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ORIGINAL RESEARCH

Analysis of human papilloma virus in oral squamous cell carcinoma using p16: An immunohistochemical study

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ABSTRACT

Background: The study was conducted for the analysis of human papilloma virus in oral squamous cell carcinoma using p16.

Material and methods: A total of sixty biopsied samples, embedded in paraffin and preserved in formalin, were obtained and histopathologically identified as OSCC. Four-micrometer serial sections had been cut, with one section undergoing H and E staining to determine the histological grades and the subsequent sections undergoing p16 IHC staining. Twenty of the sixty cases were well-differentiated (WDOSCC), twenty were moderately-differentiated (MDOSCC), and the remaining twenty were poorly-differentiated (PDOSCC) oral squamous cell carcinoma. To test the reliability of the IHC kit as well as the precision of the procedure, positive control slides of cervical cancer harbouring HPV were obtained. The main antibodies had been left out as a negative control. The occurrence of brown precipitate at the site of cytoplasm, nucleus or both had been suggestive of p16 positive immunoreactivity despite of staining intensity.

Results: Out of 60 OSCC cases, 20 cases were Well differentiated OSCC, 20 were moderately differentiated OSCC and 20 were poorly differentiated OSCC. 1/20 well differentiated cases showed p16 positivity, 2/20 moderately differentiated cases showed p16 positivity whereas 3/20 poorly differentiated cases showed p16 positivity. In total, 60 samples of OSCC were evaluated. The results revealed p16 positivity in 54 cases (90%) of 60. 6/20 poorly differentiated cases showed diffuse pattern of staining. 2/20 well as well as poorly differentiated cases showed patchy pattern of staining. 4/20 moderately differentiated cases showed patchy pattern of staining. 4/20 well differentiated cases showed rare singly dispersed cells. 1/20 moderately differentiated cases showed rare singly dispersed cells.

Conclusion: A link between HPV as well as OSCC was found in this study. The PDOSCC was shown to have a diffuse staining pattern, which indicates an increase in viral burden and may be related to its aggressive character.

Keywords: Human Papilloma Virus, Oral Squamous Cell Carcinoma.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most frequent type of head and neck squamous cell carcinoma (HNSCC), with >500,000 new cases annually worldwide.¹ OSCC is more likely to invade local tissues and spread to the lymph nodes, and has a mortality rate of ~50% within five years.² Despite the increasing knowledge into the etiology of OSCC and the advances in chemotherapy, radiation and surgery, there has been little improvement in the relative survival time in patients with OSCC in recent decades. Smoking and drinking are major risk factors for OSCC.³ In addition, infection with human papillomavirus (HPV) has been identified as another risk factor for developing carcinoma in the oral cavity.⁴

HPV is a circular double-stranded DNA molecule, ~8 kb and over 100 genotypes have been reported.⁵ The high-risk types, HPV-16 and 18 have been associated with 90% of uterine cervical cancers.⁶⁻⁸

This study was conducted for the analysis of human papilloma virus in oral squamous cell carcinoma using p16.

MATERIAL AND METHODS

A total of sixty biopsied samples, embedded in paraffin and preserved in formalin, were obtained and histopathologically identified as OSCC. Four-micrometer serial sections had been cut, with one section undergoing H and E staining to determine the histological grades and the subsequent sections undergoing p16 IHC staining. Twenty of the sixty cases were well-differentiated (WDOSCC), twenty were moderately-differentiated (MDOSCC), and the remaining twenty were poorly-differentiated (PDOSCC) oral squamous cell carcinoma. To test the reliability of the IHC kit as well as the precision of the procedure, positive control slides of cervical cancer harbouring HPV were obtained. The main antibodies had been left out as a negative control. The occurrence of brown precipitate at the site of cytoplasm, nucleus or both had been suggestive of p16 positive immunoreactivity despite of staining intensity.

RESULTS

Table 1: Criteria for evaluating p16 staining.

Grades	Pattern of staining
0	No
1	Rare singly dispersed cells
2	Patchy

Table 2: Prevalence of p16 positivity among OSCC cases.

p16 positivity	Number of cases	Percentage
Absent	06	10%
Present	54	90%
Total	60	100%

In total, 60 samples of OSCC were evaluated. The results revealed p16 positivity in 54 cases (90%) of 60.

Table 3: Demonstrating p16 positive cases in histological grades of OSCC.

Histological grades of OSCC	Number of cases	Number of p16 positive cases	Percentage of p16 positive cases
Well differentiated OSCC	20	1	16.6%
Moderately differentiated OSCC	20	2	33.3%

Poorly differentiated OSCC	20	3	50%
Total	60	06	100%

Out of 60 OSCC cases, 20 cases were Well differentiated OSCC, 20 were moderately differentiated OSCC and 20 were poorly differentiated OSCC. 1/20 well differentiated cases showed p16 positivity, 2/20 moderately differentiated cases showed p16 positivity whereas 3/20 poorly differentiated cases showed p16 positivity.

Table 4: Different grades of p16 staining in different grades of OSCC.

