SCIENTIFIC REPORT

Human papillomavirus in normal conjunctival tissue and in conjunctival papilloma: types and frequencies in a large series

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Aim: To examine conjunctival papilloma and normal conjunctival tissue for the presence of human papillomavirus (HPV).

Methods: Archival paraffin wax-embedded tissue from 165 conjunctival papillomas and from 20 histological normal conjunctival biopsy specimens was analysed for the presence of HPV by PCR. Specimens considered HPV positive using consensus primers, but with a negative or uncertain PCR result using type-specific HPV probes, were analysed with DNA sequencing.

Results: HPV was present in 86 of 106 (81%) β -globin-positive papillomas. HPV type 6 was positive in 80 cases, HPV type 11 was identified in 5 cases and HPV type 45 was present in a single papilloma. All the 20 normal conjunctival biopsy specimens were β -globin positive and HPV negative.

Conclusion: There is a strong association between HPV and conjunctival papilloma. The study presents the largest material of conjunctival papilloma investigated for HPV and the first investigation of HPV in normal conjunctival tissue. HPV types 6 and 11 are the most common HPV types in conjunctival papilloma. This also is the first report of HPV type 45 in conjunctival papilloma.

Human papillomavirus (HPV) is associated with the occurrence of conjunctival papilloma.¹ The individual HPV types are trophic for cutaneous or mucosal epithelium and have different oncogenic potentials.² So far, low-risk HPV types 6 and 11 and high-risk HPV types 16 and 33 have been identified in conjunctival papilloma.^{1,3–6} To our knowledge, similar studies on normal conjunctiva have not been reported to date. Therefore, it is still unclear whether HPV plays a crucial role in the development of conjunctival papilloma.

The purpose of this study was to analyse the largest material of conjunctival papilloma to date (fig 1) and normal conjunctival tissue for the presence of various HPV types. The aim was also to investigate for HPV types not described previously in conjunctival papilloma, and to determine whether HPV is a common pathogen on the normal ocular surface.

MATERIALS AND METHODS

We studied 165 cases of conjunctival papilloma from the files of the Eye Pathology Institute, Copenhagen, Denmark. The formalin-fixed, paraffin wax-embedded specimens were registered during the period 1988–97. A histological review of the diagnosis was made in each case. The material did not include our previous series of 55 conjunctival papillomas.¹

A total of 20 normal conjunctival biopsy specimens from patients undergoing strabismus surgery served as controls. Before excision biopsy, the conjunctiva underwent slit-lamp evaluation to exclude patients with clinical signs of ocular surface disease. All biopsy specimens were taken from the nasal and inferior part of the conjunctiva, which is known to be the most frequent location of papilloma.⁷ The investigation was

Figure 1 (A) Conjunctival papilloma (arrow) showing the characteristic exophytic, papillary growth. (B) The epithelium of the conjunctival papilloma is acanthotic (asterisk) and wrapped over the stems of fibrovascular cores (arrow) (H&E, magnification $\times 12.5$). (C) PCR investigation of conjunctival papilloma. Agarose gel with MY09/11 PCR products viewed in UV-light transillumination. Lane M contains the DNA band marker and lane 1 contains the positive control. Lanes 2–6 are considered HPV positive because of the presence of a DNA band of the appropriate size. Lane 7 contains the negative control.

approved by the Danish National Committee on Biomedical Research Ethics, Denmark.

PCR and DNA sequencing

PCR was carried out as described earlier.^{1,8} Specimens considered HPV positive using consensus primers, but with a negative or uncertain PCR result using type-specific probes, were analysed with DNA sequencing as described previously⁹ using GP5+/6+ primers.

Abbreviation: HPV, human papillomavirus

Table 1 Previous investigations of conjunctival papilloma for the presence of human papillomavirus using the PCR technique

Study	Cases	HPV types	HPV frequency (%)
McDonnell (1987) ¹⁰	23	6/11	65
Saegusa <i>et al</i> (1995) ⁶	16	16	75
Sjö <i>et al</i> (2001) ¹	55	6/11, 16	92
Eng <i>et al</i> (2002) ¹¹	24	6/11	58
This study	164	6, 11, 45	81

HPV, human papillomavirus.
Case reports are not included.

Tissue control

From each block from which sections were used for PCR analysis, an extra final cut was made for histological examination to ensure the presence of sufficient tissue.

RESULTS

The papillomas were excised from patients aged 17–82 years (mean age 37 years) and the normal controls originated from patients aged 18–65 years (mean age 27 years).

The β -globin fragment could not be amplified in 59 cases (36%) of the papilloma series and these specimens were excluded from further analysis.

HPV was detected in 86 of the remaining 106 papillomas (81%) using MY09/11 and GP5+/6+ consensus primers (fig 1). Using type-specific primers and DNA sequencing, HPV type 6 was positive in 80 cases, HPV type 11 was identified in 5 cases and HPV type 45 in a single papilloma (table 1).

All the 20 normal conjunctival biopsy specimens were β -globin positive and HPV negative.

DISCUSSION

This investigation confirms that most conjunctival papillomas harbour HPV (81%). In accordance with the benign nature of conjunctival papilloma, the low-risk HPV types 6 and 11 were found to be predominant among HPV-positive papillomas. Only in one case was an HPV type other than 6 or 11 identified—that is, HPV type 45. This is the first report of HPV type 45 in conjunctival papilloma. HPV type 45 is a high-risk HPV type primarily associated with low-grade cervical intraepithelial neoplasia of the uterus.¹² Histopathological examination of the HPV 45-positive conjunctival papilloma in this study did not disclose any signs of dysplasia. The HPV 45-positive papilloma recurred twice. However, it is known that 11% of the papillomas recur one or more times.⁷

In our material, 36% of the specimens could not be used for PCR investigations because amplifiable DNA could not be demonstrated. Formalin and paraffin wax contain large amounts of PCR inhibitors.¹³ Furthermore, it is known that the formalin-induced DNA degradation begins after a few hours of formalin fixation.¹³ At the Eye Pathology Institute, specimens are received by mail and thus formalin fixation time is never <24 h and in some cases is >3 days. This could explain why DNA extraction was unsuccessful in 36% of our cases.

This study is the first investigation of normal conjunctival tissue for the presence of HPV, and we found that all normal conjunctival biopsy specimens were HPV negative. This indicates that HPV is associated with conjunctival papilloma. Furthermore, our study shows that the conjunctiva is not an HPV reservoir, in contrast with the genital mucosa.¹⁴ This is in accordance with the outcome of similar studies on the neighbouring nasal and oral cavity, where HPV is likewise not present in normal mucosa.^{15 16}

In conclusion, our study presents the largest material of conjunctival papilloma investigated for HPV and the first investigation of a putative HPV presence in normal conjunctival tissue. Our investigation shows that HPV is associated with conjunctival papilloma.

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