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Original Research Article

CORRELATION OF HISTOPATHOLOGIC PROGNOSTIC INDICATORS WITH P16 AND CD56 IMMUNOHISTOCHEMISTRY IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

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ABSTRACT

Background: Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide and attributed to consumption of tobacco, alcohol abuse or infection with human papilloma virus (HPV). **Objective:** This research aims to study the histomorphological spectrum of different grades of HNSCC and correlate the histopathologic prognostic indicators of HNSCC with p16 and CD56 immunohistochemistry (IHC).
Materials and Methods: 60 cases of resected or excised specimens of HNSCC were included in this study. Cases with history of immunotherapy/chemotherapy or radiotherapy were excluded. Histomorphological spectrum was studied from formalin fixed, paraffin embedded and H&E stained tissue sections following which p16 and CD56 IHC was perfomed. Results were recorded and statistically analysed.
Results: Statistically significant positive correlations of higher histologic grades with greater depth of invasion ($p = 0.037$), WPOI 5 ($p = 0.012$), and the presence of perineural invasion ($p = 0.012$) were demonstrated. The analysis for p16 expression revealed an inverse association with lymphovascular invasion that approached statistical significance ($p=0.055$). CD56 was positive in 25.0% of cases and showed limited utility as a standalone prognostic indicator in this study.
Conclusions: The study supports the prognostic significance of traditional histopathologic indicators in HNSCC. It also points out the association of p16 expression (associated with viral etiology) with better prognosis as compared to HNSCC attributed to etiologies other than viral oncogenesis. This study also highlights that by further research towards the development of newer therapies, there still lies more possibility of improving survival of HNSCC patients.
Keywords: squamous cell carcinoma, histopathologic indicators, head and neck cancer, p16, CD56, prognosis.

INTRODUCTION

Head and neck cancers are attributable to consumption of tobacco, alcohol abuse and also infection with human papilloma virus (HPV), primarily HPV-16.[1] It has been established that the overall survival in patients of HNSCC is found to be related to some histopathologic prognostic factors such as histologic type, histologic grade (well, moderately, or poorly differentiated), depth of invasion(DOI), lymphovascular invasion(LVI), PNI(perineural invasion) ,WPOI (Worst Pattern Of Invasion) , regional lymph node status and pathologic stage.[2] Accurate prognosis is paramount in HNSCC management, influencing treatment planning, patient surveillance, and informed decision-making.[3] This study investigates the correlation between established histopathological prognostic indicators in

HNSCC with immunohistochemical markers p16 and CD56.

MATERIALS AND METHODS

This observational cross-sectional study was conducted at the Department of Pathology, Subharti Medical College and associated Chhatrapati Shivaji Subharti Hospital (CSSH), Meerut, from July 2023 to February 2025. The study included 60 cases of resected or excised specimens of HNSCC and excluded cases with a history of prior immunotherapy, chemotherapy, or radiotherapy. Ethical approval and informed consent of patients were obtained. Data on patient demographics, clinical characteristics, and a comprehensive panel of histopathological prognostic parameters were systematically collected. The parameters for study included histologic grade, depth of invasion, lymphovascular invasion, perineural invasion, worst pattern of invasion, lymph node metastasis, and extranodal extension. Immunohistochemical staining was performed on representative sections for p16 and CD56. The specimens were fixed using 10% neutral buffered formalin and subjected to histopathological processing followed by embedding in paraffin wax. Sectioning was done with the help of microtome and sections stained using H&E stain. Mounting (with DPX) and labelling of slides was done. During histopathology examination of H&E stained sections, blocks for IHC application were selected. P16 and CD56 IHC was performed on slides prepared from selected blocks. The Chi-square test or Fisher's exact test was used to analyse the association between histopathological prognostic indicators and IHC markers. A p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 60 cases of HNSCC were studied for histopathologic prognostic indicators and their correlation with each other was established. Relationship between histomorphology and IHC was also analysed. The study population was constituted predominantly by males, that is 90.0% (54 patients), while only 6 patients (10.0%) in the study were females. The majority of the patients (83.3%, n=50) in the study had a history of either smoking and/or tobacco use and/or alcohol abuse while only 10 patients (16.7%) reported no history of either.