Pattern of staining	Differentiated OSCC		
	Well differentiated OSCC	Moderately differentiated OSCC	Poorly differentiated OSCC
No	0	0	0
Rare singly dispersed cells	4	1	0
Patchy	2	4	2
Diffuse	0	0	6

6/20 poorly differentiated cases showed diffuse pattern of staining. 2/20 well as well as poorly differentiated cases showed patchy pattern of staining. 4/20 moderately differentiated cases showed patchy pattern of staining. 4/20 well differentiated cases showed rare singly dispersed cells. 1/20 moderately differentiated cases showed rare singly dispersed cells.

DISCUSSION

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide with an incidence of 550,000 cases annually.^{9,10} Oral cavity squamous cell carcinoma (OSCC) constitutes a majority of HNSCCs, including tumors of the oral anterior tongue and buccal mucosa. The major known risk factors for OSCC are use of tobacco and alcohol and infection with human papillomavirus (HPV).^{11,12} Unlike oropharyngeal tumors, in which HPV incidence is reported to be high (up to 90%),^{13,14} the prevalence of HPV in OSCC is generally accepted to be low.^{15,16}

In addition, unlike with oropharyngeal tumors, the role of HPV in disease prognosis and response to therapy in patients with OSCC is equivocal. Despite the fact that HPV RNA is shown to function as a better screening and patient management tool,^{17,18} the presence of HPV DNA is routinely used as a measure of HPV infection in tumors. HPV DNA results do not always match those for HPV RNA, especially in OSCC.

This study was conducted for the analysis of human papilloma virus in oral squamous cell carcinoma using p16.

Out of 60 OSCC cases, 20 cases were Well differentiated OSCC, 20 were moderately differentiated OSCC and 20 were poorly differentiated OSCC. 1/20 well differentiated cases showed p16 positivity, 2/20 moderately differentiated cases showed p16 positivity whereas 3/20 poorly differentiated cases showed p16 positivity. In total, 60 samples of OSCC were evaluated. The results revealed p16 positivity in 54 cases (90%) of 60. 6/20 poorly differentiated cases showed diffuse pattern of staining. 2/20 well as well as poorly differentiated cases showed patchy pattern of staining. 4/20 moderately differentiated cases showed patchy pattern of staining. 4/20 well differentiated cases showed rare singly dispersed cells. 1/20 moderately differentiated cases showed rare singly dispersed cells. Patil S et al (2014)¹⁹ evaluated the expression of human papilloma virus (HPV) in oral squamous cell carcinoma (OSCC) and to correlate the association of HPV in histological grades of OSCC using p16 (p16INK4a) immunohistochemistry (IHC). This study consists of 30 histological diagnosed cases of OSCC (10-well-differentiated oral squamous cell carcinoma [WDOSCC], 10-moderately differentiated oral squamous cell carcinoma [MDOSCC] and 10-poorly

differentiated oral squamous cell carcinoma [PDOSCC]). The sections were subjected to IHC procedure using p16. Two parameters in immunohistochemical p16 expression were evaluated by 3 observers based on the criteria by Galgano M. Tetal (2010) (a) percentage of p16 positive cases (b) pattern of p16 staining in various grades of OSCC. Totally, 30 samples of OSCC, p16 positivity was noted in 26/30 (86.66%). Of 26 positive cases, p16 staining was positive in 7/10 (70%) of WDOSCC, 9/10 (90%) in MDOSCC and, 10/10 (100%) PDOSCC. Incidentally, we also found single dispersed cell staining in WDOSCC, patchy staining in MDOSCC and more diffuse staining pattern predominant in PDOSCC. Their study revealed an association between HPV and OSCC. Diffuse staining pattern was noted in PDOSCC, which in turn depicts the increase viral overload, which might have an influence on its aggressive behaviour. Das R et al (2024)²⁰ examined the 5-year survival of OSCC patients with HPV positive or negative status along with p16 protein expression. A total of 72 biopsy tissue specimens from histologically confirmed oral squamous cell carcinoma (OSCC) patients were collected. HPV detection and genotyping were performed using HPV E6/E7 and HPV- type-specific multiplex primer for nested-PCR. Immunohistochemistry evaluation of p16 was conducted. SPSS statistical software (ver 20) was used for data analysis. High risk-HPV (hr-HPV) DNA positivity was found in 27.7% (n = 20) of OSCC patients. Stage III OSCC patients were 7.80 times more likely to survive 5 years than stage IV patients (OR-7.80 CI-95%; P-0.03). Among the hr-HPV positive OSCC patients, we found that the median survival time for the 1st year (95%), 3 years (78.5%), and 5 years (38.5%) was significantly higher than that of the hr-HPV negative [1st year (78.6%), 3 years (45.2%) and 5 years (38.5%)] OSCC patients (P-0.03). The survival of male patients with hr-HPV positive OSCC is 9.75 times greater than the survival of patients with HPV negative OSCC (OR-9.75; CI-95%; P-0.05). The p16 expression level (low to overexpression) group and negative P16 expression group of OSCC patients have not demonstrated a significant association with 5-year survival. We conclude that in OSCC cases of North-East India, the presence of hr-HPV in OSCC cases could be a good predictor of 5-year survival rate. Expression of p16 does not appear to have any significant association with 5-year survival.

CONCLUSION

A link between HPV as well as OSCC was found in this study. The PDOSCC was shown to have a diffuse staining pattern, which indicates an increase in viral burden and may be related to its aggressive character.

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