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Pathology Section

Immunohistochemical Expression of p16 and p53 as Prognostic Indicator in Oral Squamous Cell Carcinoma: A Cross-sectional Study

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ABSTRACT

Introduction: Head and Neck Squamous Cell Carcinoma (HNSCC) is the sixth most common cancer globally and the seventh most common cause of cancer-related mortality. Tobacco use, alcohol consumption, and Human Papillomavirus (HPV) infection are prominent risk factors for HNSCC. HPV-positive Oral Squamous Cell Carcinoma (OSCC) differs from HPV-negative OSCC in terms of risk factors, preferential site of origin, age, histomorphological features, molecular genetic alterations, and prognosis. The prominent basaloid morphology and lobular growth of OSCCs are associated with p16 positivity and p53 negativity, respectively.

Aim: To establish the immunohistochemical expression of p16 (p16INK4a) and p53 in OSCC and to assess their relationship with specific histomorphological features, in the form of solid growth of cells in a lobular configuration, small crowded cells with scant cytoplasm, dark hyperchromatic nuclei without nucleoli.

Materials and Methods: The cross-sectional study involved fifty cases of OSCC over a two-year period from January 2017 to January 2019 at Army Hospital (R and R) Delhi Cantt. The intensity of p16 and p53 protein expression was graded as follows: no staining (0), weak staining (1), moderate staining (2), and strong staining (3). The proportion/percentage of staining for p16 and p53 protein expression was calculated as follows: 1-4% (1), 5-19% (2), 20-39% (3), 40-59% (4), 60-79% (5), and 80-100% (6) cells stained. A quick score of 0-1 (negative), 2-3 (weak positive), 4-5 (moderate positive), and >6 (strong positive) was assessed. Cross tables were generated and the Chi-square test was used for testing associations. The Statistical Software for Data Science (STATA)-14 was used for statistical analysis.

Results: A total of 50 cases of OSCC were analysed for histomorphological features and immunohistochemical patterns of p16 and p53. The age distribution showed that 8 (16%), 9 (18%), 18 (36%), 13 (26%), and 2 (4%) of the patients were in the age groups of 31-40 years, 41-50 years, 51-60 years, 61-70 years, and above 70 years, respectively. The gender distribution noted 42 (84%) males and 8 (16%) females. Genital and non-genital mucosa are usually involved by HPV subtypes 6, 11, 16, 18, and 16, 18, 11, 13, 2, respectively. HPV-16 has been demonstrated in 90-95% of all HPV-positive HNSCC cases, followed by HPV-18, HPV-31, and HPV-33. p53 is considered the guardian of the genome and controls the expression and activity of proteins involved in cell cycle regulation, DNA repair, cellular senescence, and apoptosis. More than 50% of all primary HNSCC exhibit p53 mutation.

Conclusion: A significant correlation was observed between age, dysplasia, keratinisation, basaloid morphology versus p16 expression, and lobular growth, histological grade versus p53. An inverse relationship between p16 and p53 expressions was observed. The immunohistochemical expression of p16 as an immunohistochemical marker of HPV, along with p53, is recommended. Due to the constraint of the study period, the survival of the patients could not be assessed in correlation with p16 and p53 expression.

Keywords: Basaloid pattern, Head and neck squamous cell carcinoma, Immunohistochemistry, Lobular growth

INTRODUCTION

The HNSCC is extremely heterogeneous and the sixth most common cancer worldwide with variable clinical presentations, and it is the seventh most common cause of cancer-induced mortality [1-3]. According to the World Health Organisation (WHO), Squamous Cell Carcinoma (SCC) accounts for more than 90% of malignant tumours of the oral cavity and oropharynx [4].

