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Original Research Article

Role of P16 and E Cadherin in Head and Neck Squamous Cell Carcinoma

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Abstract:

Background: The vast majority of head and neck cancers are oral squamous cell carcinomas (OSCCs) that arise from the epithelial lining of oral cavity, including tongue and lips.[4]E-cadherin is a transmembrane protein involved in cellular adhesion and polarity maintenance. E cadherin is expressed in almost all epithelial cells. Loss of E cadherin is associated with gain of tumor cell motility and invasiveness. Negative IHC in tumors occurs with loss of p16 protein function/expression. Therefore, there is dire need to understand relationship of HNSCC with various molecular markers that have been discovered in the last few years and which have offered potential new and early diagnosis and treatment therapeutic modalities. alternatives. Hence, this study was undertaken to evaluate expression of p16 and E cadherin in HNSCC cases.

Methods: The present study was conducted in pathology department, during the period 2021-2022 among 50 cases of histopathologically diagnosed HNSCC. A pretested proforma was used to collect the clinicopathological parameters including age of patient, history of tobacco use, pan chewing, alcohol abuse, other relevant irritants, involvement of abnormal sexual habits, site of lesion, grade of tumor and lymph node metastasis. Histological assessment was done as slides prepared after biopsy were stained with hematoxylin and eosin stain and then studied for histological changes. Immunohistochemical evaluation included tissue processing where by automatic tissue processor and then paraffin blocks were prepared. In our study intensity of p16 and E cadherin staining was tabulated in accordance with Jang et al., and Simioneseu et al., respectively. A descriptive study was carried out for all the variables included in the study. The whole data was be entered in Microsoft Excel master sheet and analyzed using Statistical Package for Social Sciences (SPSS) version 20 software.

Results: Our study comprises an analysis of 50 diagnosed cases of head and neck squamous cell carcinoma of which 44 (88%) lesions pertain to the oral cavity. In the present study it was noted that peak incidence of Head and neck Squamous cell carcinoma was 51-60 years. In our study, most common site of HNSCC was buccal mucosa which accounted for 36% and second being tongue, which accounted for 26%. Most of the cases in our study were well differentiated squamous cell carcinoma (52%) followed by moderately differentiated squamous cell carcinoma (42%).In our study, we correlated p16 positivity with relation to site of lesion, maximum positivity was found in buccal mucosa (77.8%), followed by tongue (84.6%).

Conclusion: In our study enrolled 50 patients with histopathology-proven HNSCC and all the cases were assessed for the expression of p16 and E-cadherin by immunohistochemical study. We found that with increasing grade of tumor, p16 positivity decreased.

Keywords: squamous cell carcinomas, p 16, E-cadherin, immunohistochemical, grade of tumor.

Introduction

Head and neck squamous cell carcinoma (HNSCC) consist of a heterogenous group of lesions that arise in the upper aero digestive tract. It is one of the most common cancers worldwide with approximately 600,000 cases reported annually with a much higher incidence in developing countries. Prevalence of HNSCC in India is around 45% and its incidence is expected to increase from 1 million in 2012 to greater than 1.7 million in the year 2035. The death rate in this period caused due to this malignancy will also increase from 6.8 lakhs to 1 million.[1] World Health Organization (WHO) Global Report on "Tobacco Attributable Mortality" 2012 has recognized that 7% of all deaths in India (≥30 years of age) are due to tobacco intake.[2] Tobacco use and alcohol consumption are independent risk factors for development of HNSCC. However, with combined intake they have a synergistic effect in its development.[3] Squamous Cell Carcinomas (SCC) are histologically graded into well, moderate or poorly differentiated carcinomas. Though histological grading system is essential for the classification of HNSCC, but is not necessary for the treatment as clinical outcome or treatment response is not strongly associated with differentiation grade. Tumor Lymph Node and Metastasis (TNM) Staging is most useful in predicting prognosis and therefore helps to determine tumor progression, recurrence, disease free survival(DFS) and response to therapy. However, it has been noted that patients with tumors of same clinico-pathological stage do not have similar disease progression, response to therapy, rate of disease recurrence and survival. This is on account of molecular heterogeneity of HNSCC not being incorporated in conventional TNM classification. The vast majority of head and neck cancers are oral squamous cell carcinomas (OSCCs) that arise from the epithelial lining of oral cavity, including tongue and lips.[4]E-cadherin is a transmembrane protein involved in cellular adhesion and polarity maintenance. E cadherin is expressed in almost all epithelial cells. Loss of E cadherin is associated with gain of tumor cell motility and invasiveness.

