

Study of Assessment of P16 Expression in Various Grades of Squamous Cell Carcinoma of Head and Neck

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Abstract:

Introduction: p16 is a tumor suppressor gene, over expression of which is considered as a surrogate marker of oncogenic human papillomavirus (HPV) infection. Moreover, p16 over expression correlates with good prognosis in head and neck squamous cell carcinoma (HNSCC). In the present study, we aimed to evaluate the frequency of p16 overexpression in HNSCC in our institute and its association with clinicopathologic parameters.

Methods: We performed p16 immunohistochemistry (IHC) on 40 cases of HNSCC in a tertiary care teaching hospital. Association of p16 over expression with various clinicopathologic parameters was evaluated.

Results: In our study the most common site involved was oral cavity in 11 patients followed by sino-nasal region in 7 patients. Ca Nasopharynx was diagnosed in 6 patients, Ca oropharynx in 5 patients and lip was involved in 4 patient. In our study p16 over expression was noted in 40% (14 cases), while 60% (26 cases) were negative for p16 over expression. The relationship between histopathological type of squamous cell carcinoma and p16 immunohistochemistry is statistically significant.

Conclusion: HPV16 is the subtype most every now and again connected with HNC, and current clinically pertinent recognition strategies center around distinguishing the presence of HPV16 by PCR or ISH, or identification of p16 protein articulation in tumor tests by IHC. Moreover, p16 expression was found to be associated with some good prognostic parameters like lack of nodal metastasis; however, no significant association was noted with overall disease-free survival.

Keywords: head and neck, squamous cell carcinoma, p16 expression.

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Introduction

The aim of cancer classification is to better understand prognosis of cancer, to improve diagnosis and compare outcome results for a consecutive improvement of treatment recommendations of distinct cancers at a specific stage of disease.

Current staging of head neck squamous cell carcinoma is primarily based on clinical primary tumor extension, lymph node involvement and distant metastasis. For surgically treated tumors, two histopathological parameters tumor grading and radicality of resection are added [1-3]. Risk factors for locoregional relapse such as vascular invasion; lymph node capsular spread and tumor-free margin size of the resected tumor were recently identified. Locoregional control is significantly improved when these head neck squamous cell carcinoma patients are treated with postoperative concurrent radiotherapy with platinum-based chemotherapy. Recently a variant of squamous cell carcinoma of head and neck has

been identified which is thought to be associated with high risk human papilloma virus .These tumors have been shown to have favourable outcome despite advanced stage at presentation .More over there is increase in incidence HPV related head and neck squamous cell carcinoma. P16 staging of tumor tissue by IHC is a surrogate marker of HPV infection [4].

Head and neck cancer in India has a different demographic profile, risk factors, eating habits and personal and family history (1 to 13). They are emerging as the main public health problems in India, which are related to lifestyle, have a long latency period and need dedicated infrastructure and human resources for treatment. Coordinated and needs-based research is needed to understand threats to the nation from chronic diseases such as head and neck cancers, and these threats will become increasingly important. Extent of the problem in India: overall, 57.5% of head and neck cancers occur in Asia, particularly India. In India

Head and neck cancers accounted for 30% of all cancers.

In India, 60 to 80% of patients have advanced disease compared to 40% in developed countries. A slow decline in the incidence of most head and neck cancers has been documented in India. The problem of head and neck cancer management in India is slightly different than in the west.

The main disadvantage is loss to be followed up, making it difficult to carry out and report the final results of clinical trials. The top priority for cancer control should be given to the burden of head and neck cancer in India.

The emphasis should be on prevention of the onset and early detection of the disease. In developing countries such as India, priority should be given to the implementation of cancer control activities, making optimal use of limited resources to provide maximum benefit to the largest number of people.

Based on this aim of our study is to study the expression of p16 in various head and neck squamous cell carcinoma. Also to correlate the

incidence with various grades and specific site of squamous cell carcinoma and to assess the number of cells stained and confluence of p16 staining.