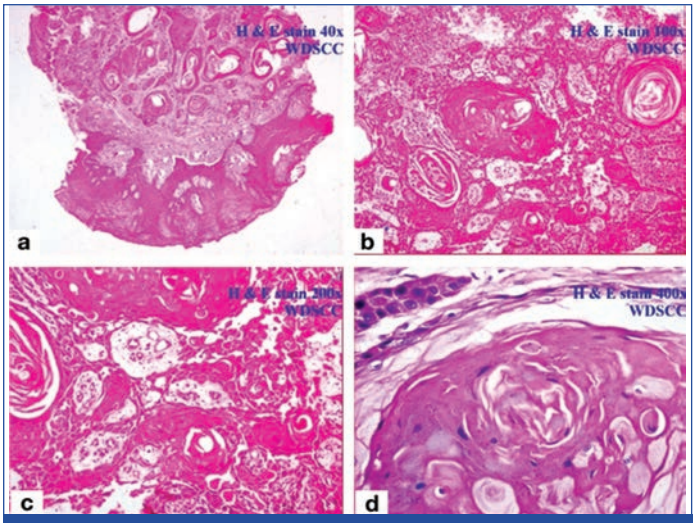
The p53 is considered the guardian of the genome and controls the expression and activity of proteins involved in cell cycle regulation, Deoxyribose Nucleic Acid (DNA) repair, cellular senescence, and apoptosis [5]. Recently, there has been an increasing trend of OSCC in young age, less tobacco and alcohol consumption, with more poorly differentiated histopathology. However, such cases usually respond to conventional radiotherapy treatment and have better survival, as reported by Gupta S et al., [6-9]. This study aims to establish the immunohistochemical expression of p16 (p16INK4a) along with p53 in OSCC and assess their relationship with specific histomorphological features.

MATERIALS AND METHODS

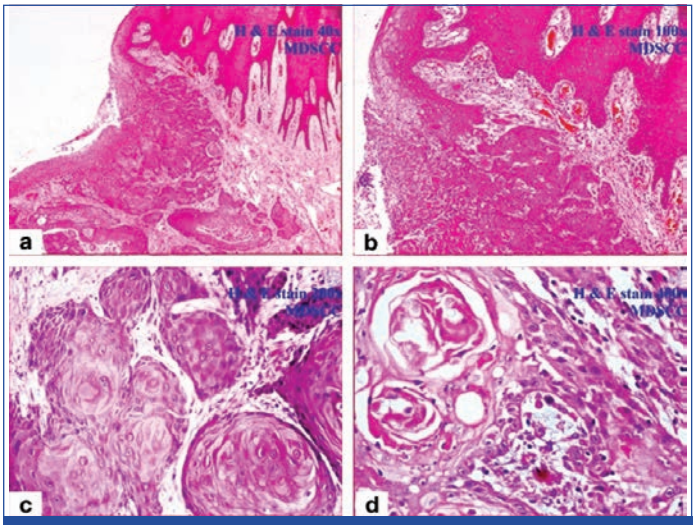
This cross-sectional study comprised fifty cases of OSCC conducted from January 2017 to January 2019 at Army Hospital (R&R) Delhi Cantt, New Delhi, India after taking Ethics Committee Approval from the institute (IEC: AHRR/PG/Pathology/NS 2017).

Procedure

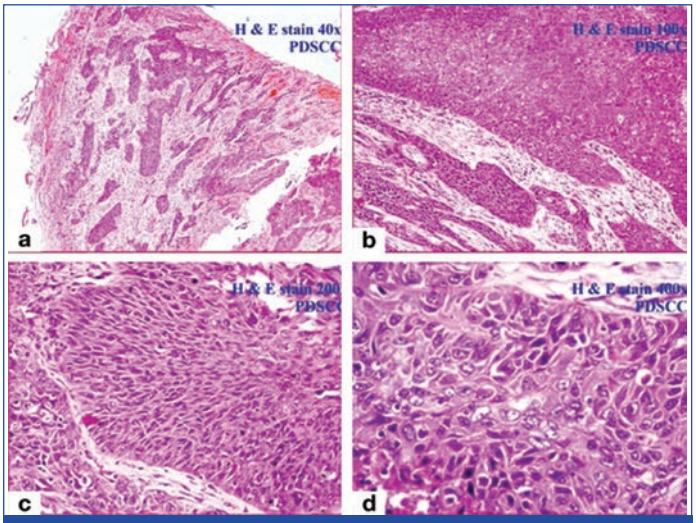
Detailed relevant clinical data for the study was collected from the data register of the "Department of Pathology". Haematoxylin and Eosin (H&E) stained slides of the respective patients were reassessed for histopathological parameters, including tumour type and grade according to Broder's criteria, and are depicted as follows: Well -Differentiated Squamous Cell Carcinoma (WDSCC)- [Table/Fig-1a-d], Moderately Differentiated Squamous Cell Carcinoma (MDSCC)- [Table/Fig-2a-d], Poorly Differentiated Squamous Cell Carcinoma (PDSCC)- [Table/Fig-3a-d], and Basaloid SCC- [Table/Fig-4a-d] [4]. All newly diagnosed/recurrent cases of OSCC were included, while cases with inadequate specimens/post-radiotherapy were excluded. Depth of invasion was categorised as T1 (<5 mm),



[Table/Fig-1]: Well Differentiated Squamous Cell Carcinoma (WDSCC) 1a: 40x; 1b: 100x; 1c: 200x; 1d: 400x.