Normal pattern is strong circumferential membranous staining. Abnormal pattern is loss or decrease/attenuation of membranous staining.[5] Negative IHC in tumors occurs with loss of p16 protein function/ expression. Immunohistochemistry positivity is considered valuable surrogate marker for HPV in oropharyngeal SCC. Positive staining is defined as "block" staining: strong nuclear and cytoplasmic expression in a continuous segment of cells (at least 10-20 cells); in squamous epithelium, block positivity needs to involve basal and parabasal cells. Cytoplasmic-only staining, diffuse blush/weak intensity staining and other focal/patchy patterns should be considered negative.[6] Therefore, there is dire need to understand relationship of HNSCC with various molecular markers that have been discovered in the last few years and which have offered potential new and early diagnosis and treatment therapeutic modalities. alternatives. Hence, this study was undertaken to evaluate expression of p16 and E cadherin in HNSCC cases.

Materials and Methods

The present study was conducted in pathology department, GSVM medical college, Kanpur. The tissue material for the study was obtained from various out patients and inpatients admitted in surgery department. The present study was conducted in our hospital during the period 2021-2022. In this study, 50 cases of histopathologically diagnosed HNSCC pertaining to oral cavity, oropharynx, larynx, and hypopharynx were studied. Those patients were considered who had undergone surgical therapy as a primary mode of treatment. Patients with other than HNSCC (such as adenocarcinoma, melanoma, sarcoma, metastasis), inadequate biopsies and those patients who have undergone radiotherapy as a primary mode of treatment are excluded. A pretested proforma was used to collect the clinicopathological parameters including age of patient, history of tobacco use, pan chewing, alcohol abuse, other relevant irritants, involvement of abnormal sexual habits, site of lesion, grade of tumor and lymph node metastasis.

Laboratory Procedure

Histological assessment was done as slides prepared after biopsy were stained with hematoxylin and eosin stain and then studied for histological changes. All cases with oral cavity and oropharynx were separated histologically into premalignant and malignant lesions and malignant lesions were graded into- well differentiated type(grade I),moderately differentiated (grade II) and poorly differentiated (grade III). Immunohistochemical evaluation included tissue processing where by automatic tissue processor and then paraffin blocks were prepared. Sections were cut at 5 microns thickness. For histological grading, hematoxylin and eosin stain was used. Then histopathological features for every case were studied. The consecutive H&E-stained slides were evaluated. Subsequently p16 and E-cadherin-stained slides were compared with H&E-stained slides to establish a relationship between stained areas and respective histopathological diagnosis. Section-stained omitting primary antibody were taken as negative control.p16 nuclear and cytoplasmic staining was taken as positive "block staining" in epithelium. E cadherin-Strong circumferential membranous staining is taken to be positive.

Immunohistochemical Analysis Of P16

IHC profile of the tumor was assessed by subjecting one representative section from tumor block to p16. Immunohistochemistry was performed on 4 µm thick sections from 10% formalin-fixed paraffin-embedded specimens, according to the streptavidin-biotin immunoperoxidase technique. Positive and negative controls were run simultaneously. Strong brown nuclear immunoreactivity was considered as positive staining. According to Jang et al., p16 was considered positive if>5% cells are stained and negative <5% cells or not at all stained. The fraction of stained cells was scored as weak staining:+(6-25% cells take staining); moderate staining: ++(26-50% cells take staining) and strong staining: +++(51-100% cells take staining).[7]

Immunohistochemistry Of E Cadherin

Sections were pretreated in CC1-buffer at 95°C for 36 min (E-cadherin). Slides were then be incubated with primary antibodies at appropriate dilution for 30 min at 36°C and detected using Ultra View Universal DAB Detection kit. Slides were counterstained with Hematoxylin and Bluing Reagent. In our study intensity of E cadherin was tabulated in accordance with Simioneseu et al., as 0= under 10% positively stained cells; 1+ = 10-25% positively stained cells: weak expression; 2+= 25-50% positively stained cells: mild to moderate expression; 3+= 50-75% positively stained cells: moderate to strong

expression; and 4+= >75% positively stained cells: very strong expression.[8]

Statistical Analysis

A descriptive study was carried out for all the variables included in the study. The whole data was be entered in Microsoft Excel master sheet and analyzed using Statistical Package for Social Sciences (SPSS) version 20 software. Association between these parameters was assessed. A value of $P < 0.05$ was taken as significant and <0.01 as highly significant; whereas, $P > 0.05$ was taken as nonsignificant.

Results

In the present study it was noted that peak incidence of Head and neck Squamous cell carcinoma (HNSCC) was 51-60 years (36%).

In our study cases from rural areas (64%) predominated over urban areas (36%). Also, three fourth of patients belonged to the lower socioeconomic strata (72%). In our study, 84% were tobacco users(Table 1).