The histological grading of the tumors in the 60 cases revealed that the majority (85.0%, n=51) of tumors were moderately differentiated. A smaller proportion (11.7%, n=7) were well differentiated, and only 2 cases (3.3%) were classified as poorly differentiated.

Table 1: The interpretation of IHC / positivity criteria

Immunomarkers	Interpretation
P16	Positive staining is defined as *block staining*, i.e., when there is a continuous segment of atleast 10-20 cells with strong nuclear and cytoplasmic expression. In squamous epithelium, block positivity needs to involve basal and parabasal layers Overexpression correlates with oncogenic HPV infection. Cytoplasmic only staining , diffuse weak intensity staining and other focal / patchy patterns was considered negative.
CD56	Membranous expression is interpreted as positive staining.

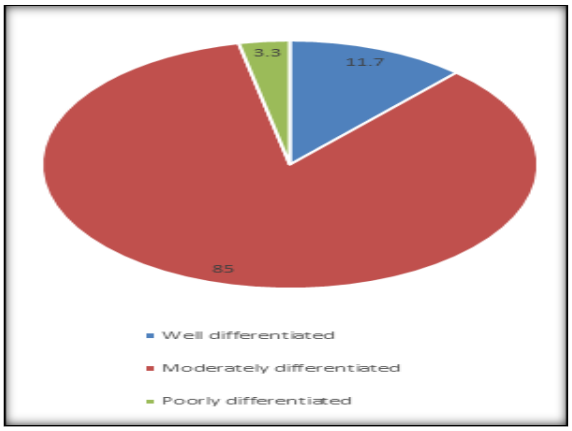

Figure 1. Pie chart showing distribution of cases according to the histologic grade of tumour

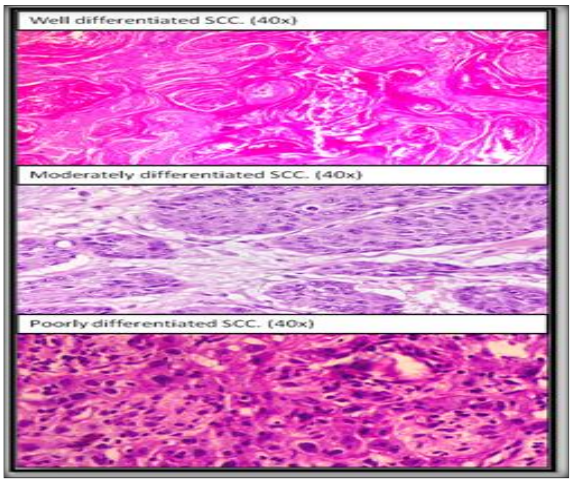

Figure 2: Photomicrographs of grades of HNSCC (40x)

The following tables depict the relationship between histologic grades and histopathologic prognostic indicators. [Table 2,3]

Using Spearman's rank correlation coefficients (ρ) for examining the relationships between several variables and histologic grade, the analysis reveals that Depth of Invasion in mm ($\rho = 0.269$, $p = 0.037$), WPOI ($\rho = 0.322$, $p = 0.012$), and PNI ($\rho = 0.323$, $p = 0.012$) show statistically significant positive correlations with histologic grade. This indicates that higher histologic grades are significantly associated with greater Depth of Invasion, higher WPOI, and the presence of PNI. In contrast site of tumour, focality, largest tumour dimension (cms.), lymph node

metastasis, LVI and ENE did not demonstrate statistically significant correlations with histologic grade ($p > 0.05$) in our study, suggesting these factors are not significantly associated with histologic grade in this sample. [Table 2, 3]

