Inverted papilloma of the conjunctiva

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ABSTRACT

Objective The purpose of the present study is to describe the clinical and histopathological features of conjunctival inverted papilloma, to analyse for the presence of human papillomavirus (HPV), and to determine if HPV infection is associated with this type of tumour and its inverted growth pattern.

Methods and Analysis Cases of conjunctival inverted papillomas were retrieved from the archives of the Department of Pathology, Rigshospitalet, Copenhagen, Denmark. Patient records and pathology reports were reviewed. Formalin-fixed and paraffin-embedded tissue was analysed for the presence of HPV by immunohistochemistry, in situ hybridisation (ISH), PCR and HPV typed by sequencing.

Results A total of four cases were retrieved. The age at diagnosis ranged from 41 to 77 years, with an equal sex distribution. All lesions were localised to the bulbar conjunctiva and two of the cases were pigmented. Histopathological examination did not reveal areas of dysplasia. All lesions were p16-positive and p53-positive by immunohistochemistry. High-risk HPV 58 was demonstrated in one lesion by ISH and PCR.

Conclusion Here we present four cases of conjunctival inverted papilloma, which is an exceedingly rare tumour with only 11 previously reported cases in the literature. Both clinically and histopathologically, the tumours show distinct features compared with exophytic conjunctival papillomas. Furthermore, this is the first description of high-risk HPV 58 in a conjunctival tumour. The biological behavior of the tumour is uncertain due to its rarity. However, a complete removal of the lesion and a careful observation are recommended. The finding of HPV 58 underlines the necessity of this precaution.

INTRODUCTION

Papillomas are benign epithelial lesions of the mucous membranes. Conjunctival papillomas are histopathologically divided into exophytic and inverted papillomas. The inverted papilloma consists of folds of papillomatous epithelium that invaginate into the underlying stroma, rather than growing in a purely exophytic fashion that is characteristic of the far more common exophytic squamous papilloma.1 In the neighbouring regions, inverted papilloma usually originates in the nasal cavity and paranasal sinuses.2

Inverted conjunctival papilloma is exceedingly rare. To date, only 11 cases of inverted conjunctival papilloma have been reported.3–10 Due to its rarity, the aetiology and underlying biology are uncertain. Recurrence and malignant transformation occurred in 2 of the 11 cases described.3–10

Low-risk human papillomavirus (LR-HPV) is associated with the occurrence of exophytic conjunctival papilloma.11 HPV is a DNA virus with a double-stranded DNA genome, and variations in the DNA sequence define the more than 200 different genotypes,12 typically stratified into two groups targeting either the mucosal or cutaneous tissue (International Agency for Research on Cancer [IARC] Monograph, 2012). The IARC has defined 15 HPV genotypes as carcinogenic to humans based on sufficient evidence of carcinogenicity, hereafter referred to as high-risk HPV (HR-HPV).

The purposes of the present study are to describe the clinical and pathological features of conjunctival inverted papillomas, and furthermore to determine if HPV is associated with this tumour type and its inverted growth pattern.

MATERIALS AND METHODS

Sample selection

Cases of inverted conjunctival papillomas were retrieved from the archives of the Eye Pathology Section, Department of Pathology,
Rigshospitalet, which is the centralised, national ophthalmic pathology laboratory in Denmark. Histological review of the original diagnosis was performed for each specimen to confirm the diagnosis. Formalin-fixed and paraffin-embedded (FFPE) specimens were collected. Clinical and histopathological data including age, sex, localisation, duration of symptoms, clinical appearance, recurrence, treatment and histopathological features were gathered from the referring ophthalmologist and from pathology reports.