Table 1: Socio demographic profile of patients with HNSCC

Variables	Number	%
Mean Age (in years)	52.31±11.25	
Age group		
≤20 years	1	2.0
21-30 years	2	4.0
31-40 years	9	18.0
41-50 years	10	20.0
51-60 years	18	36.0
>60 years	10	20.0
Gender		
Male	36	72.0
Female	14	28.0
Residence		
Rural	32	64.0
Urban	18	36.0
Socioeconomic status		
Upper and Middle	14	28.0
Lower	36	72.0
Tobacco and alcohol users	42	84.0
Smoking	38	76.0
Tobacco chewing	42	84.0
Alcohol	36	72.0

An analysis of 50 diagnosed cases of head and neck squamous cell carcinoma showed that 88% lesions pertain to the oral cavity. In our study, most common site of HNSCC was buccal mucosa which accounted for 36% and second being tongue, which accounted for 26%. Most of the cases in our study were well differentiated squamous cell carcinoma (52%) followed by moderately differentiated squamous cell carcinoma (42%). In our study, 78% of the total HNSCC cases were p16 positive and 22% were p16 negative. In our study, 98% of the total HNSCC cases were E cadherin and 2% cases were E cadherin negative. Average duration of presentation of head and neck squamous cell carcinoma was6.31±4.87 months from onset of symptoms (Table 2).

Table 2: Characteristics of HNSCC among patients

Variables	Number	%
Site		
Oral cavity	44	88.0
Buccal mucosa	18	36.0
Tongue	13	26.0
Lip	1	2.0
Soft palate	2	4.0
Oropharynx	6	12.0
Larynx	1	2.0
Hypopharynx	1	2.0
Face	2	4.0
Histological grading of SCC		0.0
Well differentiated	26	52.0
Moderately differentiated	21	42.0
Poorly differentiated	3	6.0
P16 positive	39	78.0
E cadherin positive	49	98.0
Mean duration of presentation from onset of symptoms (in months)	6.31±4.87	

In our study, the association of p16 with grading of carcinoma was calculated. We found that with increasing grade of tumor, p16 positivity decreased and the association was found to be highly significant (p< 0.004).In our study, the association of E cadherin with grading of carcinoma was calculated. We found that with increasing grade of tumor E cadherin staining decreased and the association was found to be highly significant (p< 0.0001) (Table 3).

Table 3: Intensity of staining in HNSCC with p16 and E cadherin immunoreaction in relation to histological grading

Variables	Number (%)		
	WDSCC(n=26)	**MDSCC(n=21)**	**PDSCC(n=3)**
p16 Intensity*			
Negative (n=11)	8 (30.8%)	3 (14.3%)	0 (0.0%)
Weak + (n=14)	8 (30.8%)	6 (28.6%)	0 (0.0%)
Moderate ++ (n=16)	9 (34.6%)	7 (33.3%)	0 (0.0%)
Strong +++ (n=9)	1 (3.8%)	5 (23.8%)	3 (100.0%)
E cadherin Intensity**			
Negative (n=9)	0 (0.0%)	0 (0.0%)	1 (33.3%)
Weak (n=17)	3 (11.5%)	12 (57.1%)	2 (67.7%)
Mild to Moderate (n=22)	13 (50.0%)	9 (42.9%)	0 (0.0%)
Moderate-Strong (n=10)	10 (38.5%)	0 (0.0%)	0 (0.0%)
Very Strong (n=0)	0 (0.0%)	0 (0.0%)	0 (0.0%)

*P value=0.004, **P value<0.0001, WDSCC: Well differentiated squamous cell carcinoma, MDSCC: Moderately differentiated squamous cell carcinoma, PDSCC: Poorly differentiated squamous cell carcinoma

We correlated p16 positivity with relation to site of lesion, maximum positivity was found in buccal mucosa (77.8%), followed by tongue (84.6%). We correlated E cadherin positivity with relation to site of lesion, maximum positivity was found in buccal mucosa (100.0%), followed by

tongue (100.0%). Relation of P16 and E Cadherin with risk factors was tabulated and p16 positivity was found in 78.6% tobacco consumers, 76.3% cases who were smokers, and 86.1% cases who consumed alcohol. E cadherin positivity was found in 100.0% tobacco consumers, 97.4% cases who were smokers, and 97.2% cases who consumed alcohol (Table 4).