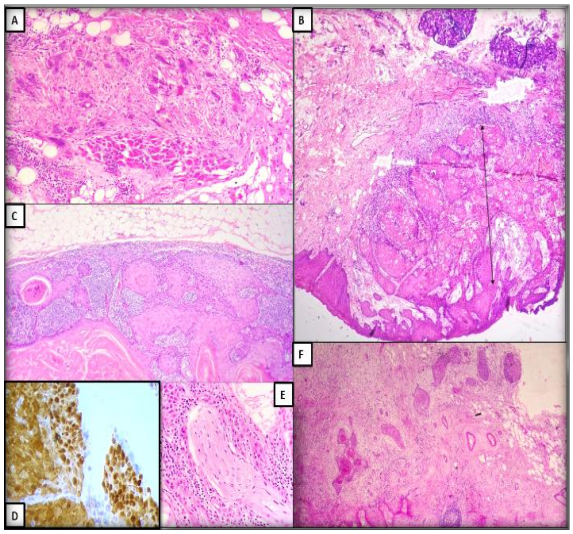

Figure 3 (A)WPOI 5(10X), (B)Measurement of Depth of invasion(10X),(C)Lymph node metastasis(10x), (D)p16 IHC showing block positivity(40x),(E)Perineural invasion(40x),(F)Extranodal extension (10X).

Positive expression for p16 was identified in 19 patients (31.7%), while the remaining 41 patients (68.3%) were negative for this marker. For the CD56 marker, positive expression was observed in 15 cases (25.0%), with the majority of cases (n=45, 75.0%) demonstrating negative expression.

The overall chi-square analysis confirmed that there was no statistically significant relationship between the histologic grade of the tumour and the expression status of the CD56 marker within the studied cohort ($\chi^2=0.721$, $p=0.697$).

The analysis revealed that the rate of p16 positivity varied across the different grades. The highest proportion of expression was observed in well-differentiated tumours, where 57.1% (n=4) of the 7 cases were p16 positive. In comparison, 29.4% (n=15) of the 51 moderately differentiated tumours were positive for p16. No p16 expression (0.0%) was observed in either of the 2 cases classified as poorly differentiated carcinoma. Although a trend of decreasing p16 positivity with increasing histologic grade was noted, the overall association was not found to be statistically significant based on the chi-square test ($\chi^2=3.146$, $p=0.207$). [Table 4]

A notable finding was observed in the correlation with lymphovascular invasion (LVI). All 7 cases (100.0%) that were positive for LVI were found to be negative for p16 expression. In contrast, among the 53 cases where LVI was absent, 19 (35.8%) were p16 positive. This inverse association approached, but did not reach, statistical significance ($\chi^2=3.672$, $p=0.055$). No statistically significant correlations were identified for any other parameters, including focality ($p=0.327$), depth of invasion ($p=0.557$), largest tumour dimension ($p=0.204$), worst pattern of invasion ($p=0.405$), lymph node metastasis ($p=0.428$), perineural invasion ($p=0.710$), or extranodal extension ($p=0.405$). [Table 5]

Table 2: Relationship between histologic grade and histopathologic prognostic indicators

		Histologic Grade				
Factor	**Category**	**Well differentiated (n, %)**	**Moderately differentiated (n, %)**	**Poorly differentiated (n, %)**	**Chi-Square Value**	**p-value**
Focality	Unifocal	7 (12.1)	49 (84.5)	2 (3.4)	0.365	0.833
	Multifocal	0 (0.0)	2 (100.0)	0 (0.0)		
Categories of DOI in mm	≤5	5 (23.8)	16 (76.2)	0 (0.0)	5.482	0.241
	>5 to ≤10	1 (4.8)	19 (90.5)	1 (4.8)		
	>10	1 (5.6)	16 (88.9)	1 (5.6)		
Largest tumor dimension in cms	≤2.0	3 (18.8)	12 (75.0)	1 (6.3)	2.278	0.685
	>2.0 - ≤4.0	3 (10.3)	25 (86.2)	1 (3.4)		
	>4.0	1 (6.7)	14 (93.3)	0 (0.0)		
WPOI	1-4	7 (14.6)	41 (85.4)	0 (0.0)	9.755	0.008
	5	0 (0.0)	10 (83.3)	2 (16.7)		
Lymph Node Metastasis	Absent	5 (13.9)	31 (86.1)	0 (0.0)	3.394	0.183
	Present	2 (8.3)	20 (83.3)	2 (8.3)		
LVI	Absent	7 (13.2)	44 (83.0)	2 (3.8)	1.398	0.497
	Present	0 (0.0)	7 (100.0)	0 (0.0)		
PNI	Absent	7 (15.2)	39 (84.8)	0 (0.0)	8.703	0.013
	Present	0 (0.0)	12 (85.7)	2 (14.3)		
ENE	Absent	7 (13.0)	45 (83.3)	2 (3.7)	1.176	0.555
	Present	0 (0.0)	6 (100.0)	0 (0.0)		

