

# HPV may not play a role in all lacrimal transitional cell papilloma\*

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## Abstract

**Background:** Tumours of the lacrimal duct apparatus (LDA) are rare and heterogenous but knowledge of their aetiology is important for the rhinologist. A link between transitional cell papilloma/carcinoma (TCP/TCC) and human papilloma virus (HPV) has been suggested in previous studies. We aimed to add to this body of evidence by submitting 16 LDA tumour samples from our tertiary referral centre for HPV analysis.

**Methodology:** All LDA tumour samples stored in the University College London tissue bank were submitted for HPV analysis by centralised nucleic acid extraction and HPV genotyping via a sensitive polymerase chain reaction (PCR).

**Results:** Only one of six transitional cell papillomas tested positive for HPV. Two of three transitional cell carcinomas returned HPV 16 positive results. Two inverted papillomas submitted were also HPV positive.

**Conclusions:** Previously published literature has suggested a strong link between HPV and neoplasia of the lacrimal system. HPV has previously been demonstrated in all TCP and TCC. This is in contrast to our data, particularly for transitional cell papilloma where, in the largest sample of transitional cell papilloma in the literature thus far, we did not find a strong association with HPV. This casts doubt on the role of HPV in the papillomatous process in the lacrimal apparatus.

**Key words:** Papillomaviridae, human papilloma virus, lacrimal apparatus, transitional cell carcinoma

## Introduction

Benign or malignant tumours of the lacrimal drainage apparatus (LDA) are rare but important for the rhinologist, particularly those performing dacryocystorhinostomy (DCR). They can be broadly classified into epithelial, mesenchymal, lymphoproliferative and melanocytic<sup>(1)</sup>. Epithelial tumours predominate, reported at 73% by Rose et al.<sup>(2)</sup>, but ranges in the literature vary from 55-100%<sup>(3,4)</sup>. The majority of these tumours present with symptoms of nasolacrimal obstruction, primarily epiphora, and may present with a medial canthal mass resembling a dacryocoele. They can thus be misdiagnosed as dacryocystitis or simple nasolacrimal duct obstruction. They may present to the rhinologist with a visible mass on endoscopy (Figures 1 and 2).

Early recognition of the potential neoplastic diagnosis is crucial as they are locally invasive, thus delays ultimately necessitate more extensive resection. Malignant tumours classically occur in the fifth decade, whereas benign lesions present in younger adults. The epithelial subtypes involved in the lining of the lacrimal drainage apparatus are diverse and this reflects the heterogeneity of epithelial tumours arising here. A recent review by Krishna et al. identified papilloma (squamous, transitional or mixed) to be the most common benign tumour (36% of all epithelial tumours), and squamous cell carcinoma followed by transitional cell carcinoma to be the most common malignancies<sup>(3)</sup>. The transitional cell tumours represent an interesting subgroup as the cells seem histologically between squamous

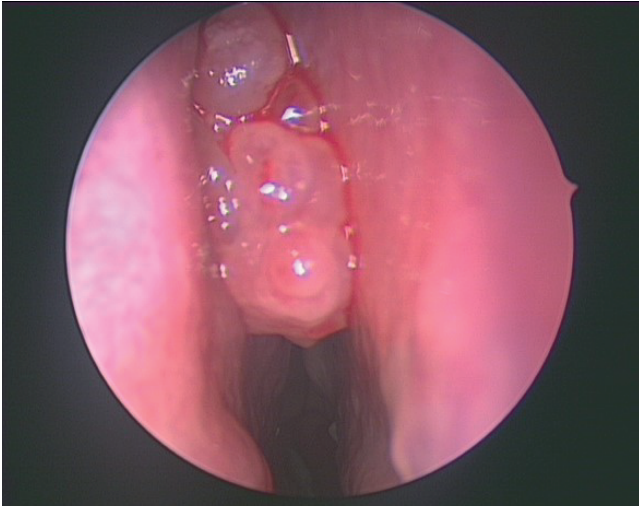


Figure 1. Endoscopic view of a transitional cell papilloma of the right nasolacrimal sac (VJL).

and columnar respiratory mucosa and appear similar to that of the vesico-ureteric epithelium. However, since the lesions do not develop from a transitional epithelium, the term has been disputed<sup>(5)</sup>. The malignant transitional cell form is also known by several synonyms, including “non-keratinising carcinoma”, “intermediate cell carcinoma” and “Schneiderian carcinoma”. LDA tumours are usually treated with a combination of primary resection with adjuvant radiotherapy and/or chemotherapy<sup>(6)</sup>. The surgical approach adopted in the literature varies from DCR and local resection<sup>(7)</sup> to dacryocystectomy for papilloma with or without intranasal resection of the lacrimal duct<sup>(8)</sup>, with a wider lateral rhinotomy approach with en-bloc resection of bone, including adjacent orbital periosteum<sup>(9)</sup>, and the entire lacrimal duct system for malignancies<sup>(6,10)</sup>.

