Original Article

Ki67 Antigen and P53 Protein Expression at Invasive Tumor Front of Oral Squamous Cell Carcinoma: An Observational Study

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Abstract

Background: Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity and is characterized by variable biological behavior. Evaluation of histopathological grading, along with immunohistochemical markers at the invasive tumor front, may provide valuable prognostic information on tumor aggressiveness and recurrence. The aim is to histologically grade OSCC using Broders' grading and Byrne's invasive front grading, and to assess the association of Ki-67 antigen and p53 protein expression at the invasive tumor front of OSCC. **Material and Methods:** This retrospective cross-sectional observational study was conducted in the Department of Pathology, Narayana Medical College and Hospital, Nellore, over a period of 2 years (October 2020 to October 2022), after obtaining Institutional Ethics Committee approval. A total of 74 biopsy-proven cases of oral squamous cell carcinoma (OSCC) received from the Departments of General Surgery, ENT, and Dental Surgery at Narayana Medical College and the Indian Red Cross Cancer Hospital were included in the study. Histopathological grading was performed using both Broder's and Byrne's grading systems. The immunohistochemical expression of Ki-67 and p53 was evaluated at the invasive tumor front. **Results:** Of 74 cases, females accounted for 60.8% of patients. Ulcer proliferative growth was the most common gross pattern (85.1%). Moderately differentiated squamous cell carcinoma was predominant (56.8%), and most tumors belonged to Bryne's Grade II (67.6%). High Ki-67 expression was observed in 71.9% of cases and was significantly associated with Broders' grade ($p < 0.0001$) and tumor recurrence ($p = 0.027$). Strong p53 expression (3+) was noted in 57.9% of cases and was significantly associated with higher histological grades ($p = 0.001$) and recurrence ($p = 0.004$). **Conclusion:** This observational study demonstrates that Ki-67 antigen and p53 protein expression at the invasive tumor front of oral squamous cell carcinoma correlate with histopathological aggressiveness and tumor behavior. Increased Ki-67 expression was significantly associated with higher Broders' grades and tumor recurrence, indicating enhanced proliferative activity in aggressive tumors. Similarly, strong p53 expression showed a significant association with advanced histological grades and recurrence, reflecting underlying genetic instability. Although the association between Ki-67 expression and Bryne's grading was not statistically significant, a progressive increase was observed with higher grades. Evaluation of Ki-67 and p53 at the invasive front provides valuable prognostic information and may aid in risk stratification and therapeutic planning.

Keywords: Oral squamous cell carcinoma; Ki-67; p53; Invasive tumor front; Bryne's grading; Broders' grading.

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Introduction

Cancer is the world's leading cause of morbidity today. Oral malignancies stand at the top six among all cancer types worldwide. India has the highest number of oral carcinomas, posing a severe health risk, and accounts for one-third of the overall global burden of oral carcinomas.[1] Over 77,000 new carcinomas and 52,000 deaths are reported in India each year, accounting for nearly one-fourth of global incidents. Because mouth cancer is one of the most common types of cancers in India, the increasing number of cases is a major issue for community health.

Oral Squamous Cell Carcinomas (OSCC) develop through a multistep process driven by genetic predisposition and environmental factors such as alcohol consumption, chronic inflammation, viral infection, and carcinogens in tobacco.[2] Tobacco use, alcohol consumption, and betel quid chewing with or without tobacco have all been implicated as major causes of oral cavity cancer. Carcinogens bind to human DNA, altering genes and causing mutations during replication and disrupting cellular growth control processes.

According to research, the tumour invasive front (TIF) is the best field for estimating growth fraction.[3] In the most advanced cancers, 3 to 6 tumour cell buds or tumour cell groups that have detached at the advancing edge of the OSCCs have been described as the deep invasive tumour front (ITF), where the

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innermost tumor buds are the most aggressive cells that influence prognosis.[4]

Bryne M. (1998) proposed that molecular and morphological characteristics at the deeper front of the invasive area of various OSCC may reflect tumor prognosis better than those in other areas of the cancer.[5]

Several investigations have found that the p53 gene and the Ki-67 protein are overexpressed in the deeper front of invasive OSCC. The goal of this study was to investigate any association between histologic tumor-front grading and Ki-67 antigen and p53 protein expression in OSCC.

