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MAIN ARTICLE

Human papillomavirus and salivary gland neoplasia: a p16^{INK4} immunohistochemical and in situ hybridisation study

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Abstract

Objective: This study aimed to evaluate the association between human papillomavirus infection and salivary gland tumours in a Scottish cohort.

Methods: Specimens from a range of salivary gland tumours operated on between 1997 and 2012 were studied. A tissue microarray constructed from tissue blocks was subjected to p16^{INK4} (cyclin-dependent kinase inhibitor 2A) immunohistochemistry and in situ hybridisation using probes specific for human papillomavirus, including types 16 and 18.

Results: A total of 61 tumours (benign and malignant) were deemed suitable for the study. p16^{INK4} staining yielded three (4.9 per cent) positive samples: one small cell carcinoma, one squamous cell carcinoma and one poorly differentiated carcinoma. Human papillomavirus in situ hybridisation demonstrated a positive signal in the latter sample only (1.6 per cent).

Conclusion: This study demonstrated a very low human papillomavirus detection rate in salivary gland tumours. It can therefore be concluded that human papillomavirus infection is unlikely to play a role in salivary gland neoplasia. Rare human papillomavirus positive cases should be carefully evaluated to exclude the possibility of a metastatic lesion.

Key words: Salivary Glands; Human Papillomavirus; Neoplasms

Introduction

The aetiopathogenic role of human papillomavirus (HPV) in cervical carcinogenesis is well established. Studies have shown that more than 90 per cent of cervical cancerous and pre-malignant lesions are HPV related.[1,2] Syrjänen *et al*. first described the presence of HPV in head and neck cancer lesions in 1983.[3] Since then, numerous studies have evaluated the association of HPV with both benign and malignant head and neck tumours using various HPV detection techniques. In the head and neck region, the association of HPV infection with squamous cell carcinoma of the oropharynx is well established; it is now accepted that more than 50 per cent of such lesions are associated with oncogenic HPV.[4–9] In previously published reviews, the overall incidence of oncogenic HPV in head and neck cancer was between 22.0 per cent and 46.5 per cent, regardless of anatomical subsite.[4,10–13] Studies have also demonstrated favourable outcomes for HPV-associated cancers, particularly oropharyngeal carcinomas, because of their increased sensitivity to radiotherapy.[14] It is therefore important to know the HPV status of a tumour, especially when making treatment decisions.

Salivary gland tumours account for 6 per cent of all head and neck neoplasms; the annual incidence ranges from 1.0 to 6.5 cases per 100 000 individuals worldwide.[15,16] Although studies evaluating HPV association with mucosal tumours of the head and neck region have been widely reported, only a handful of studies (with variable findings) have reported HPV association with salivary gland tumours.[4,16–22] Contradictory evidence means that the aetiological role of HPV in salivary gland tumorigenesis is currently unclear.

This study aimed to examine a range of benign and malignant salivary gland tumours for HPV involvement by p16^{INK4} immunohistochemical analysis of tumour tissue samples followed by in situ hybridisation analysis for HPV DNA.

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Materials and methods

Study design and data gathering

Cases were identified using the National Health Service Central Register Community Health Index ('CHI') number (a unique number allocated to each patient when they register with a family doctor in Scotland) from pathology records at Ninewells Hospital, Dundee, UK. This number was correlated with clinical data obtained from medical case notes. Archived paraffin-embedded tissue blocks were obtained for a range of primary and metastatic salivary gland tumours operated on between 1997 and 2012. Tissue samples representing all major types of benign and malignant salivary gland tumours were selected ($n = 61$).

Human papillomavirus detection

The tissue blocks were processed to construct a tissue microarray. HPV status was initially determined immunohistochemically by screening the tissue microarray for strong $p16^{INK4}$ expression. The interpretation of $p16^{INK4}$ immunohistochemical staining can be somewhat variable; thus, at our institution, samples with at least 70 per cent strong nuclear and cytoplasmic staining are considered $p16^{INK4}$ positive (this is currently the most widely used scoring system).[23] However, as $p16^{INK4}$ positivity is not an absolute indicator of HPV status, tissue sections were also processed for in situ hybridisation using probes specific for HPV, including genotypes 16 and 18. This analysis was performed at the Department of Oral Pathology, Centre for Oral Health Research, Freeman Hospital and University of Newcastle upon Tyne, UK. Positive control samples were cervical and tonsillar lesions with established evidence of HPV infection; negative control samples were chronic sialadenitis lesions with no evidence of HPV infection.

Results

Paraffin-embedded tissue samples from 61 salivary gland tumours were deemed suitable for the study. Twenty-three samples were from malignant tumours (17 primary, 6 metastatic) and 38 were from benign tumours (Tables I–III). Immunohistochemical analysis of $p16^{INK4}$ expression yielded three (4.9 per cent) positive samples: one small cell neuroendocrine carcinoma, one squamous cell carcinoma and one poorly differentiated carcinoma. In situ hybridisation assessment of HPV demonstrated a positive signal in the latter sample only (1.6 per cent). The oncogenic HPV genotype 16 was detected in all positive samples. The remaining tumours were negative for both $p16^{INK4}$ immunohistochemical staining and HPV infection. It is important to note that none of the benign tumours were $p16^{INK4}$ positive by immunohistochemical staining or HPV DNA positive by in situ hybridisation.