Persistent infection with oncogenic or “high risk” human papilloma virus (hr-HPV) is known for its causative relationship with cervical cancer, but is also associated with other anogenital malignancies and a component of oropharyngeal tumours. Further, there is evidence to suggest that HPV-associated tumours are increasing in the UK<sup>(11)</sup>. It has previously been suggested that HPV may be involved in the development of certain lacrimal and conjunctival tumours, both benign and malignant<sup>(12-18)</sup>. Low risk types of HPV (specifically 6 and 11) have been found in benign lacrimal sac transitional cell papillomas, and both low and high risk-HPV types (the latter represented by HPV 16 and 18) have been found in malignant transitional cell carcinomas of the lacrimal sac<sup>(17,18)</sup>.

Knowledge of HPV status in oropharyngeal carcinomas can provide insight into long-term prognosis for the patient and inform treatment decisions<sup>(19)</sup>. Notably, the International Collaboration on Oropharyngeal Cancer Network for Staging (ICON-S) has created a staging classification system which incorporates HPV-status and acknowledges the different treatment response and

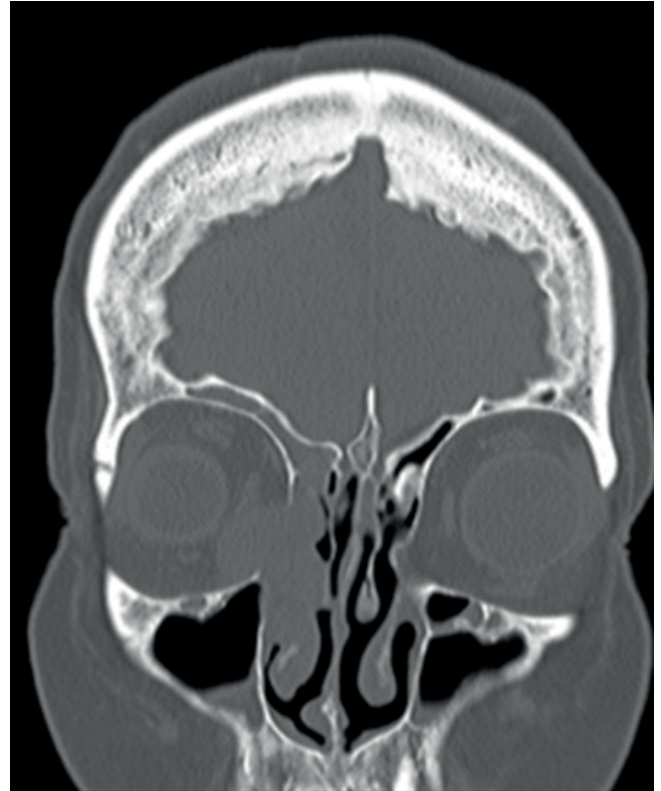


Figure 2. Coronal CT of a right nasolacrimal sac transitional cell papilloma demonstrating bony erosion and orbital infiltration.

clinical outcomes associated with HPV positive and negative tumours<sup>(20)</sup>. Additionally, quantifying the extent of HPV in transitional cell papilloma and carcinomas allows the potential impact of prophylactic HPV vaccines to be quantified.

In the light of mounting evidence implicating HPV in the aetiology of these tumours, we aimed to add the experiences of a national LDA tumour referral centre to the literature by retrospectively submitting banked tumour samples for HPV analysis.

### Material and methods

The project was reviewed and approved by the NHS Research Ethics Committee. Cases of lacrimal duct apparatus neoplasms between 2004-2011 were identified retrospectively from the departmental database for the University College London Hospitals biobanked samples. All the tumours in this series had been removed via a modified lateral rhinotomy approach and en-bloc resection of the nasolacrimal system by the senior author (VJL). All stored samples of neoplasms of the lacrimal duct apparatus in the UCL/UCLH Biobank were included. Any other tissue diagnoses were excluded. Identified samples underwent nucleic acid extraction and HPV genotyping via polymerase chain reaction (PCR) based assay.