Table 4: Relation of p16 and E Cadherin with risk factors

Variables	p 16+ (n=39)	E cadherin+ (n=49)
Site		
Buccal mucosa (n=18)	14 (77.8%)	18 (100.0%)
Tongue (n=13)	11 (84.6%)	13 (100.0%)
Tobacco and alcohol users		
Tobacco chewing (n=42)	33 (78.6%)	42 (100.0%)
Smoking (n=38)	29 (76.3%)	37 (97.4%)
Alcohol (n=36)	31 (86.1%)	35 (97.2%)

Discussion

Present study comprises an analysis of 50 diagnosed cases of head and neck squamous cell carcinoma of which 44 (88%) lesions pertain to the oral cavity. Vigneshwaranet al., and Mehrotra et al., stated that more than 90% of head and neck cancers were squamous cell carcinomas (HNSCC) that arise from the mucosal surfaces of the oral cavity, oropharynx and larynx.[9,10]In our study, most common site of HNSCC was buccal mucosa which accounted for 36% and second being tongue, which accounted for 26%. Dundy et al., stated that buccal mucosa was observed as the most common site (43.2%) followed by tongue (13.5%) oral squamous cell carcinoma.[11]

In our study, male preponderance was noted with male (72%) to female (28%) ratio of around 3:1, which is in accordance with studies by Singh et al.[12] Dhanutai et al., and Mehrotra et al., cited that the male-to-female ratio was 2.22:1 anf 3.27:1 respectively.[13,14]This may be primarily due to their addiction to tobacco chewing and smoking, which is the most important risk factor for development of precancerous lesions and oral cancers. However, in studies of western countries the ratio was significantly different.[14]

In our study cases from rural areas (64%) predominated over urban areas (36%). Also, three fourth of patients belonged to the lower socioeconomic strata (72%). Dangi et al., stated that most of the times, head and neck cancers, especially oral cancers affect people belonging to the lower socioeconomic strata of society and people in the rural areas due to a higher exposure to risk factors such as the use of tobacco.[15]

In the present study it was noted that peak incidence of Head and neck Squamous cell carcinoma (HNSCC) was 51-60 years (36%). This was in accordance with findings of Dhanutai et al., who stated that the mean age of the Asian patients 56.37±14.98 years, while that of non-Asian patients was 69.99±15.51 years.[13] A Research by National institute of Dental and Craniofacial cited that most of the oral cancer cases occur between the age of 50 to 70 years, but it could also affect children as early as 10 years. [16]The average of HNSCC in our study was 52.31±11.25 years, lower than that published by Gervasio et al., (58.6 years) and Anwer et al., (58.33±20.54 years).[17,18]

Tobacco addiction in any form has been widely implied in causative agent of oral premalignant and malignant lesions. In our study, 84% were tobacco users. These findings were consistent with Jiang et al., Agarwal et al., and Madani et al., who

suggested that increase in frequency and doses of tobacco use increases risk of developing carcinoma of oral cavity.[19,20,21]

In our study, average duration of presentation of head and neck squamous cell carcinoma was 6.31±4.87 months from onset of symptoms. Similar observations were made (presentation within six months) in 75.56% by Anwer et al.[18] It was because of early seeking nature of human for any gross discomfort to body and moreover easy and cheap availability of health services.

In our study, most of the cases in our study were well differentiated squamous cell carcinoma (52%) followed by moderately differentiated squamous cell carcinoma (42%). Our results were concordant with the study of Yuen et al., Fregonesi et al., and Ai et al., whereas wide variability and discordance was observed when the criteria for grading used were different.[22,23,24]

In our study p16 positivity was found in 78.6% tobacco consumers, 76.3% cases who were smokers, and 86.1% cases who consumed alcohol. Smith et al., and Ralli et al., found a statistically significant association of p16 expression with alcohol consumption and tobacco use ($p< 0.05$).[25,26] In studies by Lazarus et al., and Dragomir et al., found no statistically significant association between p16 expression and tobacco/alcohol use.[27,28]

In our study, we found that with increasing grade of tumor, p16 positivity decreased and the association was found to be highly significant ($p< 0.004$), which was in concordance with study by Smith et al., Ralli et al., and Muirhead et al., and who observed that there was significantly more probability of p16 overexpression in later stage and high-grade tumor ($p<0.05$).[26,26,29] But our findings were discordant with the study of with Yuen et al., and Dragomir et al.[22,28]

In our study, we found that with increasing grade of tumor E cadherin staining decreased and the association was found to be highly significant ($p< 0.0001$), which was in concordance with study by Nijkamp et al., Chaw et al., Akhtar et al., and Afrem et al., observed a decrease E-cadherin reactivity in parallel with decreasing tumor differentiation and with the increase of invasion pattern of oral carcinoma.[30,31,32,33]

Conclusion

In our study enrolled 50 patients with histopathology-proven HNSCC and all the cases were assessed for the expression of p16 and E-cadherin by immunohistochemical study. We found that with increasing grade of tumor, p16 positivity decreased. Upon assessing the expression of E-cadherin in the cases, majority of them showed mild to moderate expression of E-cadherin staining, which was suggestive of decrease in expression of E- cadherin as the tumor progressed to higher grade.

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