Human papillomavirus in conjunctival papilloma

Nicolai Christian Sjö, Steffen Heegaard, Jan Ulrik Prause, Christian von Buchwald, Henning Lindeberg

Abstract

***Aim*—To examine conjunctival papillomas for the presence of human papillomavirus (HPV) and koilocytosis.**

***Methods*—Archival paraffin embedded tissue from 55 conjunctival papillomas was analysed for the presence of HPV by polymerase chain reaction and subsequent filter hybridisation. Histological sections of the 55 papillomas were evaluated for the presence of koilocytosis.**

***Results*—HPV was present in 48 of 52 (92%) β globin positive papillomas. HPV type 6/11 were found in 40 of 47 investigated papillomas and a double infection with HPV 6/11 and 16 was identified in a single papilloma. In six papillomas the HPV type could not be identified. Koilocytosis was present in 22 of 55 papillomas (40%).**

***Conclusion*—There is a strong association between HPV and conjunctival papillomas. HPV type 6/11 is the most common HPV type in conjunctival papilloma. The sensitivity of koilocytosis as an indicator of HPV in conjunctival papilloma is low.**

(*Br J Ophthalmol* 2001;**85**:785–787)

Eye Pathology Institute, University of Copenhagen, Denmark
N C Sjö
S Heegaard
J U Prause

Department of Ophthalmology, Rigshospitalet, Denmark
J U Prause

Department of Oto-rhino-laryngology, Rigshospitalet, Denmark
C von Buchwald

Oral Pathology Laboratory, Royal Dental College, University of Aarhus, Denmark
H Lindeberg

Correspondence to:
Steffen Heegaard, Eye Pathology Institute, University of Copenhagen, Frederik V's Vej 11, DK-2100 Copenhagen, Denmark
sh@eyepath.ku.dk

Accepted for publication
26 January 2001

Conjunctival papilloma is a benign and common tumour of the stratified squamous epithelium of the conjunctiva.[1 2] The growth pattern of conjunctival papillomas may be exophytic, mixed, or of the rare inverted type.[1 3] The exophytic configuration may be sessile or pedunculated.

Conjunctival papillomas are known to occur in both children and adults, but they are most common among people aged 20–39 years.[4 5] A male preponderance (60%) has been found among patients with conjunctival papilloma.[4]

The aetiology of conjunctival papillomas is not established, but human papillomavirus (HPV) is thought to be an important factor.[6] HPV is a family of double stranded DNA virus, of which 80 types have been identified and a number of new types are under examination (http://www.stdgen.lanl.gov/). HPV types 6, 11, and 16 have been identified in conjunctival papillomas.[7–9] In addition, HPV types 16 and 18 have been found in normal conjunctival tissue with a frequency of 32%.[10] The reported frequency of HPV DNA in conjunctival papillomas as determined by polymerase chain reaction (PCR) varies from 50% to 100% in three minor investigations.[8 11 12]

HPV induced diseases may in the future be prevented or treated through vaccination, since trials investigating the efficacy of HPV vaccines have shown promising results.[13 14]

Koilocytosis is the morphological hallmark of HPV infection.[15] The koilocyte is a superficial or intermediate mature squamous cell characterised by perinuclear vacuolation, densely staining peripheral cytoplasm, and a nucleus with an undulating nuclear membrane and a rope-like chromatin pattern.[16] Viral antigen has been demonstrated in nuclei of koilocytes using broad spectrum papillomavirus antibodies.[17]

The aim of the present study was to investigate conjunctival papillomas for the presence of HPV and koilocytosis.

Materials and methods

Fifty five conjunctival papillomas were collected from the files of the eye pathology institute (33 males, 22 females, age range 18–84 years, mean age 41 years). The specimens were registered during the period 1988–97. The papillomas were reviewed histologically on haematoxylin and eosin stained sections for confirmation of the diagnoses, and it was noted whether or not koilocytosis was present in the tumour.

POLYMERASE CHAIN REACTION

Three sections from each paraffin block were cut. The sections were placed in 1.5 ml tubes and 50 µl digestion buffer (10 mM TRIS pH 7.0, 1 mM EDTA, proteinase K 200 µg/ml) was added. The specimens were digested at 65°C for 3 hours, spun, and the aqueous phase was transferred to a new tube. Boiling for 10 minutes inactivated the proteinase K. The samples, together with appropriate positive and negative controls, were amplified with primers targeting a 288 bp fragment of the single copy β globin gene in order to ensure the integrity of the DNA. β Globin negative papillomas were excluded from further analysis. PCR was performed with the universally accepted consensus primers GP5+/GP6+ and CPI/CPII.[18 19] Samples that were HPV negative with these primers, were further subjected to PCR with MY9/MY11 consensus primers.[20] These primers will amplify the majority of the known mucosatropic HPV types. (Primer GP5+/GP6+; HPV type: 6, 7, 10, 11, 13, 14D, 16, 18, 30, 31, 32, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 54, 55, 56, 58, 59, 61, 62, 66, 72, 73. Primer CPI/CPII; HPV type: 2A, 4, 5, 6, 7, 8, 10, 11, 16, 17, 18, 21, 24, 31, 33, 34, 35, 36, 44, 45, 47, 51, 52, 53, 54, 55, 56, 58, 59, 66, 68. Primer MY9/MY11; HPV type: 2A, 6, 7, 8, 9, 10, 11, 13, 15, 16, 17, 18, 21, 22, 23, 24, 25, 28, 29, 31, 32, 33, 34, 35, 36, 38, 40, 42, 44, 45, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 59, 61, 62, 63, 64, 66, 67, 68, 69, 70, 72, 73.)[21] A volume of 15 µl of the PCR product was run in