Immunohistochemistry
P16 immunohistochemistry (IHC) was performed as supplement and surrogate marker of HPV infection. P53 IHC was performed as an indicator of ultraviolet-induced tumour growth. IHC was performed on a Ventana BenchMark ULTRA IHC/ISH Staining Module (Ventana Medical Systems, Tucson, Arizona, USA) according to the manufacturer’s recommendations. P16 was detected by incubating sections with monoclonal mouse antibody CINtec p16 clone E6H4, and p53 was detected with monoclonal mouse antibody clone DO7 (both Roche Medical Systems, Tucson, Arizona, USA) according to the manufacturer’s recommendations. Slides were counterstained with haematoxylin. The threshold of p16 positivity was >70% positive and cell staining has been considered positive, according to Nordic Immunohistochemical Quality Control (http://www.NordiQC.org; Aalborg University Hospital).

In situ hybridisation
In situ hybridisation (ISH) for high-risk HPV DNA types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 66 was performed by Inform HPV III Family 16 Probe (Ventana Medical Systems) according to the manufacturer’s guidelines using a BenchMark automated slide staining system (Ventana Medical Systems). The specimens were dichotomously classified as positive or negative.

DNA extraction and detection of type-specific HPV
DNA extraction from FFPE was done using a 10 µm tissue section of the archival material. Proteinase K treatment and subsequent DNA extraction were performed using the QIAamp DNA FFPE extraction kit and the QIAcube extraction unit (both Qiagen, Hilden, Germany) in concordance with the manufacturer’s specifications.

HPV detection: nested PCR assay
Nested PCR with PGMY 09/11 L1 consensus primers and GP5+/GP6+ L1 consensus primers was used to analyse the extracted DNA samples as previously described by Rusan et al. Briefly, PCR reactions with PGMY 09/11 primers were performed in the first reaction and GP5+/GP6+ primers in the second reaction. Samples were run in duplicate. HPV plasmids for HPV 6, HPV 16 and HPV 18 were used as positive controls. Negative controls consisted of 5 µL of diethyl pyrocarbonate (DEPC)-treated H2O, instead of template. The RNase P housekeeping gene was used to verify that amplifiable DNA was present in all DNA extracts (TaQMan RNase P detection kit, Applied Biosystems). Positive samples were run on a 2% agarose gel, extracted using the QiAquick Gel Extraction Kit (Qiagen) and sent for sequencing to Eurofins MWG Operon. The positive and negative controls used in the PCR reaction were also run on the gel, and the positive controls were extracted and sent for sequencing. The one positive sample, along with several negative samples, was reanalysed to check for consistency.

RESULTS
Four cases of conjunctival inverted papillomas were retrieved from our files. Review of the patient journals and pathology files revealed a range of age at diagnosis from 41 to 77 years, with an equal sex distribution. The duration of the symptoms ranged from 1 year to more than 20 years. Two of the lesions were clinically mistaken for being a nevus due to the pigmented appearance (figure 1A). Three of the lesions were localised to the medial bulbar conjunctiva and one localised to the lateral limbus. All the cases were treated with complete excision without adjuvant therapy. Histopathological examination showed the invagination of the conjunctival epithelium into the stroma (figure 1B,C), and re-examination did not disclose any areas of intraepithelial dysplasia or invasiveness. None of the lesions recurred or progressed to malignancy within the follow-up time (minimum of 4 years). Clinical and histopathological information is listed in table 1.

All tumours were positive in p53 IHC and one tumour was positive in p16 IHC (table 2, figure 1D,E). Two of the four specimens were excluded from PCR due to insufficient tissue and DNA. HR-HPV 58 was identified by both PCR and CLART in one of the two inverted conjunctival papillomas available for PCR analysis (figure 1F). The same specimen was also positive by DNA ISH, whereas the three other tumours were HPV-negative using ISH.
DISCUSSION

