10]. Kim K Y.,, James S Lewis J S., and Zhong Chen Z., 2018.Current status of clinical testing for human papillomavirus in oropharyngeal squamous cell carcinoma.J PatholClin Res, Vol.4:pp. 213–226.

[11]. Massano, J., Regateiro, F.S., Januário, G. et al. (2006) 'Oral squamous cell

[12]. carcinoma: Review of prognostic and predictive factors'. Oral Surgery Oral Medicine Oral Pathology Oral Radiolology and Endodontology, Vol. 102, pp. 67–76.

[13]. Bortoluzzi, M.C., Yurgel, L.S., Dekker, N.P., Richard, C.K., Jordan, R.C.K. and Regezi, J.A. (2004) 'Assessment of p63 expression in oral squamous cell carcinomas and dysplasias'. Oral Surg Oral Med Oral Pathol Oral RadiolEndod , Vol. 98, pp. 698-704

[14]. Rao, S.V.K., Mejia, G., Roberts-Thomson, K. et al. (2013)ʻ Epidemiology of oral cancer in Asia in the past decade-an update (2000-2012)'. Asian Pacific journal of cancer prevention, Vol. 14(10), pp. 5567-5577.

[15]. Venkatesh, A., Wadhwan, V., Aggarwal, P. et al.(2018) 'Elevated p63 expression as an indicator for poorer prognosis in squamous cell carcinomas of the oral cavity: An immunohistochemical study'. Indian J Med PaediatrOncol, Vol.39, pp.146-52.

[16]. Lauxen, I.S., Oliveira, M.G., Rados, P.V. et al. (2014)ʻ Immunoprofiling of oral squamous cell carcinomas reveals high p63 and survivin expression'. Oral Diseases, Vol. 20, pp. 76–80.

[17]. Pindborg, J.J., Reichart, P.A., Smith, C.J. et al. (1997)ʻ Histological typing of cancer and precancer of the oral mucosa', 2nd edition, springer, pp. 11-31.

[18]. Sharada, P., Swaminathan, U., Nagamalini, B.R. et al. (2018)ʻ A Semi-quantitative analysis of immunohistochemical expression of p63, Ki-67, Cyclin-D1, and p16 in common oral potentially malignant disorders and oral squamous cell carcinoma'. J NTR Univ Health Sci, Vol.7, PP.120-8.