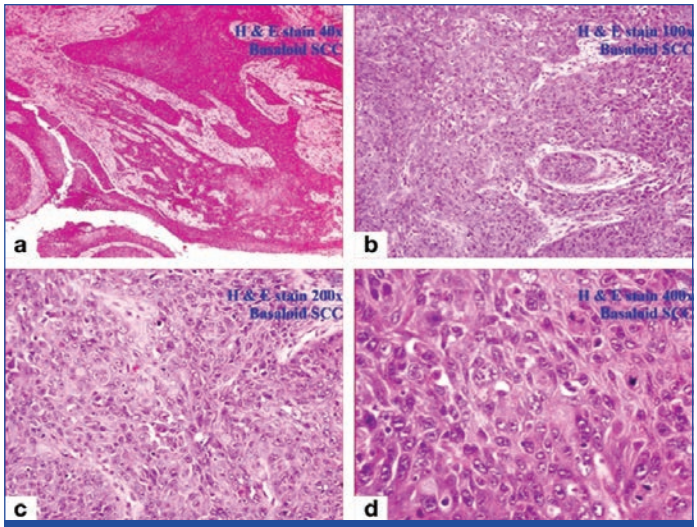
[Table/Fig-2]: Moderately Differentiated Squamous Cell Carcinoma (MDSCC) 2a: 40x; 2b: 100x; 2c: 200x; 2d: 400x.



[Table/Fig-3]: Poorly Differentiated Squamous Cell Carcinoma (PDSCC) 3a; 40x, 3b; 100x, 3c; 200x, 3d; 400x).

T2 (5-10 mm), and T3 (>10 mm), respectively, and measured using the microscope scale on representative slides.

Immunohistochemistry (IHC) was performed using p16 and p53 antibodies: p16 (Mouse monoclonal antibody; Thermo Fischer; Immunogen: Purified recombinant fragment of p16 expressed in *Escherichia coli (E.coli)*; Clone: 5A8A4; Isotype: Mouse/IgG1) and p53 (Mouse monoclonal antibody; Thermo Fischer; Immunogen: Recombinant human wild type p53 protein expressed in *E.coli*; Clone: DO-7; Isotype: IgG2b). IHC involves the binding of the



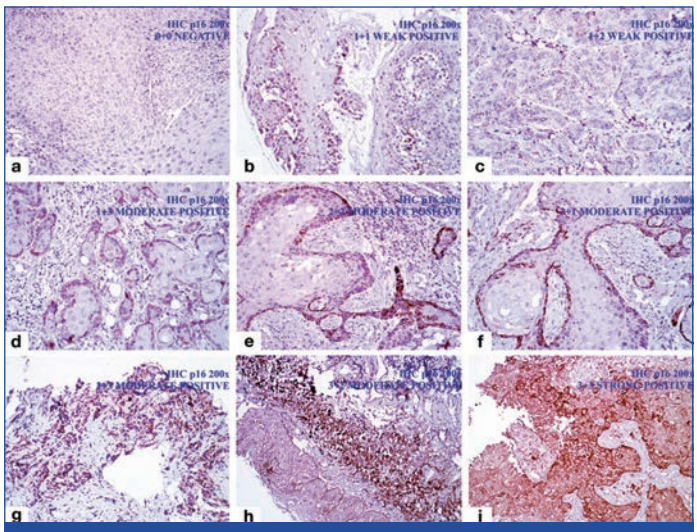
[Table/Fig-4]: Features of basaloid SCC in the form of solid growth of cells in a lobular configuration, small, crowded cells with scant cytoplasm, dark hyperchromatic nuclei without nucleoli as expressed in 4a: 40x; 4b: 100x; 4c: 200x; 4d: 400x.

primary antibody to specific tissue antigens, followed by interaction with a biotinylated secondary antibody [5,6]. The streptavidin/Horse Radish Peroxide (HRP) complex is then applied, with streptavidin attaching to the biotin on the secondary antibody and HRP acting as the indicator enzyme. Upon addition of a DAB chromogen, a coloured precipitate develops at the tissue antigen sites. Positive controls of p16 (cervical carcinoma) and p53 (human colon adenocarcinoma), as well as negative controls (tumour section without any antibody), were processed with every batch to determine the correctness of the procedure. Nuclear and cytoplasmic staining of p16 and nuclear staining of p53 were considered positive [Table/Fig-5a-i] for p16 and [Table/Fig-6a-i] for p53. The intensity of p16 and p53 protein expression was graded as follows:

