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ASSESSMENT OF P63 EXPRESSION AND ITS ASSOCIATION WITH HISTOLOGICAL GRADING 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. Pathologists have played a major role in identifying different histological and immunohistochemical markers that have a direct bearing on both the behavior and treatment of oral cancer. P63 overexpression has been studied as a prognostic and therapeutic target in oral squamous cell carcinoma. The aim of the present study was to evaluate the immunohistochemical expression of p63 and its association with histopathological grading of oral squamous cell carcinoma. **Material and Method:** 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. Haematoxylin and Eosin stained slides of each case was prepared from each paraffin block for proper evaluation of tumor type and grade. Two slides were prepared from representative tumor blocks of each specimen and stained with primary antibodies against p63 (mouse monoclonal Ab-1; DAKO Denmark). Finally the association between histological grading and p63 expression was assessed by Chi-square test and later intergroup analysis was done between different grades of squamous cell carcinoma by bonferroni test. **Result:** The mean age of the patients with oral carcinoma studied was 56.98 ± 12.96 years, years ranging from 30 to 80 years; the majority of the cases (62.1%) were of grade I. Among the 58 patients, maximum were grade I with low p63 expression in 23 cases (39.6%) but grade II and grade III were high p63 expression respectively in 17 cases (29.3%) & 3 cases (5.17%).There was statistically significant association between p63 expression and grading of OSCC whereas no significant relationship was detected between markers immunoreactivity and patients age, gender, tumor location and other risk factor. In post hoc analysis, significant association found between grade I and grade II (p=0.0001) and also significant between grade I and grade III (p=0.002). No significant association was observed between grade II and grade III (p=1.00) of OSCC. **Conclusion:** The immunohistochemical evaluation of p63 overexpression significantly associated with higher grade of OSCC. So it can be used as a prognostic marker and potential targeted therapy of OSCC. Larger sample sizes using standardized tests are needed to understand the exact biological role of p63 in this type of carcinoma.

KEYWORD:- Oral cancer, Oral squamous cell carcinoma, Poor hygiene, Poor diet.

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]

According to the most recent GLOBOCAN estimates, worldwide in 2018, there were approximately 354,864 new cases of lip/oral cavity cancer, and 177,384 deaths from oral cavity cancer per year.[3]

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.[4]

In Bangladesh more than 7000 people are newly diagnosed each year and among them¸ 6.6% people are died due to their lifestyle and other factors.[5]

In Western countries¸ oral SCC affects the tongue in 20% - 40% of cases and the floor of the mouth in 15% - 20% of the cases, and together these sites account for about 50% of all cases of oral SCC (Bagan, Sarrion and Jimenez, 2010).[6] The gingiva, palate, retromolar area and the buccal and labial mucosa are oral sites less frequently affected.[7]

Oral cancer is related to the 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, and viral infections, chronic irritation from ill-fitting dentures, rough, or fractured teeth.[4]

The development of oral squamous cell carcinoma is a multistep process requiring the accumulation of multiple genetic alterations, influenced by a patient's genetic predisposition as well as by environmental influences, including tobacco, alcohol, chronic inflammation and viral infection. Recent advances in the field of tumor suppressor genes and oncogenes have provided a tool for studying the genetic changes occurring at different stages of carcinogenesis, including transition from premalignancy to malignancy.[8]

The p63 gene, a member of the p53 family, is located on chromosome 3q27-28. It has been shown that the product encoded by these genes essential in the development of epithelial tissues, epithelial stem cell maintenance, and differentiation. It has a vital role in cell cycle regulation and tumor differentiation.[9]

It is responsible for the transcription of two groups of p63 protein (TAp63 and DNp63), both of which have a, b, and c isoforms. The TAp63 group contains an N-terminal transactivation domain and has functions similar to p53. The DNp63 group lacks the transactivation domain and acts by inhibiting both p53 and TA p63, leading to cell proliferation. P63 and p53 regulate proliferation and differentiation and may have a role in potentially malignant disorders and malignant lesions of the oral cavity.[10]

OBJECTIVE

General objective:

- To evaluate the p63 protein expression in oral squamous cell carcinoma and its association with histopathological grading.

Specific objectives:

- To see the histopathological grading of invasive oral Squamous cell carcinoma.
- To evaluate the immunohistochemical expression of p63 in oral Squamous cell carcinoma.
- To evaluate the association between p63 expression and histopathological grading of the oral Squamous cell carcinoma.