### HPV detection

A 10 micron section of the relevant formalin-fixed paraffin-

Table 1. Histological diagnosis and HPV result.

Diagnosis	HPV PCR
Transitional Cell Papilloma	Negative
Transitional Cell Papilloma	Negative
Transitional Cell Papilloma	Negative
Transitional Cell Papilloma	Negative
Transitional Cell Papilloma	Negative
Transitional Cell Papilloma	HPV 6
Transitional Cell Carcinoma	Negative
Transitional Cell Carcinoma	HPV 16
Transitional Cell Carcinoma	HPV 16
Transitional Cell Carcinoma	Invalid
Transitional Cell Carcinoma	Invalid
Squamous Cell Carcinoma	Negative
Inverted Papilloma	HPV 16
Inverted Papilloma	HPV 11
Dysplastic papilloma	Negative
Squamoproliferative papilloma	Negative

embedded (FFPE) block was obtained and subjected to nucleic acid extraction using the Qiagen Mini Kit (Qiagen, Hilden, Germany) with a protocol optimized for the downstream detection of HPV<sup>(21)</sup>. All testing was performed centrally at the Scottish HPV Reference Laboratory. HPV genotyping was performed using the Optiplex HPV Genotyping test (Diamex, Heidelberg, Germany) which is a PCR based assay with luminex detection. The assay detects 24 HPV types including HPV6 and HPV11 and all established high-risk types; it also incorporates detection of an endogenous beta-globin control, tuned to detect 50 copies per reaction, as a sample adequacy check and is the assay applied for HPV immunization surveillance in Scotland. The assay has been scored "proficient" for HPV typing as adjudicated by external quality assurance panels including that of the World Health Organisation LabNet scheme which contains cloned HPV genomes ranging from concentrations of 5 international units (IU) to 500 IU. A test is regarded as proficient in typing "if it can detect 50 International Units (IU) / 5 ul of HPV 16 and HPV 18 DNA, and 500 genome equivalents (GE) / 5 ul of the other HPV types included in the panel". The analytical sensitivity of the assay ranges from 5 IU to 100 IU depending on HPV type (including 5 IU detectable for HPV 16 and 18 and 50 IU for types HPV 6 and 11).

Samples that were HPV negative and beta-globin negative were considered technically invalid for molecular HPV detection and re-tested using a new section and a 1:10 dilution of the nucleic acid. Despite this, two of the samples remained invalid for HPV detection over the separate attempts.

## Results

The histological diagnoses of the tumours submitted for analysis are summarised in Table 1. Transitional cell papilloma/carcinoma (TCP/TCC) comprised the majority (11/16). Fourteen of the sixteen blocks analysed were suitable for HPV testing. The 2 technically invalid samples (defined by HPV negative and housekeeping control negative results) were transitional cell carcinomas and were excluded. HPV type specific testing isolated low risk HPV 6 in one of the six TCP specimens (17%), high risk HPV 16 in two of three TCC specimens (67%), high risk HPV 16 in one inverted papilloma (IP), and low risk HPV 11 in the other IP specimen. In all papillomatous lesions, HPV was isolated in three of ten specimens (30%). In all carcinomatous lesions, high risk HPV 16 was isolated in two of four (50%).

There were two papillomas that, despite histopathological reviews, remained difficult to categorise; one demonstrated papillomatous features with dysplasia, the other was papillomatous but could not be further categorized into inverted/exophytic/oncocytic. Both of these samples were negative for HPV on PCR.

## Discussion

HPV has long been associated with cervical, anogenital and oropharyngeal carcinoma. It had been hoped that vaccination programmes might thus be able to reduce the incidence of these tumours. Indeed, a significant reduction in HPV 16 and 18 prevalence in the years since vaccination has been described in several settings as summarized in a recent metanalysis<sup>(22)</sup>, and a significant reduction in anogenital warts and cervical intraepithelial neoplasia (CIN) has been demonstrated in the UK<sup>(23)</sup>. Previously published series looking specifically at HPV presence in LDA papilloma and carcinoma have suggested a strong association<sup>(17,18)</sup> and thus the suggestion that vaccination might play a role in their future management. However, this was not the case with our series, particularly for the benign papillomata. We found only one of the six transitional cell papillomas to be positive for low risk HPV 6 on PCR analysis. Both Sjo et al. and Madreperla et al. also utilized PCR to identify HPV presence and it is generally accepted that PCR is more sensitive and specific compared to DNA in-situ hybridisation (ISH)<sup>(24)</sup>. Indeed, none of the PCR negative samples in Sjo's paper demonstrated positivity with ISH. Sjo et al. demonstrated HPV positivity in all four TCPs in their sample (three HPV 11 and one HPV 6). Madreperla et al. found HPV in two of three papillomas, but did not differentiate between papilloma subtypes.