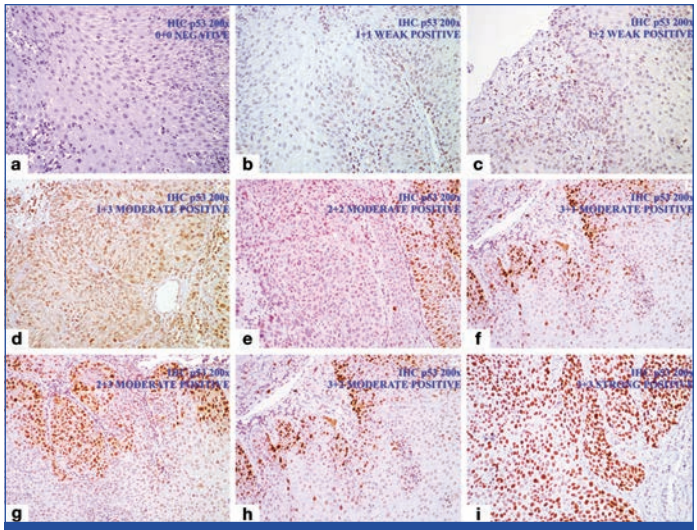
- No staining (0),
- Weak staining (1),
- Moderate staining (2),
- Strong staining (3) [7].

The proportion/percentage of staining for p16 and p53 protein expressions were calculated as follows:

- 1-4% (1),
- 5-19% (2),
- 20-39% (3),
- 40-59% (4),
- 60-79% (5),
- 80-100% (6) of cells stained [8].



[Table/Fig-5]: Immunohistochemistry Images of p16 protein expression.



[Table/Fig-6]: Immunohistochemistry Images of p53 protein expression a) 0+0 negative; b) 1+1 weak positive; c) 1+2 weak positive; d) 1+3 moderate positive; e) 2+2 moderate positive; f) 3+1 moderate positive; g) 2+3 moderate positive; h) 3+2 moderate positive; i) 3+3 strong positive.

IHC results were further evaluated using a semi-quantitative Quick-score, obtained by multiplying the intensity with the percentage of tumour cells expressing the protein [9,10]. A quick score of 0-1 (negative), 2-3 (weak positive), 4-5 (moderate positive), and >6 (strong positive) was assigned. The parameters studied included age, gender, dysplasia of the lining epithelium, histological grades of SCC, basaloid morphology, lobular growth pattern, depth of invasion, intensity of permeating lymphocytes, and lymphovascular/perineural invasion.

STATISTICAL ANALYSIS

Quantitative data are expressed as means and standard deviation, while ordinal/categorical data are expressed as absolute numbers and percentages. Cross tables were generated, and the Chi-square test was used to test associations. STATA-14 was used for statistical analysis. A p-value of <0.05 was considered statistically significant.

RESULTS

The histomorphological features and immunohistochemical patterns for p16 and p53 were analysed in 50 cases of OSCC. The age distribution showed that 8 (16%), 9 (18%), 18 (36%), 13 (26%), and 2 (4%) of the patients were in the age groups of 31-40 years, 41-50 years, 51-60 years, 61-70 years, and above 70 years, respectively, with a mean age of 54.72±11.80 years and a median age of 55.5 years. The study comprises of 42 (84%) males and 8 (16%) females.

The distribution of various parameters of the patients, including the histomorphological features, has been mentioned in [Table/Fig-7]. The comparison of various histological grades of OSCC with the four patterns (negative, weak, moderate, and strong) of p16 and p53 expression respectively has been mentioned [Table/Fig-8,9]. There was a significant statistical difference in the distribution of histological grades with the four patterns of p16 expression (p-value 0.014) and p53 expression (p-value=0.029). A statistically significant association between p16 and p53 with the histological grade of MDSCC (p-value of 0.036) was observed. However, no significant statistical association between p16 and p53 for the histological grades of WDSCC (p-value of 0.908) and PDSCC (p-value of 0.358) was noted.