METHODOLOGY

Type of study: Cross sectional observational study.
Place of study: Department of Pathology, Chittagong Medical College, Chattogram and CARE investigation, Chattogram (For Immunohistochemical study)
Study population: All patients with tumors of oral cavity histopathologically diagnosed as invasive oral squamous cell carcinoma, received at department of pathology of Chittagong Medical College, Chattogram during specified time duration.
Study period: Sept, 2018 to Aug, 2020.
Period of data collection: Dec, 2018 to Feb, 2020.
Sample size: Due to resource limitation (Time and money) a total of 58 patients were included in the study as a sample.
Sampling technique: Consecutive Sampling.

Sample selection criteria:
Inclusion criteria:

1. Patients with histopathologically diagnosed as oral squamous cell carcinoma.
2. Patients who had given written informed consent.

Exclusion criteria:

1. Lesions other than oral squamous cell carcinoma.
2. Patients having metastatic carcinoma.
3. Patients who had not given informed written consent.
4. Those who had received chemotherapy or radiation therapy for oral squamous cell carcinoma.

Data collection:
A set of questionnaire was used for each of cases.
Data collection procedure:
All the relevant information and data were systematically recorded in a pre-designed data sheet.
Data analysis:
The data was analyzed in the computer using an appropriate statistical method.

RESULTS

Table 1: Distribution of the patients according to socio-demographic variables (n=58).

Socio demographic variables	Frequency(n)	Percent (%)
Age (years)		
30 – 40	10	17.24
41 – 50	12	20.89
51 – 60	18	31.03
61 – 70	11	18.97
71 – 80	6	10.34
> 80	1	1.72

Mean ± SD (Min-Max) 56.98 ± 12.96 (30 -85)		
Sex		
Male	30	51.7
Female	28	48.3
Socioeconomic condition		
Low	34	58.62
Middle	24	41.38

Among the 58 patients, maximum (31.03%) patients were 51-60 years of age, and 20.89% of patients were 41-50 years of age. The mean age (±SD) of the patients was 56.98 ± 12.96 years. Male and female were 51.72% and 48.28%, respectively. 51.7% were from low socio-economic status, and 48.3% were from middle socio-economic status.

Table 2: Distribution of the patients according to personal history (n=58).

Personal history	Frequency (n)	Percent (%)
Smoking		
Yes	28	48.28
No	30	51.72
Alcohol		
Yes	07	12.07
No	51	87.93
Pan Chewing & betel nut		
Yes	44	75.86
No	14	12.07

Maximum patients had the habit of the pan, betel nut chewing (75.86%).The habit of smoking were 48.28% & alcohol intake 12.07%.

Table 3: Distribution of patients according to the duration of smoking (n=58).

Duration of smoking (Years)	Frequency(n)	Percent (%)
10 -20	07	25
20 -30	14	50
30 -40	07	25
Total	28	100 %
Mean ± SD (min-max)	23.75 ±7.31	

Among the 28 smokers, 50%, patients had a history of smoking for 20-30 years and mean duaration of smoking was 23.75±7.31.

Histopathological grading of oral squamous cell carcinoma (OSCC)

Figure 1 shows, among the 58 patients, 62.1% were histologically reported as grade I, 29.3% as grade II & 8.6% were grade III, according to El-Naggar et al. 2017.

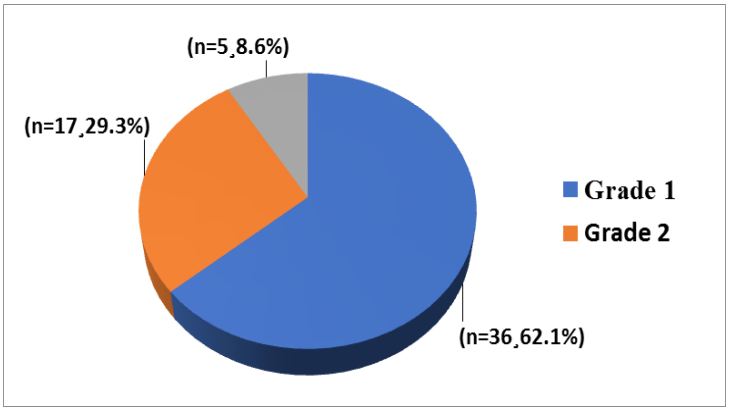

Figure 1: Distribution of patients according to histopathological grading of OSCC (n=58).