Table 1 Correlation between koilocytosis and HPV in conjunctival papillomas

		Positive	*HPV negative*	*Total*
Koilocytosis	Positive	19	2	21
	Negative	29	2	31
	Total	48	4	52

a 4% submerged 3:1 NueSieve agarose (Medinova, Hellerup, Denmark) in TAE buffer, stained with GelStar and viewed in ultraviolet transillumination.

The sample was considered HPV positive when a DNA sample of a predicted size (for example, ~140 bp for the GP5+/GP6+ primer) was identified. HPV positive samples were analysed by dot-blot hybridisation with alkaline phosphatases labelled oligoprobes for HPV types 6/11, 16, 18, 31, 33, 35, and 45.[21] Visualisation was performed by chemiluminescence (CSPP, Roche Molecular Biochemicals).

Results

Of the 55 papillomas investigated, the β globin fragment was amplified from 52 (94.5%). The three β globin negative papillomas were excluded from further analysis. HPV was detected in 48 of the 52 (92%) investigated papillomas. The GP5+/GP6+ primer identified 32 HPV positive samples. Using the CPI/CPII primer a further eight HPV positive papillomas were found and the MY9/MY11 primer identified eight further HPV positive specimens.

Dot-blot analysis was conducted on 47 of the 48 papillomas and demonstrated HPV 6/11 in 40 papillomas and HPV types 6/11 and 16 in one papilloma.

Koilocytosis was present in 22 of the 55 papillomas (40%).

The results of the PCR analysis for HPV were compared with the results of the histological examination for koilocytosis. Nineteen of the 48 (40%) papillomas that tested positive for HPV with PCR contained areas with koilocytosis (Table 1).

Discussion

We found like other investigators that the presence of koilocytosis has a low diagnostic value for HPV infection.[22 23] Consequently, increasing interest has been focused on methods of direct detection of HPV.

Various authors have analysed conjunctival papillomas for presence of HPV, and HPV types 6, 11, and 16 have been demonstrated (Table 2). The previously published material has either been small or less sensitive techniques have been applied (Table 2). Using the PCR technique with consensus primers we demonstrated HPV in 48 of 52 papillomas (92%). The consensus primers employed in this study each identified different proportions of the papillomas as being HPV positive. This may well depend upon the fact that the employed consensus primers have different sensitivity and specificity for HPV types, and this stresses the need to use more than one consensus primer when investigating archival paraffin embedded tissue for HPV.

Table 2 HPV DNA in conjunctival papillomas. Comparison of published data

Study	*Methods*	*HPV types*	*HPV*	*Cases*
Fierlbeck (1990)[36]	ISH	6	—	1
Michel (1996)[37]	ISH	6/11	—	1
Mincione (1992)[38]	ISH	6/11	50%	4
Mantyjarvi (1989)[7]	ISH	11	—	1
McDonnell (1987)[6]	ISH	6/11	65%	23
Naghashfar (1986)[9]	SB, ISH	6	—	1
Lass (1983)[17]	SB	11	50%	2
Saegusa (1995)[8]	PCR	16	100%	5
Nakamura (1997)[11]	PCR	6	50%	8
Assadoullina (1999)[12]	PCR	6	—	1
Present study	PCR	6/11,16	92%	52

ISH = in situ hybridisation, SB = Southern blotting, PCR = polymerase chain reaction.

This study revealed that the HPV types most commonly found in papillomas of the conjunctiva in Denmark are HPV 6/11. HPV infection with multiple types is known to occur[24] and indeed we were able to demonstrate a single papilloma with a double infection with HPV types 6/11 and 16. Naturally, we cannot rule out the possibility of other HPV types also being present.

HPV types can be classified according to their potential for inducing anogenital carcinomas.[25] HPV types rarely found in cancers are defined as low risk types and those often found in cancers are high risk types.[25] In our material only one of 48 papillomas contained high risk HPV (HPV 16) and this might substantiate the fact that carcinoma rarely develops from a conjunctival papilloma.[26] Furthermore, the high risk HPV DNA was detected in a papilloma also harbouring low risk HPV. Perhaps simultaneous infection of conjunctiva with low and high risk HPV types is necessary for the rare development of carcinoma in a papilloma.