A statistically significant negative correlation was observed between age and histological grade (r=-0.298, p=0.036), between dysplasia and p16 (r=-0.499, p<0.001), has been observed [Table/Fig-10]. However, positive correlation was observed between dysplasia and p53 (r=0.425, p=0.002). There was a statistically significant positive correlation between lobular growth and p16 (r=0.543, p<0.001), and a negative correlation was observed between lobular growth and p53 (r=-0.481, p<0.001). A significant positive correlation was found between keratinisation and histological grade (r=0.443, p=0.001), and negative correlations were observed between keratinisation and p16 (r=-0.395, p<0.001) as well as p53 (r=-0.290, p=0.041). There was a significant positive correlation between prominent basaloid morphology and p16 (r=0.543, p<0.001), and negative correlations were observed between prominent basaloid morphology and p53 (r=-0.481, p<0.001) and p53 (r=-0.290, p<0.001). The respective correlation coefficient values are mentioned in [Table/Fig-10].

Parameters		Frequency (Total N=50)	%
Gender	Male	42	84%
	Female	8	16%
Histological grades	WDSCC	14	28%
	MDSCC	27	54%
	PDSCC	9	18%
p16 IHC expression	Negative	31	62.0%
	Weak	4	8.0%
	Moderate	2	4.0%
	Strong	13	26.0%
p53 IHC expression	Negative	26	52.0%
	Weak	5	10.0%
	Moderate	7	14.0%
	Strong	12	24.0%
Dysplasia		41	82.0%
Lobular growth patterns		11	22.0%
Permeation by infiltrating lymphocytes	Dense	1	2.0%
	Mild	28	56.0%
	Moderate	21	42.0%
Prominent Basaloid morphology		11	22.0%
Lymphovascular invasion		7	14.0%
Perineural invasion		6	12.0%

[Table/Fig-7]: Distribution as per various parameters and histomorphological features.

Histological grades	Total	p16				p-value
		Negative	Weak	Moderate	Strong	
		Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)	
WDSCC	14	9 (33.3%)	3 (60.0%)	1 (50.0%)	1 (6.2%)	0.014*
MDSCC	27	17 (63.0%)	1 (20.0%)	1 (50.0%)	8 (50.0%)	
PDSCC	9	1 (3.7%)	1 (20.0%)	0 (0.0%)	7 (43.8%)	
Total	50	27 (100%)	5 (100%)	2 (100%)	16 (100%)	

[Table/Fig-8]: Comparison of histological grades and p16 Immunohistochemistry (IHC).

Histological grades	Total	p53				p-value
		Negative	Weak	Moderate	Strong	
		Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)	
WDSCC	14	11 (42.3%)	2 (40.0%)	1 (14.3%)	0 (0.0%)	0.029*
MDSCC	27	11 (42.3%)	3 (60.0%)	6 (85.7%)	7 (58.3%)	
PDSCC	9	4 (15.4%)	0 (0.0%)	0 (0.0%)	5 (41.7%)	
Total	50	26 (100%)	5 (100%)	7 (100%)	12 (100%)	

[Table/Fig-9]: Comparison of histological grades and p53 Immunohistochemistry (IHC).

DISCUSSION

Oropharyngeal cancers are the most common cancers worldwide, accounting for nearly 40% of all malignancies in India and South East Asian countries, primarily due to the habit of chewing tobacco and areca nut (pan/gutka) among the population [8]. The divergence in epidemiologic trends among OSCCs arising from different anatomic

	Parameters		Histological grades	p16	p53
Spearman's rho	Age (31 to >70 years)	Correlation coefficient	-0.298*	0.165	0.106
		p-value	**0.036**	0.252	0.465
		N	50	50	50
	Dysplasia (n=41)	Correlation coefficient	0.152	-0.499**	0.425**
		p-value	0.293	**<0.001**	**0.002**
		N	50	50	50
	Exhibit Lobular growth (n=11)	Correlation coefficient	-0.05	0.543**	-0.481**
		p-value	0.73	**<0.001**	**<0.001**
		N	50	50	50
	Permeated by infiltrating lymphocytes (n=50)	Correlation coefficient	-0.057	0.065	-0.148
		p-value	0.692	0.652	0.306
		N	50	50	50
	Keratinisation WDSCC=14 MDSCC=27 PDSCC=9	Correlation coefficient	0.443**	-0.395**	-0.290*
		p-value	**0.001**	**0.005**	**0.041**
		N	50	50	50
	Prominent Basaloid morphology (n=11)	Correlation coefficient	-0.05	0.543**	-0.481**
		p-value	0.73	**<0.001**	**<0.001**
		N	50	50	50
	Depth (T1=5 mm T2=5-10 mm T3=>10 mm)	Correlation coefficient	-0.058	-0.125	**0.049**
		p-value	0.688	0.387	0.737
		N	50	50	50
	PNI (n=6)	Correlation coefficient	0.092	-0.066	0.125
		p-value	0.525	0.647	0.385
		N	50	50	50
	Histological grades WDSCC=14 MDSCC=27 PDSCC=9	Correlation coefficient	1	0.012	-0.297*
		p-value	-	0.936	**0.036**
		N	50	50	50
	p16 Positive=19 Negative=31	Correlation coefficient	**0.012**	1	-0.197
		p-value	0.936	-	0.137
		N	50	50	50
	p53 Positive=24 Negative=26	Correlation coefficient	-0.297*	-0.197	1
		p-value	**0.036**	0.137	-
		N	50	50	50