Table 4: Distribution of the patients according to p63 expression.

Percentage of tumor cells	Frequency	Percentage
1-24%	9	15.5
25-75%	32	55.2
>75%	17	29.3
Total	58	100
Staining intensity		
Mild	21	36.2
Moderate	29	50.0
High	8	13.8
Total	58	100
Total score		
Low	25	43.1
High	33	56.9
Total	58	100

Table 4 showing among the 58 patients, maximum (32) percentage of p63 positive tumor cells was 25-75%, maximum (29) intensity of p63 was moderate, and high expression of p63 was found 56.9% cases in final score. In contrast, low expression of p63 was found among 43.1% cases.

Table 5: Association between the percentage of p63 expression and histological grading.

Percentage of P63 positive cell	Histopathological grading of tumor			P value
	Grade I	Grade II	Grade III	
<1-24%	8(13.8)	0(0.0)	1(1.7)	0.0001[s]
25-75%	28(48.3)	4(6.9)	0(0.0)	
>75%	0 (0.0)	13(22.4)	4(6.9)	
Total	36(62.1)	17(29.3)	5(8.6)	

*Chi-square test was done to measure the level of significance.
Figure within parentheses indicates in percentage.
s=statistically significant.
Among grade I cases, 28 patients were expressed p63 in between 25-75%, 8 patients were 1-24% expression, none of the grade I patient had expressed >75%. On the other hand, in grade II, maximum number of cases (13) were found to be >75% expression, and similar results also found in grade III cases (4). There was statistically significant ($p<0.05$) result observed between histopathological grade and percentage of p63 expression.

Table 6: Comparison of Mean ± SD of percentage of p63 expression among different grades of oral squamous cell carcinoma.

Grade	Mean ± SD
Grade I	42.16 ± 15.81
Grade II	79.17 ±16.81
Grade III	73.00 ± 33.54
P-value	0.0001

ANOVA test was done to measure the level of significance.
Data was expressed Mean ± SD of percentage of p63
In the study, the mean ± SD of percentage of p63 positive cells were 42.16 ±15.81, 79.17 ±16.81 and 73.00 ± 33.54 in grade I, grade II and grade III SCC respectively which was statistically significant. As post hoc analysis there was found to be significant association between grade I and grade II (p=0.0001) and also significant between grade I and grade III (p=0.002). No significant association was observed between grade II and grade III (p=1.00).

Table 7: Post hoc analysis.

Grade	P-value
Grade I vs Grade II	0.0001
Grade I vs Grade III	0.002
Grade II vs Grade III	1.00

Bonferroni test was done to measure the level of significance.

Table 8: Distribution of patient according to p63 staining intensity by histological grading.

Staining intensity of p63	Histopathological grading of tumor			P value
	Grade I	Grade II	Grade III	
Mild	19(32.8)	0(0.0)	2(3.4)	0.0001[s]
Moderate	17(29.3)	11(19.0)	1(1.7)	
High	0(0.0)	6(10.3)	2(3.4)	
Total	36(62.1)	17(29.3)	5(8.6)	

*Chi-square test was done to measure the level of significance.
Figure within parentheses indicates in percentage.
s=statistically significant.

Among 36 patients with grade I, maximum cases (19) were found mild staining intensity and out of 17 cases of grade II, maximum patients (11) with moderate staining intensity, but in grade III cases, similar number of patients (2) were stained with mild and high intensity.

Table 9: Association between histopathological grading and final scoring of P63 expression.

Histopathological grading	Final scoring of P63 expression			P-Value*
	Grade I	Grade II	Grade III	
Low	23(39.6)	0(0.0)	02(3.45)	0.0001[s]
High	13(22.4)	17(29.3)	03(5.17)	
Total	36(62.1)	17(29.3)	5(8.6)	

*Chi-square test was done to measure the level of significance
Figure within parentheses indicates in percentage.
s=statistically significant.
Among the 58 patients, maximum were grade I with low P63 expression (39.6%) ¸but grade II and grade III carcinoma were high P63 expression respectively (29.3%) & (5.17%).So, there was Statistically significant association between histopathological grade and P63 expression.