The previously reported cases of inverted conjunctival papillomas have shown a broad variety regarding the clinical appearance, in accordance to our results. Inverted papillomas are described as pigmented, partly pigmented or completely unpigmented lesions, enlarging over a few months to several years and affecting females and males equally. Yet it is worth noticing that pigmentation of the papilloma by clinical evaluation has been described in 5 out of 15 lesions, in contrast to exophytic papilloma where pigmentation is rarely described. In the present series, this correlates to the histopathological finding of hyperpigmentation of the basal layers of the epithelium. The localisation of the lesions is scattered all over conjunctiva, however more frequently reported in the nasal part of the conjunctiva, which is seen in 10 out of 15 reported cases. Ten out of 15 cases were epibulbar. Unlike exophytic papillomas which are most often localised inferiorly, a superior location is more common in inverted papillomas (table 1). All present cases have been treated exclusively by surgical removal. This is the case also in previously reported cases, except in two cases where adjuvant cryotherapy and neoadjuvant local chemotherapy with Mitomycin were applied. In table 3, we have summarised the clinical characteristics of inverted conjunctival papillomas compared with the exophytic papillomas.

Histopathologically, no lesion has been reported with a keratinising epithelium, which was also the finding in the present cases. None of the present cases showed cellular
<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Sex</th>
<th>Duration of symptoms</th>
<th>Clinical appearance and/or clinical diagnosis</th>
<th>Localisation</th>
<th>Treatment</th>
<th>Histopathology</th>
<th>Recurrence</th>
</tr>
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<tbody>
<tr>
<td>Bhushan et al</td>
<td>11</td>
<td>M</td>
<td>4 years</td>
<td>Well-defined, pigmented lesion, 1.5x1 cm.</td>
<td>Limbus, superotemporal conjunctiva, left eye.</td>
<td>Excisional biopsy. Subepithelial and intraepithelial inflammation. No nuclear atypia, mitosis or necrosis. HPV testing not performed.</td>
<td>None (follow-up 2 years).</td>
</tr>
<tr>
<td>Chang et al</td>
<td>34</td>
<td>F</td>
<td>Unknown</td>
<td>Papillomatous appearance with no significant pigmentation, enlarged over 2 months and then excised.</td>
<td>Tarsal conjunctiva of the upper lid, nasal one-third, left eye.</td>
<td>Excision (incomplete). Mixed exophytic and endophytic. Goblet cells and cyst formation. HPV positive by ISH.</td>
<td>Lost to follow-up.</td>
</tr>
<tr>
<td>Heuring et al</td>
<td>96</td>
<td>F</td>
<td>At least 6 months</td>
<td>25x15x15 mm, reddish tumour with papillary surface.</td>
<td>Nasal bulbar conjunctiva, right eye.</td>
<td>Excision. Carcinomatous foci in the peripheral areas. HPV testing not performed.</td>
<td>Recurrence after 4 months.</td>
</tr>
<tr>
<td>Jakobiec et al</td>
<td>42</td>
<td>F</td>
<td>2 years</td>
<td>Cystic lesion.</td>
<td>Nasal epibulbar conjunctiva.</td>
<td>Excision, free margins. Goblet cells and cysts. HPV testing not performed.</td>
<td>None (follow-up 1 year).</td>
</tr>
<tr>
<td>Kalantzis et al</td>
<td>31</td>
<td>M</td>
<td>1 year</td>
<td>Fleshy mass, 1.1x0.9x0.3 cm, coexisting with an exophytic papilloma in the same eye.</td>
<td>Superior epibulbar conjunctiva, right eye.</td>
<td>Excision. Numerous mucin-secreting goblet cells. Negative for the presence of HPV 6, 11 and 16 by PCR.</td>
<td>None (follow-up 1 year).</td>
</tr>
<tr>
<td>Lassalle et al</td>
<td>48</td>
<td>M</td>
<td>Unknown</td>
<td>10x8x7 mm, sessile tumour.</td>
<td>Inferior palpebral conjunctiva, right eye.</td>
<td>Local chemotherapy with Mitomycin followed by surgical resection. Carcinomatous transformation within the papilloma with infiltration of the orbicularis muscle. Few goblet cells and cysts. HPV negative by microarray.</td>
<td>None (2 years follow-up).</td>
</tr>
<tr>
<td>Stagner et al</td>
<td>63</td>
<td>M</td>
<td>Unknown</td>
<td>Red-pink papillary lesion, 1.5x0.6x0.1 cm, extending up to the corneal limbus.</td>
<td>Inferonasal epibulbar conjunctiva, right eye.</td>
<td>Excision and cryotherapy. No goblet cells or cystic formation. HPV negative by PCR for high-risk HPV types.</td>
<td>None (follow-up 6 months).</td>
</tr>
<tr>
<td>Present case 1</td>
<td>41</td>
<td>F</td>
<td>&gt;20 years</td>
<td>Unpigmented, cystic lesion.</td>
<td>Inferonasal bulbar conjunctiva, left eye.</td>
<td>Excision, free margins.</td>
<td>Many goblet cells. No signs of dysplasia. No mitotic activity. HPV-negative.</td>
</tr>
<tr>
<td>Present case 4</td>
<td>51 years</td>
<td>F</td>
<td>Several years</td>
<td>Clinically diagnosed as a pinguecula.</td>
<td>Lateral limbus.</td>
<td>Excision, free margins.</td>
<td>Small cystic cavities. No sign of dysplasia. HPV-negative.</td>
</tr>
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F, female; HPV, human papillomavirus; ISH, in situ hybridisation; M, male.
atyria, dysplasia or invasive growth. However, of 11 previously reported cases in the literature, 2 showed an aggressive behaviour.\textsuperscript{5,10} Both of these conjunctival papillomas had carcinomatous foci at the time of diagnosis, and one of them recurred 4 months after primary excision.\textsuperscript{5} Studies of the regionally close sinonasal mucosa have shown that at least 15% of all cases of sinonasal papillomas have developed synchronously or metachronously have shown that at least 15% of all cases of sinonasal papillomas have developed synchronously or metachronously squamous cell carcinoma,\textsuperscript{2} and that sinonasal inverted papillomas have developed synchronously or metachronously.