[Table/Fig-10]: Comparison of histological grades and p53 Immunohistochemistry (IHC).

subsites represents its heterogeneity [1]. The index study included 50 fresh and retrospective cases of OSCCs treated at a tertiary care hospital of the Armed Forces in Northern India over a two-year period from January 2017 to January 2019. The age range of the patients was 31-86 years, with 84% of the cases occurring in the 41-80 years age group, which is similar to the findings observed by Shinohara S et al., [11]. The mean age of the patients in the present study was 54.7±11.80 years, which concurs with a corresponding value of 51.28±12.14 reported by Hashmi AA et al., [7].

In the present study, 84% of the cases were males and 16% were females. The higher proportion of males can be attributed to the study being conducted in a tertiary care hospital of the Armed Forces. The reasons for the relative protection observed among females remain unknown but may be associated with anatomical, reproductive, or hormonal factors [11].

Non-traditional, behavioural, and environmental risk factors, including having multiple sexual partners and a history of oral-genital/oral-anal sex, have been evolved to the etiology of oropharyngeal cancers [6,7,10,12]. Human papillomavirus (HPV) is a causative agent of head and neck cancer, similar to its role in genital cancers, owing to similarities in the epithelia. The prevalence of HPV in normal oral mucosa ranges from 0.6% to 81% [5,6,12]. Different HPV subtypes, such as 6, 11, 16, 18, and 13, are involved in genital and non-genital mucosa. HPV-16 has been demonstrated in 90-95% of all HPV-positive HNSCC cases, followed by HPV-18, HPV-31, and HPV-33. Kulkarni SS et al., observed that 96% of cervical and 70.59% of OSCC cases were positive for HPV, respectively [12]. Variable central keratinisation and horn pearl formation were noted, depending on the tumour differentiation of the tumour as per Broder's classification [13]. Sarwath H et al., studied the immunoexpression of p16 and p53 proteins as biomarkers of oral carcinogenesis and their correlation with histomorphological parameters [5-8].

The present study includes 14 (28%) well-differentiated OSCC, 27 (54%) MDSCC, and 9 (18%) PDSCC cases. Basaloid SCC, described by Wain SL et al., is characterised by a follicular or lobular pattern of invasion with peripheral, elongated palisade cells surrounding each lobule [14]. The characteristic histopathological features of the basaloid component of the tumour include: (a) solid growth of cells in a lobular configuration closely opposed to the surface mucosa; (b) small crowded cells with scant cytoplasm; (c) dark hyperchromatic nuclei without nucleoli; and (d) small cystic spaces containing material resembling mucin that stains with periodic acid-Schiff or Alcian blue [14].

The present study reveals 19 (38%) p16-positive cases (inclusive of all grades) and 31 (62%) p16-negative cases. Among the 38% p16-positive cases, four cases (8%) showed weak positivity, two (4%) showed moderate positivity, and 13 (26%) showed strong positivity. Furthermore, the comparison of various histological grades of OSCCs with patterns of p16 expression exhibits strong positivity was more associated with higher grades of OSCC, in accordance with Ralli M et al., In OSCC, increased expression of p53 intensity is related to the clinical severity of the disease [9,15-17]. Worldwide, variable p53 overexpression ranging from 31% to 85.6% in HNSCC cases has been observed, as reported by Khan H et al., from India and 63.3% expression by Tandon P et al., in a Brazilian population [18-20]. In the present study, it was observed that 52% of the patients had negative p53, 24% had strong p53, 14% had moderate p53, and 10% had weak p53 expressions. The comparison of various histological grades of OSCC with the four grades (negative, weak, moderate, and strong) of p53 expression in the index study showed that five out of nine cases of PDSCC were positive for p53, 16 out of 27 cases of MDSCC were positive for p53, and only three out of 14 cases of WDSCC were positive for p53. These results are similar to those reported by Ghanghoria S et al., [21]. Variable expression of p53 in OSCCs may be due to different techniques used, methods of interpretation, or differences in ethnicity and risk factors involved in OSCC pathogenesis. The present study showed strong p53 positivity in PDSCC than MDSCC, which indicates that with the increase in OSCC pathological grading, the rate of mutant p53 positivity also increases, consistent with the results of previous studies [21].