Materials and Methods

Study Design: Retrospective, cross-sectional, Observational study

Place of Study: Narayana Medical College and Hospital, Nellore

Period of Study: It is conducted for a period of two years (Oct 2020 to Oct 2022)

Study Variables

- Ø Sex Distribution: Male and Female
- Ø Gross Tumor Morphology:
- Ø Histopathological Grading (Broders' Grade):
- Ø Invasive Tumor Front Grading (Bryne's Grade):
- Ø Ki-67 Immunohistochemical Expression:
- Ø p53 Immunohistochemical Expression:
- Ø Tumor Recurrence Status:
- Ø Recurrent cases
- Ø Non-recurrent cases

Sample Size: This study included a total of 74 cases of biopsy specimens of OSCC sent for histopathological examination

Inclusion Criteria

Biopsy and surgery specimens of oral carcinomas from ENT, General Surgery, and the IRCS Cancer Hospital, Nellore.

The specimens included OSCC ITF

Exclusion Criteria

Inadequate sampling and autolyzed samples.

Inadequate history in requisition form

Statistical Analysis: Data were entered and analyzed using SPSS version 25.0. Continuous variables were expressed as mean ± standard deviation, and categorical variables as frequencies and percentages. The prevalence of refractive errors was calculated, and associations with screen time and other risk factors were assessed using chi-square tests for categorical variables and independent t-tests or ANOVA for continuous variables. Correlation between screen time and severity of refractive errors was evaluated using Pearson's correlation coefficient. A p-value <0.05 was considered statistically significant.

Results

Table 1: Distribution with all parameters

		Frequency	Percentage (%)
Sex	Male	29	39.2
	Female	45	60.8
	Total	74	100
Gross	Exophytic Growth	4	5.4
	Fungating Growth	7	9.5
	Ulceroproliferative	63	85.1
	Total	74	100
Broders	Mod Diff Scc	42	56.8
	Poorly Diff Sarcomatoid Scc	2	2.7
	Poorly Diff Scc	5	6.8
	Vercus Varient Well Diff Scc	3	4.1
	Well Diff Scc	22	29.7
	Total	74	100
Brynes_Grade	Grade-I	7	9.5
	Grade-II	50	67.6
	Grade-III	17	23
	Total	74	100

Table 2: Association between Bryne's Grade and Ki-67 Grade (n = 57)

Bryne's Grade	Bryne's Grade	Low Ki-67	Intermediate Ki-67	High Ki-67	Total (n, %)	P Value
	Grade I	0 (0.0%)	2 (28.6%)	5 (71.4%)	7 (12.3%)	0.161
	Grade II	5 (12.8%)	9 (23.1%)	25 (64.1%)	39 (68.4%)	
	Grade III	0 (0.0%)	0 (0.0%)	11 (100.0%)	11 (19.3%)	
	Total	5 (8.8%)	11 (19.3%)	41 (71.9%)	57 (100.0%)	
Broders' Grade	Moderately Differentiated SCC	0 (0.0%)	0 (0.0%)	30 (100.0%)	30 (52.6%)	< 0.0001
	Poorly Differentiated Sarcomatoid SCC	0 (0.0%)	0 (0.0%)	2 (100.0%)	2 (3.5%)	
	Poorly Differentiated SCC	0 (0.0%)	0 (0.0%)	3 (100.0%)	3 (5.3%)	
	Verrucous Variant Well Differentiated SCC	0 (0.0%)	2 (66.7%)	1 (33.3%)	3 (5.3%)	
	Well Differentiated SCC	5 (26.3%)	9 (47.4%)	5 (26.3%)	19 (33.3%)	
	Total	5 (8.8%)	11 (19.3%)	41 (71.9%)	57 (100.0%)	
Recurrent Cases	No	5 (11.6%)	11 (25.6%)	27 (62.8%)	43 (75.4%)	0.027
	Yes	0 (0.0%)	0 (0.0%)	14 (100.0%)	14 (24.6%)	
	Total	5 (8.8%)	11 (19.3%)	41 (71.9%)	57 (100.0%)	