Table 3: Spearman's Rank Correlation Coefficients with Histologic Grade of HNSCC

Variable	Correlation Coefficient (ρ)	Significance (p-value)
Site of tumour	0.099	0.454
Focality	0.043	0.743
Largest tumour dimension (cms.)	0.059	0.655
Depth of Invasion in mm	0.269	0.037
Worst Pattern of Invasion	0.322	0.012
Lymph node metastasis	0.174	0.183
Lymphovascular Invasion	0.085	0.52
Perineural Invasion	0.323	0.012
Extranodal Extension	0.078	0.556

Table 4: Association of Histologic Grade with p16 Immunohistochemical Expression

Factor	Category	P16		Chi square value	p-value
		Negative (n, %)	Positive (n, %)		
Grade of tumour	Well differentiated	3 (7.3)	4 (21.1)	3.146	0.207
	Moderately differentiated	36 (87.8)	15 (78.9)		
	Poorly differentiated	2 (4.9)	0 (0.0)		
Total		41 (100.0)	19 (100.0)		

Table 5: Association of prognostic indicators with p16 Expression in HNSCC

Variable	Category	Total Cases (N)	p16 Negative (n, %)	p16 Positive (n, %)	Chi-Square (χ^2) Value	p-value
Focality	Unifocal	58	39 (67.2%)	19 (32.8%)	0.959	0.327
	Multifocal	2	2 (100.0%)	0 (0.0%)		
Depth of Invasion (DOI)	≤5 mm	21	13 (61.9%)	8 (38.1%)	1.17	0.557
	>5 to ≤10 mm	21	14 (66.7%)	7 (33.3%)		
	>10 mm	18	14 (77.8%)	4 (22.2%)		
Largest Tumour Dimension	≤2.0 cm	16	9 (56.3%)	7 (43.8%)	3.176	0.204
	>2.0 - ≤4.0 cm	29	23 (79.3%)	6 (20.7%)		
	>4.0 cm	15	9 (60.0%)	6 (40.0%)		
Worst Pattern of Invasion (WPOI)	1-4	48	34 (70.8%)	14 (29.2%)	0.693	0.405
	5	12	7 (58.3%)	5 (41.7%)		
Lymph Node Metastasis	Absent	36	26 (72.2%)	10 (27.8%)	0.629	0.428
	Present	24	15 (62.5%)	9 (37.5%)		
Lymphovascular Invasion (LVI)	Absent	53	34 (64.2%)	19 (35.8%)	3.672	0.055
	Present	7	7 (100.0%)	0 (0.0%)		
Perineural Invasion (PNI)	Absent	46	32 (69.6%)	14 (30.4%)	0.138	0.71
	Present	14	9 (64.3%)	5 (35.7%)		
Extranodal Extension (ENE)	Absent	54	36 (66.7%)	18 (33.3%)	0.693	0.405
	Present	6	5 (83.3%)	1 (16.7%)		

DISCUSSION

In the present study significant associations were established between the histologic grade of the tumours and parameters such as increased depth of invasion, WPOI 5, and the presence of perineural invasion. The analysis of p16 and CD56 expression further elucidate the relationship between these markers and the observed histopathologic parameters, with the potential to refine prognostic stratification in HNSCC.

Chang et al. (2019),[4] studied DOI as an adverse prognostic factor in oral squamous cell carcinoma. Another study on a DOI of 4-5 mm in tongue SCC as a predictor for occult metastases was conducted by Brandwein-Gensler et al. (2010).[5] Dolens et al. (2021),[2] conducted a meta-analysis to study if DOI shows any significant association with poor survival. In the present study, DOI >5mm was found in a substantial proportion of cases (65%) and such tumours were more frequently moderately differentiated.

Lymphovascular invasion was identified in 11.7% of the HNSCC cases in our study. Studies by Chang et al. (2019),[4] Dolens et al. (2021),[2] also attempted to study whether LVI was significantly associated with an increased risk of poor survival outcomes or not.