Out of the 50 cases, 41 showed dysplasia of the stratified squamous lining epithelium, with 58.5% and 33.6% of cases having positive expression for p53 and p16, respectively. This indicates that dysplasia is not associated with p16 expression. Eleven cases exhibited a pattern of lobular growth, with nine of them (81.8%) showing positive p16 expression. This observation is similar to the findings of Shinchara S et al., All eleven cases with lobular growth showed negative p53 expression, indicating an inverse relationship [11].

Among the 11 cases that showed basaloid morphology, nine cases (81.8%) showed strong p16 expression, and none had p53 expression. This strongly indicates an inverse relationship between

p16 and p53 markers. This observation is in correlation with Wain SL et al., who highlighted that HPV-positive cases consistently exhibited prominent basaloid morphology [13,14]. Within the basaloid subtype, the detection of HPV is a highly favourable prognostic factor, helping to identify a subset of cancers that deviate from the highly aggressive behaviour associated with this variant.

A significant positive correlation was found between keratinisation and histological grade (r=0.443, p=0.001), and a negative correlation was observed between keratinisation and p16 (r=-0.395, p<0.001), as well as p53 (r=-0.290, p=0.041). A significant negative correlation/inverse relationship was found between p16 and p53 (r=-0.297, p=0.037). The association of p16 and p53 across the various histological grades, including MDSCC (n=27), PDSCC (n=9), and WDSCC (n=14), was assessed. It was observed that there was a statistical significance between p16 and p53 under the histological grade of MDSCC (p-value of 0.036). However, there was no significant association between p16 and p53 for the histological grades of PDSCC (p-value of 0.358) and WDSCC (p-value of 0.908). Similar findings were observed by Shinohara S et al., Ghanghoria S et al., and Ralli M et al., [15,21].

A statistically significant difference in the distribution of histological grades versus the four patterns of p16 and p53, with an increase in their intensity corresponding to clinical severity and higher histological grades, was observed. A significant negative correlation was found between dysplasia versus p16, keratinisation versus p16, lobular growth versus p53, or vice versa.

Limitation(s)

The analysis of data from a single centre, and there is a need for validation of p16 IHC through DNA detection-based studies.

CONCLUSION(S)

HPV-positive oropharyngeal cancer is a unique subtype that dominates the landscape of head and neck oncology, and its increasing incidence is impacting diagnostic, preventive, and therapeutic practices. We observed a significant negative correlation between age versus histological grades, dysplasia of the lining squamous epithelium, keratinisation, versus p16 expression, and lobular growth and histological grade versus p53. A positive correlation between lobular growth and p16 expression, and keratinisation and histological grade. Furthermore, we noted an inverse relationship between the expressions of p16 versus p53. Therefore, we recommend the use of immunohistochemical expression of p16 as a surrogate marker of HPV, along with p53.

REFERENCES

[1] Ausoni S, Boscolo-Rizzo P, Singh B, da Mosto MC, Spinato G, Tirelli G, et al. Targeting cellular and molecular drivers of head and neck squamous cell carcinoma: Current options and emerging perspectives. Cancer Metastasis Rev. 2016;35(3):413-26.

[2] Sgaramella N. Squamous cell carcinoma of the oral tongue: Studies of biomarkers connected to human papillomavirus infection, epithelial to mesenchymal transition, and locoregional metastasis. Canc and Onco. 2017;1-2. https://www.diva-portal.org/smash/get/diva2:1094089/SPIKBLAD01.pdf.

[3] Gupta N, Gupta R, Acharya AK, Patti B, Goud V, Reddy S, et al. Changing trends in an oral cancer-a global scenario. Nepal J Epidemiol. 2016;6(4):613-19.

[4] Broders AC. Carcinoma: Grading and practical application. Arch Pathol. 1956;2:376-81.

[5] Sarwath H, Bansal D, Husain NE, Mohamed M, Sultan AA, Bedri S. Introduction of p16INK4a as a surrogate biomarker for HPV in women with invasive cervical cancer in Sudan. Infectious Agents and Cancer. 2017;12:50.