All the cases available to p53 IHC (one missing due to lack of tumour tissue) were positive. There is a hypothesis that p53 overexpression correlates with ultraviolet-induced tumour growth,\textsuperscript{14} however, it is not a specific marker, and in concordance with a previous reported case of conjunctival inverted papilloma the p53 expression in our series is lower than the expression frequently seen in carcinoma of the conjunctiva.\textsuperscript{5}

In the present study, we have shown HR-HPV 58 in one of two investigated conjunctival inverted papillomas available for PCR analysis. This lesion was also positive by p16 IHC and HPV-positive by ISH. This is, to our knowledge, the first study demonstrating the presence of an HR-HPV 58 in a conjunctival lesion. A test for the presence of HPV has been done in 4 of the 11 reported cases of inverted conjunctival papillomas.\textsuperscript{4,7,8,10} HPV was detected in one case, but the viral genotype was in that case not determined.\textsuperscript{4}

In the more common exophytic conjunctival papilloma, there is a strong association to HPV, as HPV has been detected in 81%–92% in large case series.\textsuperscript{11,20} LR-HPV 6 and 11 are the most common types detected in these lesions, in accordance to their benign clinical nature. Only rarely, there are reported high-risk subtypes 16, 33 and 45 in exophytic conjunctival papilloma.\textsuperscript{11,20–22} These oncogenic HPV types are more often detected in epithelial malignant lesions of the conjunctiva, although the aetiological role of the virus in conjunctival squamous cell carcinoma remains controversial.

In sinonasal inverted papilloma, LR-HPV 6 and 11 and HR-HPV 16, 18 and 57 have been demonstrated.\textsuperscript{23} However, the detection rate is highly variable, with a reported range of 0%–100%.\textsuperscript{19,24} The possible oncogenic role of HPV in the progression of sinonasal inverted papillomas has been proposed in many studies.\textsuperscript{25}