Brandwein-Gensler et al. (2010),[5] included PNI in their proposed Risk Model, and Chang et al. (2019),[4] studied PNI as a prognosticator of overall survival. Perineural invasion was observed in 23.3% out of total patients in our study and was found to be significantly associated with poorer grades of HNSCC.

Brandwein-Gensler et al. (2010),[5] highlighted WPOI as a component of their multiparameter histological predictive model, and Raja Sekhar et al. (2022),[6] incorporated WPOI into their prognostic H-score for OSCC. In our analysis, WPOI was significantly associated with histologic grade (p=0.008). All cases demonstrating the most aggressive WPOI 5 were either moderately (83.3%) or poorly differentiated (16.7%), while no well-differentiated tumours were found in this category.

Regional lymph node metastasis was present in 40.0% of the HNSCC patients in our research and poorly differentiated tumours were only observed in node-positive patients (8.3%). Khan et al. (2022),[7] also observed a significant correlation between higher histological grades and LNM. Chang et al. (2019),[4] and Dolens et al. (2021),[2] conducted research on ENE for its prognostic value.In the present study, all 6 cases exhibiting ENE were moderately differentiated tumours.

Spearman's rank correlation revealed that higher histologic grades were associated with greater depth of invasion ($\rho = 0.269$, $p = 0.037$), WPOI 5 ($\rho = 0.322$, $p = 0.012$), and the presence of perineural invasion ($\rho = 0.323$, $p = 0.012$). Furthermore, Chi-square analysis confirmed significant associations between histologic grade and both WPOI ($p = 0.008$) and PNI ($p = 0.013$). Similar findings showing clustering of adverse features with higher histologic grade were seen in the study by Lin et al. (2020).[8]

In the current study, p16 positivity was observed in 31.7% of cases (n=19). p16 positivity was noted in 22.9% cases in the study by Hashmi et al. (2020).[9] Researches by Pandey et al. (2021),[10] and Ralli et al. (2016),[11] respectively showed p16 positivity in 60% and 78.7% cases. A notable finding in our analysis was the inverse association between p16 expression and lymphovascular invasion (LVI), which approached statistical significance (p=0.055). All the 7 cases exhibiting LVI were negative for p16, suggesting that p16-positive tumours may be less likely to exhibit this specific mode of invasion, a finding that aligns with more favourable prognosis associated with HPV-positive HNSCC.

A key component of the TME is the presence of tumour-infiltrating lymphocytes, which reflect the host's immune response against the cancer. Several studies have been conducted to look for a link between immune infiltrate and prognosis in HNSCC. For instance, the research conducted by Suzuki et al. (2022),[12] on patients treated with chemoradiotherapy, to study the interplay between tumour morphology and the host immune response and its role in determining patient outcomes. CD56 (NK cell marker and indicator of tumor microenvironment) expression was identified in 25.0% of the HNSCC cases (n=15) in our study.

CONCLUSION

HNSCC constitutes a significant global health burden, with its incidence influenced by risk factors such as tobacco and alcohol use. Prognostication in HNSCC is complex, as traditional staging systems often fail to capture the full spectrum of tumour behaviour and biological heterogeneity. This limitation highlights a critical need for reliable prognostic biomarkers that can refine risk stratification and guide personalized treatment strategies. Therefore, the primary aim of this study was to correlate established histopathological prognostic indicators in HNSCC with the immunohistochemical expression of p16 and CD56.

A statistically significant positive correlation was found between a higher histologic grade and several adverse prognostic indicators. Using Spearman's rank correlation, histologic grade was significantly associated with greater depth of invasion (p=0.037), a worse pattern of invasion (p=0.012), and the presence of perineural invasion (p=0.012). Chi-square analysis further confirmed the significant association between histologic grade and both WPOI (p=0.008) and PNI (p=0.013).

The investigation of immunohistochemical markers provided additional, nuanced information. Expression of p16 was found in 31.7% of cases, and its inverse association with lymphovascular invasion (p=0.055) suggests a potential link to a less invasive phenotype. CD56 was positive in 25.0% of cases and showed limited utility as a standalone prognostic indicator. Further research with larger sample size is needed to find correlations between tumor microenvironment and HNSCC prognosis.

Ethics -This study was approved by the university ethical review committee . Informed consent was obtained from all patients for being included in the study.

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Conflicts of interest: There are no conflicts of interest.

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