TABLE I
BENIGN TUMOUR SUB-TYPES AND HISTOPATHOLOGY*

Characteristic	Value
Gland sub-type (*n*)	
– Parotid	25
– Submandibular	11
– Minor	2
Histopathological classification	
– Pleomorphic adenomas	29
– Warthin's tumours	9
$p16^{INK4}$ staining	All negative
HPV DNA†	All negative

*$n = 38$ patients (16 men, 22 women); mean age, 45.4 (range 17–79) years. †In situ hybridisation. HPV = human papillomavirus

Discussion

This Scottish study demonstrated no significant association between HPV infection and salivary gland neoplasia. Although three tissue samples were $p16^{INK4}$ positive, two were metastatic carcinomas and the other was a mixed or poorly differentiated carcinoma that was subsequently found to be HPV16 positive by in situ hybridisation assessment. It is therefore reasonable to suggest that primary salivary gland tumours (benign and malignant) do not exhibit an association with HPV and that in the rare cases in which HPV is detected, the patient should be assessed very carefully for the possibility of a metastatic carcinoma.

Although this study included very limited sample numbers, previous studies have reported variable HPV detection rates in salivary gland tumours (range 0–77.8 per cent).[16–22] In 2007, Vageli and colleagues were the first to detect HPV in salivary gland tumours.[20] They analysed nine parotid tumours (benign and malignant) by polymerase chain reaction and identified oncogenic HPV in six (66.7 per cent) cases and low-risk (benign) HPV in one (11.1 per cent). $p16^{INK4}$ over-expression has been reported in three studies. Skálová *et al.* evaluated a mixed group of benign and malignant tumours ($n = 55$) and found $p16^{INK4}$ over-expression in 81.8 per cent.[17] However, only 9 per cent of these had strong nuclear and cytoplasmic staining (score of greater than 3) and there was no detection of oncogenic HPV DNA by polymerase chain reaction in any of the cases. Brunner *et al.* studied 38 minor salivary gland carcinomas of different histological sub-types and demonstrated $p16^{INK4}$ over-expression in 71 per cent.[19] However, only two (5.3 per cent) cases

TABLE II
MALIGNANT TUMOUR SUB-TYPES*

Gland sub-type	*n*
– Parotid	16
– Submandibular	3
– Minor	4

*$n = 23$ patients (8 women, 15 men); mean age, 54.8 (range 21–93) years

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TABLE III
MALIGNANT TUMOUR HISTOPATHOLOGY

Characteristic	*n*	$p16^{INK4}$ staining	HPV DNA*
Primary tumours			
– Acinic cell carcinomas	6	–	–
– Carcinoma ex-pleomorphic	3	–	–
– Adenoid cystic carcinomas	3	–	–
– Adenocarcinoma	2	–	–
– Mucoepidermoid carcinomas adenomas	2	–	–
– Mixed/poorly differentiated	1	+ve	+ve
Metastatic tumours			
– SCC	3	+ve (1/3)	–
–Renal cell carcinoma	1	–	–
–Lymphoepithelioma-like carcinoma	1	–	–
–Small cell neuroendocrine carcinoma	1	+ve	–

*In situ hybridisation. HPV = human papillomavirus; +ve = positive

(mucoepidermoid carcinoma) had strong or diffuse nuclear and cytoplasmic staining. Interestingly, both were positive for oncogenic HPV by in situ hybridisation (and none were positive for benign HPV genotypes). In addition, Descamps *et al.* reported a very low incidence of $p16^{INK4}$ over-expression (weak positivity in 7.6 per cent of cases).[22]

- **There was a very low human papillomavirus DNA detection rate in 61 salivary gland neoplasms**
- **Human papillomavirus is unlikely to play a significant role in salivary gland tumour development**
- **Human papillomavirus positive salivary gland tumours should be carefully assessed to exclude the possibility of a metastatic carcinoma**

Different rates of HPV detection by various DNA analysis techniques (including polymerase chain reaction) have been reported for benign and malignant tumours. Hafed *et al.* examined 34 salivary gland tumours (19 malignant and 15 benign) and 7 control salivary tissue specimens (chronic sialadenitis) by in situ hybridisation for the presence of oncogenic HPV genotypes (16 and 18).[16] Only one (5.3 per cent) malignant and seven (46.7 per cent) benign cases were HPV positive, whilst all control specimens were HPV negative. These findings differ from those of the current study, especially regarding benign tumours. Worldwide, there is wide variation in the reported incidence of HPV-associated head and neck cancers from various countries.[24] This is also true for salivary gland tumours, as discussed in the literature review included in this report. Hafed *et al.* examined an Egyptian patient cohort in which the prevalence of HPV infection is likely to be very different from that of a Scottish cohort.[16] Anderson *et al.* previously studied 100 head and neck cancer cases from south-east Scotland and reported a relatively low incidence of HPV-positive cases (10 per cent).[25] The current study was also performed in south-east Scotland, where the overall HPV infection prevalence in the population may be low, as reflected in the results presented here. Descamps *et al.* studied 79 parotid lesions (40 benign and 39 malignant tumours) for the presence of benign and oncogenic HPV genotypes using real-time quantitative polymerase chain reaction: four (7.5 per cent) benign tumours were positive for HPV DNA and one (2.6 per cent) malignant tumour was positive for oncogenic HPV genotype 16.[22]

Conclusion

This study demonstrated a very low HPV DNA detection rate in 61 salivary gland neoplasms of a range of different histological sub-types. From this Scottish cohort, it is reasonable to conclude that HPV infection is unlikely to play a significant role in salivary gland neoplasia. The rare samples positive for $p16^{INK4}$ staining or HPV DNA should be carefully evaluated to exclude the possibility of a metastatic carcinoma.

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