Materials and Methods

The study is undertaken in the department of Pathology of a tertiary care teaching hospital during the study period (March 2019 to June 2020). The study sample includes histopathological specimens of head and neck diagnosed as squamous cell carcinoma, from the Department of Surgery. The received specimens will be fixed in 10% buffered formalin, routinely processed and embedded in paraffin blocks. The 3-4 micron thick sections will be taken for hematoxylin and eosin staining, and then histological grading of the tumor is done. Immunohistochemical staining procedures are done with P16 (CDKN2A). The percentage of cells that are p16 positive and the confluence of p16 staining defined as groups of contiguous cells demonstrating staining) are analysed and correlated with grade of tumors as well as with specific region of head and neck. The results are analysed tabulated and statistical significance is evaluated.

Table 1: Interpretation of p16

S. No	Percentage of positive tumor cells	Result
1	Diffuse nuclear and cytoplasmic staining in > 70% of tumor cells	Positive
2	Diffuse nuclear and cytoplasmic staining in < 70% of tumor cells	Negative
3	Complete absence of staining	Negative

The squamous cell carcinoma of head and neck with various histological types analyses with Fischer's exact test. The test result P value less than 0.05 was considered statistically significant.

Results

The total percentage of positivity among the different grades of Head and Neck squamous cell carcinoma obtained from our study: On examining 40 cases HEAD AND NECK squamous cell carcinoma the immunopositivity within different grades of oral squamous cell carcinoma noted as follows. Well differentiated squamous cell carcinoma –WDSCC Moderately differentiated squamous cell carcinoma - MDSCC, Poorly differentiated squamous cell carcinoma-PDSCC.

In our study the most common site involved was oral cavity in 11 patients followed by sino-nasal

region in 7 patients. Ca Nasopharynx was diagnosed in 6 patients, Ca oropharynx in 5 patients and lip was involved in 4 patients. While other cases was having cancer in other areas.

Coming to the age distribution patients were most commonly above 40 years age group, 13 patients were between 40-50, 12 patients were between 51-60, 11 patients were between 61-70 years. In our study population 24 were male and rest 16 were female. Above 60 years males were most common compare to females. Next coming to type of squamous cell carcinoma 12 were well differentiated, 18 were moderately differentiated and 10 were poorly differentiated. Among well differentiated cases was equal in both male and female. Moderately differentiated was double the time in males compared to females. Poorly differentiated was bit high in males.

Table 2: Type of SCC

Type of SCC	Total	Percentage
WD SCC	12	30%
MD SCC	18	45%
PD SCC	10	25%
Total	40	100%

Further we analyzed the p16 positivity and a total of 14 patients was had p16 positive expression and rest had negative expression, we also compared the type with positivity, the positive expression was comparatively higher in well differentiated squamous cell carcinoma. By Chi square test, the p value was calculated and it was

more than 0.005. The relationship between histopathological type of squamous cell carcinoma and p16 immunohistochemistry is statistically significant.

Table 3: p16 IHC in SCC of Head and Neck

Type of SCC	P16		Total	Positive Percentage
	Positive	Negative		
WD SCC	4	8	12	33%
MD SCC	8	10	18	44%
PD SCC	2	8	10	20%
TOTAL	14	26	40	35%
Percentage	40%	60%	100%	