[6] Gupta S, Gupta S. Role of human papillomavirus in oral squamous cell carcinoma and oral potentially malignant disorders: A review of the literature. Indian J Dent. 2015;6(2):91-98.

[7] Hashmi AA, Hussain ZF, Hashmi SK, Irfan M, Khan EY, Faridi N, et al. Immunohistochemical overexpression of p53 in head and neck squamous cell carcinoma: Clinical and prognostic significance. BMC Res Notes. 2018;11(1):433.

[8] Sgaramella N, Coates PJ, Strindlund K, Loljung L, Colella G, Laurell G, et al. Expression of p16 in squamous cell carcinoma of the mobile tongue is independent of HPV infection despite presence of the HPV-receptor syndecan-1. Br J Cancer. 2015;113(2):321-26.

[9] Gupta AK, Lee JH, Wilke WW, Quon H, Smith G, Maity A, et al. Radiation response in two HPV-infected head-and-neck cancer cell lines in comparison to a non-HPV-infected cell line and relationship to signaling through AKT. Int J Radiat Oncol Biol Phys. 2009;74(3):928-33.

[10] Gatoo MA, Dar AM, Siddiqui M. Correlation of P53 expression with different histological grades in oral squamous cell carcinoma patients from northern India. J Cancer and Oncol. 2018;6(1):01-14.

[11] Shinohara S, Kikuchi M, Tona R, Kanazawa Y, Kishimoto I, Harada H, et al. Prognostic impact of p16 and p53 expression in oropharyngeal squamous cell carcinomas. Jpn J Clin Oncol. 2014;44(3):232-40.

[12] Kulkarni SS, Kulkarni SS, Vastrad PP, Kulkarni BB, Markande AR, Kadakol GS, et al. Prevalence and distribution of high-risk human papillomavirus (HPV) types 16 and 18 in carcinoma of cervix, saliva of patients with oral squamous cell carcinoma and the general population in Karnataka, India. Asian Pac J Cancer Prev. 2011;12(3):645-48.

[13] Huang K, Chen L, Zhang J, Wu Z, Lan L, Wang L, et al. Elevated p53 expression levels correlate with tumour progression and poor prognosis in patients exhibiting esophageal squamous cell carcinoma. Oncol Lett. 2014;8(4):1441-46.

[14] Wain SL, Kier R, Wollmer RT, Bossen EH. Basaloid-squamous carcinoma of the tongue, hypopharynx, and larynx: Report of 10 cases. Hum Pathol. 1986;17(11):1158-66.

[15] Ralli M, Singh S, Yadav SPS, Sharma N, Verma R, Sen R. Assessment and clinicopathological correlation of p16 expression in head and neck squamous cell carcinoma. J Cancer Res Ther. 2016;12(1):232-37.

[16] Patil S, Rao RS, Amrutha N, Sanketh DS. Analysis of human papillomavirus in oral squamous cell carcinoma using p16: An immunohistochemical study. J Int Soc Prev Community Dent. 2014;4(1):61-66.

[17] Liu SZ, Zandberg DP, Schumaker LM, Papadimitriou JC, Cullen KJ. Correlation of p16 expression and HPV type with survival in oropharyngeal squamous cell cancer. Oral Oncol. 2015;51(9):862-69.

[18] Barasch S, Mohindra P, Hennrick K, Hartig GK, Harari PM, Yang DT. Assessing p16 Status of oropharyngeal squamous cell carcinoma by combined assessment of the number of cells stained and the confluence of p16 staining: A validation by clinical outcomes. Am J Surg Pathol. 2016;40(9):1261-69.

[19] Tandon P, Dadhich A, Saluja H, Bawane S, Sachdeva S. The prevalence of squamous cell carcinoma in different sites of oral cavity at our Rural Health Care Centre in Loni, Maharashtra-a retrospective 10-year study. Contemp Oncol (Pozn). 2017;21(2):178-83.

[20] Khan H, Gupta S, Husain N, Misra S, Negi MPS, Naseem J, et al. Correlation between expressions of cyclin-D1, EGFR, and p53 with chemoradiation response in patients of locally advanced oral squamous cell carcinoma. BBA Clin. 2015;21(3):11-17.

[21] Ghanghoria S, Ghanghoria A, Shukla A. p53 expression in oral cancer: A study of 50 cases. Nep JOL. 2015;5:747-51.

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