Table 3: Association between Bryne's Grade and p53 Grade (n = 57)

		p53 Negative n (%)	p53 1+ n (%)	p53 2+ n (%)	p53 3+ n (%)	Total n (%)	P-Value
Bryne's Grade	Grade I	2 (28.6%)	0 (0.0%)	3 (42.9%)	2 (28.6%)	7 (12.3%)	
	Grade II	14 (35.9%)	2 (5.1%)	2 (5.1%)	21 (53.8%)	39 (68.4%)	
	Grade III	1 (9.1%)	0 (0.0%)	0 (0.0%)	10 (90.9%)	11 (19.3%)	
	Total	17 (29.8%)	2 (3.5%)	5 (8.8%)	33 (57.9%)	57 (100.0%)	
Broders' Grade	Moderately Differentiated SCC	5 (16.7%)	0 (0.0%)	0 (0.0%)	25 (83.3%)	30 (52.6%)	0.001
	Poorly Differentiated Sarcomatoid SCC	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (100.0%)	2 (3.5%)	
	Poorly Differentiated SCC	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (100.0%)	3 (5.3%)	
	Verrucous Variant Well Differentiated SCC	2 (66.7%)	0 (0.0%)	0 (0.0%)	1 (33.3%)	3 (5.3%)	
	Well Differentiated SCC	10 (52.6%)	2 (10.5%)	5 (26.3%)	2 (10.5%)	19 (33.3%)	
	Total	17 (29.8%)	2 (3.5%)	5 (8.8%)	33 (57.9%)	57 (100.0%)	
		p53 Negative n (%)	p53 1+ n (%)	p53 2+ n (%)	p53 3+ n (%)	Total n (%)	

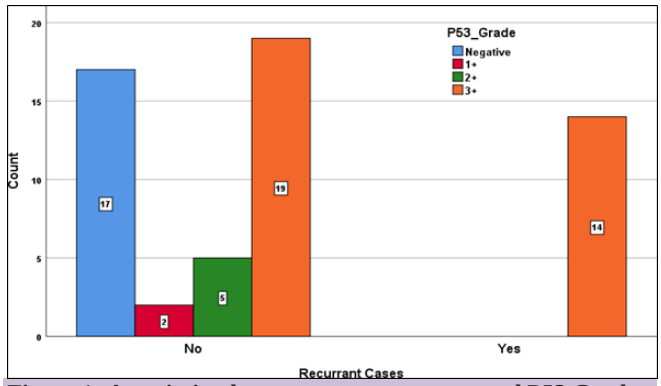

Figure 1: Association between recurrent cases and P53 Grade

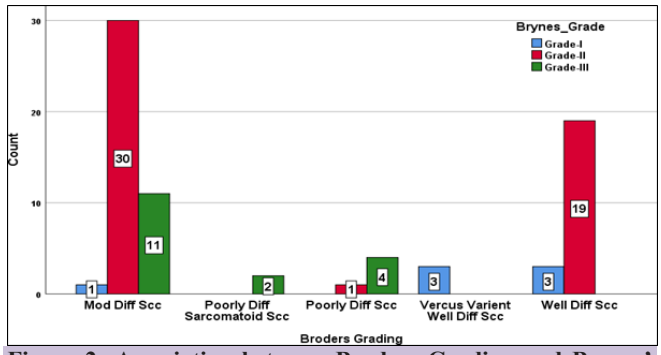

Figure 2: Association between Broders Grading and Brynes' Grade

In the current study, 74 patients were included. Most of the study population was female, with 50 cases (60.8%) and 29 cases (39.2%) male.

Regarding gross tumor morphology, the most prevalent was ulceroproliferative (63 patients, 85.1%). 7 patients had fungating growth. (9.5%), while exophytic growth was the least common presentation, observed in 4 patients (5.4%).

The most common histological subtype, according to Broders' grading, was moderately differentiated squamous cell carcinoma in 42 cases (56.8%). 22 patients (29.7%) had well-differentiated squamous cell carcinoma. Cases of poorly differentiated squamous cell carcinoma totalled 5 (6.8%), and 3 (4.1%) were squamous cell carcinoma, verrucous type. Sarcomatoid squamous cell carcinoma, a poorly differentiated subtype, was the least common subtype, with 2 patients (2.7%) showing it.

According to Byrne's grading system, most patients were Grade II, totaling 50 (67.6%). The highest number, 17 patients (23.0%), was Grade III, and the lowest, 7 patients (9.5%), was Grade I.