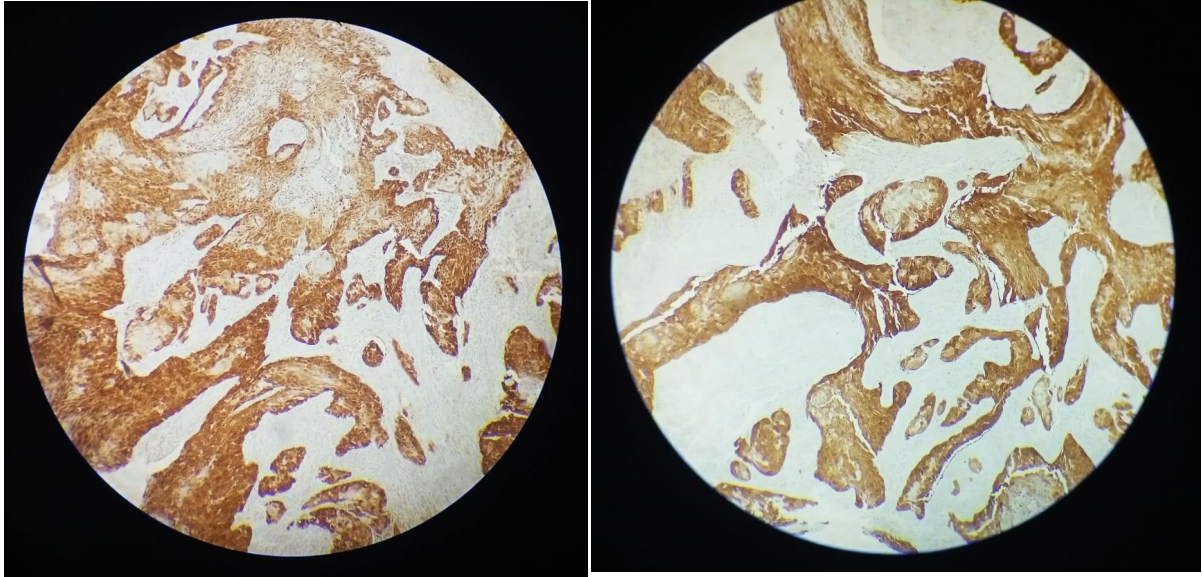


Figure 1: Diffuse p16 positivity

Discussion

Squamous cell carcinoma contributes more than 90% of total tumors of head and neck cancer. It is usually seen in 4th to 5th decade and has male preponderance. Tobacco, smoking and betel quid are common risk factors with recent increase in incidence associated with HPV infection. This HPV related SCC is more common in oral cavity than the other sites of head and neck cancer. In present study 40 cases of SCC are taken and its age, sex, site wise, histopathological difference and p16 association has been done.

In present study majority cases are seen during 4th decade. In study by Jagannath DAV Sharma Et al [5] and Datta et al [6], increased incidence was seen during 4th decade. There was an increase in the incidence of cases with SCC variant in the oral cavity compared to the laryngeal region (27.5% and 5% respectively). Similar distribution of tumors was seen in the study by Boslooper et al. [7] while the larynx was the most common site of involvement in most of the studies. [8] The difference in site involved could be because of different geographical distribution and occurrence of risk factors. Overall SCC of Head and neck (whether HPV / Non HPV SCC) is more common

in male and in study by Rajesh Vaidya incidence in male was 78%, in female 22% and M : F ratio was 3.5 : 1. In study by S. Nair et al [9] incidence was 77% in males, 23% in females and the ratio was 3.3:1.

In present study out of 40 cases, 18 cases were MDSCC (45%) followed by 12 cases were WDSCC (30%) and 10 cases PDSCC (25%). MDSCC is more common than WDSCC and PDSCC. Grade III tumors were less frequently observed in our study when compared to study by Solomon et al. [10] Studies by Wilson et Al [8]. and Smilek et al [11] observed moderately differentiated carcinoma/Grade II forming the most common group

In present study out of 40 cases only 14 cases are positive for p16. P 16 positives for only 40% of cases, 44% of p16 positive cases are MDSCC. This tumors p16 status can guide clinicians in tailoring therapy as well as promote use of specific inhibitors in combination chemotherapy. Hence HPV has strong correlation to SCC and p16 can be used for determination of HPV status. Our results were concordant with the study of Fregonesi et al., Yuen et al. and Ai et al., whereas wide variability and discordance was observed when the criteria for

grading used were different. [12,13,14] While our findings were similar with the study of Ralli et al [15] who observed that there is more probability of p16 overexpression in later stage and high-grade tumor

Conclusion

HPV status assessment may be helpful in early identification of cancer, determination of prognosis and post treatment follow-up. HPV status should be included as an important risk and prognostic factor in future trials. Along with it, trials should be performed to get a proper treatment regime for HPV-positive and HPV-negative HNSCC in order to provide better treatment and lower relapse rates. As HPV integration with the transcription of viral oncoprotein induces overexpression of p16, we can use p16 immunohistochemistry as a surrogate marker of HPV. Significant expression of p16 in node-negative patients may guide the type and intensity of the therapy in patients with HNSCC. Overexpression of p16 has been significantly seen in male patients of HNSCC who had tumors in the oral cavity and had a history of abnormal sexual habits. Vaccination programs may provide prevention from HPV infection in high-risk population.

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