The volume of TCP/TCC samples analysed reflects the tertiary nature of our unit and close relationship with our national Ophthalmology hospital; our unit has previously published the largest series of TCP and TCC tumours<sup>(6)</sup>. Although our series is the largest published series of TCPs, the sample size is still small and we accept that the local disease is heterogeneous. While the

analytical sensitivity of the assay applied in the present study was high (5 IU for HPV 16/18 and 50 IU for 6/11) the analytical sensitivities of HPV assays in the related literature are either not reported or are dissimilar in “read out” (for example ISH) so cannot be compared easily. Thus, it is feasible that differences in assay analytical sensitivity, and sample heterogeneity, could affect between-study observations as could nucleic acid input and quality in the case of amplification assays. Nucleic acid yield from 10 micron sections using the method applied in the current study is on average ~100 ng/ul but can range depending on the cellularity and age of the particular sample. Nevertheless, pooling the specific TCP data from our series and that of Sjo et al. only yields a 50% HPV positivity rate, so we suggest caution in attributing causality without further, complementary research – particularly as the DNA PCR based detection technology applied in the present study, while sensitive, does not indicate transcriptionally active HPV so it is feasible that an HPV is present but not necessarily driving or active within a lesion. Additionally it is possible that in a component of apparently “HPV negative” tumours, HPV may have caused the lesion at an earlier stage but then subsequently been fragmented and/or lost during later stages of lesion progression. This “hit and run” theory is gaining traction for cervical cancers where no HPV is detected using molecular technologies<sup>(25)</sup>. State of the art next generation sequencing technologies may lend further insight into the role of HPV in TCP through their high resolution and sensitivity for low copy number HPV sequences, as well as their ability to determine transcriptional activity of relevant host and viral genes. In addition, the application of markers of oncogenic transcriptional activity such as reverse transcription PCR of key HPV oncogenes E6 and E7 may provide additional information on viral activity. Furthermore, p16INK4a is known to be a secondary marker of deregulated E6/E7 activity and is used extensively to determine the HPV component of oropharyngeal cancer<sup>(26)</sup>. While its application to transitional cell papilloma of the LDA is not established or indicated clinically, it would be of interest to use it for research in this context to determine the concordance of p16INK4a and HPV status. Our data for transitional cell carcinoma did demonstrate high risk HPV 16 in 2 of the 3 technically valid samples; this finding correlates with that of Sjo et al, who demonstrated HPV 16 in three of four TCCs and HPV 11 in the fourth. The only other positive results were in both IP specimens; one for high risk HPV 16,

the other low risk HPV 11. Again, data on the role of HPV in IP is not strongly conclusive. In a recent review, Wang et al. explored the possible aetiological factors, and although HPV infection may play a role, the pathogenesis is likely multifactorial as HPV is not detectable in all tumours<sup>(27,28)</sup>.

Clearly this study is limited by the small sample size. Given the rarity of LDA tumours, and the heterogeneity of the subtypes, we would endorse a large multicentre study, where complementary HPV detection technologies reflecting key different chemistries are applied concurrently. Such a study would provide more robust insight into the likelihood and implications of HPV infection in LDA tumours.

## Conclusion

There may be multiple pathways for LDA neoplasia to develop; the human papilloma virus may drive one, as is the case for other epithelial tumours including those of the oropharynx, vulva, vagina and penis<sup>(11)</sup>. However, our data suggests we should remain cautious in attributing a causative role for HPV in transitional cell papillomas of the LDA. Additional complementary work that goes beyond the determination of HPV presence/absence in lacrimal transitional cell lesions and focuses on HPV activity could provide further insight into the extent and nature of the role of HPV in these neoplasms.

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## Authorship contribution

HJ & SG collated, prepared and analysed the data, and prepared the manuscript; SG collected data; KC organized HPV testing; JR designed the study, obtained ethical approval and collected data; VL supervised and provided the tissue samples; All authors reviewed the manuscript.

## Conflict of interest

None of the authors have conflicts to declare.

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