The correlation between the grade of Bryne and Ki-67 was tested in case 57. In Grade I tumors, only 5 cases (71.4%) had high Ki-67 expression, and only 2 cases (28.6%) had intermediate Ki-67 expression; no cases had low Ki-67 expression. High Ki-67 expression was observed in Grade II tumours, with 25 cases (64.1) showing high expression, 9 cases (23.1) showing intermediate expression, and 5 cases (12.8) showing low expression. All Grade III tumors showed high Ki-67 expression (11 cases, 100.0%). However, the association between Byrne's grade and Ki-67 expression was not statistically significant (p = 0.161).

A statistically significant association was observed between Broders' histopathological grade and Ki-67 expression (p < 0.0001). All cases of moderately differentiated squamous cell carcinoma demonstrated high Ki-67 expression (30 cases, 100.0%). Similarly, all poorly differentiated sarcomatoid squamous cell carcinoma (2 cases, 100.0%) and poorly differentiated squamous cell carcinoma (3 cases, 100.0%) showed high Ki-67 expression. In contrast, the verrucous variant of well-differentiated squamous cell carcinoma predominantly showed intermediate Ki-67 expression in 2 cases (66.7%), with high expression in 1 case (33.3%). In poorly differentiated squamous cell carcinomas, Ki-67 staining was irregular, with 9 cases (47.4%) exhibiting intermediate expression, 5 cases (26.3%) low expression, and 5 cases (26.3%) high expression.

In 57 patients, the correlation between tumor recurrence and Ki-67 expression was studied. In non-recurrent cases, the expression of Ki-67 was found to be high in 27 cases (62.8), intermediate expression was observed in 11 cases (25.6), and low expression was observed in 5 cases (11.6). Interestingly, all recurring cases exhibited high Ki-67 expression (14 cases, 100.0%), and none showed low or intermediate expression. This correlation between recurrence and Ki-67 proficiency was statistically significant (p = 0.027).

The correlation of Bryne grade and the p53 expression was studied in 57 cases. The p53 expression in Grade I tumors was heterogeneous: 3 cases (42.9%) were p53-positive, whereas 2

cases (28.6% each) were p53-negative and 2 cases (28.6% each) were p53 3+. In Grade II tumors, 21 cases (53.8) were covered in the p53 3+ and 14 cases (35.9) in the p53-negative expression, but 2 cases (5.1) in p53 1+ and 2 cases (5.1) in p53 2+. Strong p53 expression was observed in Grade III tumors, with 10 cases (90.9%) showing p53 3+ positivity and none showing p53 negativity. On the whole, the better the grades were, the greater the p53 expression, which suggested a tendency toward more severe tumors.

The correlation between p53 expression and Broders' histopathological grade was statistically significant (p = 0.001). The majority of moderately differentiated squamous cell carcinoma cases demonstrated strong p53 positivity, with p53 3+ expression observed in 25 cases (83.3%), while the remaining 5 cases (16.7%) were p53-negative. All cases of poorly differentiated sarcomatoid squamous cell carcinoma (2 cases, 100.0%) and poorly differentiated squamous cell carcinoma (3 cases, 100.0%) exhibited p53 3+ expression. In contrast, the verrucous variant of well-differentiated squamous cell carcinoma predominantly showed p53-negative expression in 2 cases (66.7%), with p53 3+ expression in 1 case (33.3%). Among well-differentiated squamous cell carcinoma cases, p53 expression was variable: p53-negative in 10 cases (52.6%), p53 2+ in 5 cases (26.3%), and p53 1+ or 3+ in 2 cases (10.5%).

Of 17 cases with negative P53 grade, none were recurrent. In 2 cases with 1+ P53 grade, none had recurrent disease. For 5 cases with 2+ P53, none were recurrent. For 33 cases of 3+ P53 grade, 42.4% of cases were tobacco use. The association between P53 grade and recurrent cases was statistically significant (P = 0.004).

According to Byrne's grade, among the 7 cases of Grade I, 42.9% were verrucous variant well-differentiated squamous cell carcinoma (SCC), while 42.9% were well-differentiated SCC. Among the 50 cases of Grade II, the majority (60.0%) had moderately differentiated SCC, followed by well-differentiated SCC. Among the 17 cases of Grade III, 64.7% of cases had moderately differentiated SCC, followed by poorly differentiated SCC. The association between Broders' grading and Bryne's grade was found to be statistically significant (p < 0.0001).