DISCUSSION

In this study¸ it was observed that the age of the patients varied from 30-85 years. Out of 58 patients¸ 31% of samples belonged to age 51-60 years and mean ± SD age was 56.98 ± 12.96 years. Similarly¸ in Brazil, Lin, W.J., 2011 found in their study that the age range of 51-60 years (35.4%).[11] In India, Venkatesh et al., 2018 showed the age distribution ranged between 50-59 years. Some other investigators found a little higher age distribution.[12]

In the present study, it was observed that 51.7% of patients belong to males with a male-female ratio 1.07:1. These differences may be due to combined exposure of mutagenic substances like tobacco and betel nut in men. Similar findings were observed in some other studies. Saghravaniana et al., 2017 and Muzio et al., 2005, found most of the patient is male with a male-female ratio 1.04:1 and 1.7:1 respectively.[13,14] Monteiro et al., 2016 also observed male predominance with a male female ratio of 2.8:.[15]

In the present study, out of 58 cases, 28 patients (48.2%) had habits of smoking. Among them¸ 14 patients had habits of 20-30 years¸ and 7 patients used to smoke for more than 30 years. In the present study¸ 75.8% of patients had a habit of pan chewing¸ and betel nut; only 7% of patient had habit of alcohol consumption. In their study, Madani et al, 2010 found most of the patients had a smoking habits (35.7%), the habit of betel leaf (12.6%), alcohol consumption (30.3%).[16] Habit of chewing betel leaf and betel nut was observed more than drinking alcohol suggested that these habits are more common in our subcontinent.

In the present study, 62.1 % of patients belongs to grade I, 29.3% grade II and 8.6% grade III. Venkatesh et al., 2018 found in their study that 37.03 % of patients belong to grade I, 37.03 % grade II and 7% grade III. May be most of the patients diagnosed at grade I in our subcontinent due to early developed signs & symptoms of oral carcinoma¸ improved quality of life of the patient and the public awareness. But in some studies, Shetty, S.S., 2014 included samples 35 % grade I, 37% grade II and 26 % grade III patients. Muzio et al, 2005 included 34% samples of grade I, 39% grade II¸ and 26 % grade III patients.[17]

In this study among 58 patients, percentage of positive p63 expression showed 1-24% in 9 cases (15.5%), 25-75% in 32cases (55.2%) and >75% in 17cases (29.3%). 27 OSCC cases, <50% in 5cases (18.52%), 50-75% in 9 cases (33.33%) and >75% of positive tumor cells in 13cases (48.15%). Muzio et al., 2005 also found 25% in 33 cases, 30% in 36cases and >50% of positive p63 expression in 20 cases.[14]

In this study among 58 patients, intensity of p63 expression was mild in 21cases (36.2%), moderate in 29cases (50.0%) and high in 8 cases (13.8%). Patel et al., 2017 found, Out of 30 OSCC cases, 11 cases (36.7%) showed mild, 13 cases (43.3%) showed moderate, and 6 cases (20.0%) showed intense p63 expression.[18]

In final scoring of p63 expression, 56.9% cases found with high expression. In contrast¸ low expression of p63

was found among 43.1% cases. In this study, there was no negative p63 expression found in any cases.

In the present study, Among the 58 patients, maximum were grade I with low P63 expression in 23 cases(39.6%), but grade II and grade III carcinoma were high P63 expression respectively in 17 cases (29.3%) & 3cases (5.17%). The p-value was found to be highly significant ($p<0.001$) with the applied chi-square test.

In the present study, percentage of p63 immunopositive cells was evaluated in grade I, grade II and grade III SCC. The mean ± SD percentage of p63 positive cells was 42.16 ±15.81, 79.17 ±16.81 and 73.00 ± 33.54 in grade I, grade II and grade III SCC respectively. ANOVA was applied on the results to compare the percentage of p63 positive cells in different grades of OSCC. The p value was found to be highly significant ($p<0.001$).

In this study, grade 3 neoplasm showed two different patterns: 2 cases of completely dedifferentiated cancer generally exhibited low expression for p63 labelling, while high expression of p63 was generally observed in 3case of infiltrative cancer.

The reason behind this dissimilarity might be due to different isoform of p63 gene, small sample size, 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 higher grade of OSCC, whereas no significant relationship was detected between p63 expression and patient's age, gender, tumor location and other risk factors. These results can possibly advance our understanding of the initiating mechanisms, pathogenesis, and prognosis of OSCC and also result in novel therapeutic targets in cancer treatment.

CONCLUSION

In this study it was observed that, p63 overexpression significantly associated with higher grade of OSCC. So, p63 expression can be as reliable indicator considered of histological grading and an early marker of poor prognosis. It also highlighted the necessity of modification of therapies for the proper management of these carcinomas.

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