Different theories may explain why the presence of HPV differs in inverted and exophytic papillomas. First, the hyperkeratotic superficial epithelium of exophytic papillomas is susceptible for HPV replication because reinfection of the papilloma by assembled virions may occur. In contrast, inverted papillomas tend to be non-keratinising, and as superficial epithelium is shed, HPV is lost, accounting for the lower detection rate.\textsuperscript{19} Second, HPV assays used for these types of studies are most often without defined clinical cut-offs, rendering any analysis dependent on the performing laboratory’s ability to run high-definition HPV assays. Here, we use a well-characterised, quality-controlled, clinical HPV array assay with a WHO Laboratory Network proficiency study-validated detection of all 13 HR-HPV genotypes plus HPV 6 and 11.\textsuperscript{20,27} Therefore, the inability to detect HPV DNA does not necessarily abolish a viral role in the tumour development of inverted papillomas.

In conclusion, HR-HPV 58 was identified in one of two inverted conjunctival papillomas with a successful DNA extraction. To our knowledge, this is the first study demonstrating HPV 58 in a conjunctival papilloma. HPV 58 is characterised as a high-risk HPV due to its oncogenic potential in other tissue types. Due to the rarity of inverted conjunctival papilloma, their biological

<table>
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<th>Table 2</th>
<th>Results of the HPV analyses</th>
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<tr>
<td>Present case</td>
<td>P16 IHC*</td>
</tr>
<tr>
<td>1</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>Negative</td>
</tr>
<tr>
<td>3</td>
<td>Positive</td>
</tr>
<tr>
<td>4</td>
<td>Negative</td>
</tr>
</tbody>
</table>

* Nuclear and cytoplasmic staining of tumor cells by immunohistochemistry. ** Nuclear staining of tumor cells by immunohistochemistry. HPV, human papillomavirus; IHC, immunohistochemistry; NA, not available; PCR, polymerase chain reaction.

<table>
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<th>Table 3</th>
<th>Clinical features of exophytic papilloma versus inverted papilloma of the conjunctiva</th>
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<tbody>
<tr>
<td></td>
<td>Inverted papilloma\textsuperscript{3–10}</td>
</tr>
<tr>
<td>Age (mean, range)</td>
<td>53.2 years (11–85)</td>
</tr>
<tr>
<td>Sex</td>
<td>Even gender distribution</td>
</tr>
<tr>
<td>Appearance</td>
<td>Commonly pigmented</td>
</tr>
<tr>
<td>Localisation</td>
<td>Most often nasal and superiorly</td>
</tr>
<tr>
<td>Malignancy</td>
<td>Described in 2 of 15 at the time of diagnosis</td>
</tr>
<tr>
<td>Human papillomavirus (HPV)</td>
<td>2 of 6 of tested lesions HPV-positive</td>
</tr>
</tbody>
</table>

Human papillomavirus (HPV)
behaviour is uncertain. However, since HPV 58 has a significant oncogenic potential and because of the high incidence of recurrence and association to malignancy of inverted papilloma in the neighbouring sinonasal region, inverted papillomas of the conjunctiva should be removed completely and the patients carefully observed. Our finding of HR-HPV 58 underlines the necessity of this precaution.

Contributors IR: contributed to data collection and interpretation, and manuscript drafting. NCS: contributed to conception and design, data collection, and manuscript drafting. JHB: contributed to data analysis and interpretation, and has revised the manuscript critically. SH: contributed to conception, design and data interpretation, and has revised the manuscript critically. All authors have approved the final version of the manuscript.

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Competing interests IR: none declared. NCS: none declared. JHB attended meetings with various HPV device manufacturers. JHB has received honoraria from Hologic/Gen-Probe, Roche, Qiagen, Genomica and BD Diagnostics for lectures. Hvidovre Hospital has ongoing contracts with BD Diagnostics, Genomica, Self-Screen and EU Horizon 2020. SH: none declared.

Patient consent for publication Not required.

Ethics approval The study was conducted according to the Declaration of Helsinki, and was approved by the Regional Scientific Ethics Committee of the Capital Region, Denmark (H-16044879) and the Danish Data Protection Agency (RH-2013-30-1035).

Provenance and peer review Not commissioned; externally peer reviewed.

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REFERENCES