Discussion

In the present study, female patients constituted the majority (60.8%), which contrasts with the traditionally reported male predominance in oral squamous cell carcinoma. However, similar trends of increasing female incidence have been reported by Sharma et al,[6] and Gupta et al,[7] who attributed this shift to rising tobacco chewing habits and betel quid use among females in South Asian populations. This is a demographic trend that points to the changing epidemiology of oral cancer.

The predominant gross appearance in the current research was the ulceroprolliferative growth (85.1%). This observation is consistent with those of Patel et al. [8] and Rao et al. [9], who reported that over 70% of cases of oral squamous cell carcinoma exhibited ulcerobacterial lesions. Morphology of this type is considered a marker of aggressive local invasion and late clinical presentation.

Histological subtype imaging showed that moderately differentiated squamous cell carcinoma was the most common (56.8%), followed by well-differentiated carcinoma (29.7%). Singh et al,[10] and Kaur et al,[11] also reported similar distributions, but identified moderately differentiated tumors as the most common. This prevalence might represent tumor progression at diagnosis, which should place greater emphasis on early detection practices.

The Grade II tumors accounted for the majority (67.6) of the current study, with Grade III tumors accounting for the lesser (23.0) part. Similar results were reported by Anneroth et al,[12] who found that more intermediate grades occur in oral squamous cell carcinoma. By adding invasive front features, Byrne's higher grading provides better prognostic value than traditional grading systems.

Although there was an increase in Ki-67 expression as the grade of Bryne increased, this difference was not statistically significant (p = 0.161). The same was found by Verma et al,[13] who showed that proliferative indices were higher in advanced Bryne grade, non-significantly, as observed. This implies that although Ki-67 is a marker of proliferative activity, other tumor microenvironmental factors may have influenced its expression.

A statistically significant difference between Broders' grade and high Ki-67 expression (p < 0.0001) was observed, and all children with poorly or moderately differentiated carcinoma had high Ki-67 expression. This observation is consistent with the literature of Sharma et al,[6] and Patel et al,[8] who reported high Ki-67 indices with high histological grades, indicating that it can be used as an indicator of tumor aggressiveness.

High Ki-67 expression (100%) was observed in all recurrent cases, and there was a statistically significant relationship (p = 0.027). Gupta et al,[7] and Singh et al,[10] also reported similar results, and the findings revealed that high Ki-67 expression correlates with worse recurrence rates and serves as an effective prognostic biomarker in practice.

There was an increasing trend of p53 overexpression associated with increasing grades of Bryne, namely Grade III, with mucinous p53 3+ expression predominant (90.9%). Similar findings have been reported by Anneroth et al,[7] and Kaur et al,[6] who claim that p53 is associated with tumor aggressiveness and invasiveness.

A significant correlation between Broders' grade and p53 expression was found (p = 0.001), with higher grades showing stronger p53 negativity. Similar findings from Rao et al,[4] and Verma et al,[8] support the presence of p53 changes during dedifferentiation and tumor progression.

In the current research, no cases with p53-negative expression, p53 1+, or p53 2+ expression were recurrent, whereas 42.4% of p53 3+ cases were recurrent, and this association was statistically significant (p = 0.004). These results are in line with those of Gupta et al,[2] and Patel et al,[3] who found that aggressive tumor behavior and recurrence are predictive of strong p53 expression.

The grade and the grading of Bryne and Broders were also found to be statistically significantly associated (p < 0.0001). Grade I tumours were mainly well-differentiated and verrucous, whereas Grade III tumours had a greater proportion of

moderately and poorly differentiated carcinomas. The complementary prognostic utility of these grading systems when used together was also demonstrated by Simar Drive and Anneroth et al.[7]

Conclusion

This observational study reveals that Ki-67 antigen and p53 protein expression at the invasive tumor front of oral squamous cell carcinoma are associated with histopathological aggressiveness and tumor behaviour. The high Ki-67 expression rate was strongly associated with high Broders grades and tumor recurrence, indicating high proliferation in aggressive tumors. Likewise, high p53 expression was strongly correlated with high histological grade and recurrence, indicating inherent genetic instability. Although the correlation between Ki-67 expression and the grades assigned by Byrne was not significant, there was a progressive increase with the grades. Intrinsic assessment of Ki-67 and p53 at the invasive front will provide useful prognostic data and may support risk stratification and treatment planning.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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