HPV is rarely present in normal oral and nasal mucosa.[27 28] However, HPV has been found in cytomorphologically normal cervical smears with a prevalence up to 25%.[29] In the only study of normal conjunctiva HPV was found in 32% of the samples, but this study only looked for two different HPV types.[10] The finding of HPV in normal conjunctival tissue may indicate that the neoplastic development of a papilloma is initiated by multiple factors—among these HPV. Immune suppressed organ transplanted patients suffer particularly high incidences of HPV infections that are directly associated with the extent and duration of immune suppression, and an association between HIV and an increase in the incidence of conjunctival carcinomas has been found.[30 31]

The transmission route of HPV to the conjunctiva has not yet been clarified.

Fetal passage through an infected birth canal might be one of the more common methods of direct transmission. Nine out of nine papillomas from patients aged 1–10 years contained HPV in one study.[6] Autoinoculation certainly seems as a probable transmission mode and McDonnell *et al* found HPV in ocular swabs from women with HPV induced lesions of the genital tract.[32]

HPV vaccines with both prophylactic and therapeutic potential are being developed and are presently undergoing phase I and phase II clinical trials.[13 14] This potential treatment would especially benefit patients experiencing

multiple recurrences of conjunctival papilloma and patients with malignant tumours associated with HPV such as conjunctival carcinoma and squamous cell tumours of the lacrimal system.[33–35] Moreover, the development of HPV vaccines may lead to a decrease in HPV associated tumours.

Landsforeningen Værn om Synet; The John and Birte Meyer Foundation; Betzy, Dagny og Caja Bojesens Mindefond; Synoptik Fonden; and The Danish Eye Research Foundation supported this study.

1 Conlon MR, Alfonso EC, Starck T, *et al.* In: Albert DM, Jakobiec FA, eds. *Principles and practice of ophthalmology*. Pennsylvania: Saunders, 1994:278–9.
2 Santos A, Gomez-Leal A. Lesions of the lacrimal caruncle. Clinicopathologic features. *Ophthalmology* 1994;**101**:943–9.
3 Streeten BW, Carrillo R, Jamison R, *et al.* Inverted papilloma of the conjunctiva. *Am J Ophthalmol* 1979;**88**: 1062–6.
4 Sjö N, Heegaard S, Prause J. Conjunctival papilloma. *Acta Ophthalmol Scand* 2000;**78**:663-6.
5 Elsas FJ, Green WR. Epibulbar tumors in childhood. *Am J Ophthalmol* 1975;**79**:1001–7.
6 McDonnell PJ, McDonnell JM, Kessis T, *et al*. Detection of human papillomavirus type 6/11 DNA in conjunctival papillomas by in situ hybridization with radioactive probes. *Hum Pathol* 1987;**18**:1115–9.
7 Mantyjarvi M, Syrjanen S, Kaipiainen S, *et al.* Detection of human papillomavirus type 11 DNA in a conjunctival squamous cell papilloma by in situ hybridization with biotinylated probes. *Acta Ophthalmol (Copenh)* 1989;**67**: 425–9.
8 Saegusa M, Takano Y, Hashimura M, *et al.* HPV type 16 in conjunctival and junctional papilloma, dysplasia, and squamous cell carcinoma. *J Clin Pathol* 1995;**48**:1106–10.
9 Naghashfar Z, McDonnell PJ, McDonnell JM, *et al.* Genital tract papillomavirus type 6 in recurrent conjunctival papilloma. *Arch Ophthalmol* 1986;**104**:1814–5.
10 Karcioglu ZA, Issa TM. Human papilloma virus in neoplastic and non-neoplastic conditions of the external eye. *Br J Ophthalmol* 1997;**81**:595–8.
11 Nakamura Y, Mashima Y, Kameyama K, *et al*. Detection of human papillomavirus infection in squamous tumours of the conjunctiva and lacrimal sac by immunohistochemistry, in situ hybridisation, and polymerase chain reaction. *Br J Ophthalmol* 1997;**81**:308–13.
12 Assadoullina A, de Villiers EM, Bialasiewicz AA, *et al.* [Detection of human papillomavirus types in tumors and inflammations of the eye.] *Klin Monatsbl Augenheilkd* 1999; **215**:10–18.
13 Balsley JF, Connor EM, Reichman RC, *et al.* Progress in the development of human papillomavirus vaccines for HPV-11 and HPV-16/18 and mapping of a critical neutralizing epitope. 18th International Papillomavirus Conference, Barcelona, Spain, 2000:366.
14 Fife KH, Brown DR, Wheeler CM, *et al.* A dose-ranging study of the safety and immunogenicity profiles of an HPV 11 L1 VLP candidate vaccine in young health women. 18th International Papillomavirus Conference, Barcelona, Spain, 2000:364.
15 Koss LG. Cytologic and histologic manifestations of human papillomavirus infection of the uterine cervix. *Cancer Detect Prev* 1990;**14**:461–4.
16 Rosai J. In: Rosai J, ed. *Ackerman's surgical pathology*. St Louis: Mosby, 1996:1358.
17 Lass JH, Grove AS, Papale JJ, *et al.* Detection of human papillomavirus DNA sequences in conjunctival papilloma. *Am J Ophthalmol* 1983;**96**:670–4.
18 Roda Husman AM, Walboomers JM, van den Brule AJC, *et al.* The use of general primers GP5 and GP6 elongated at their 3' ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. *J Gen Virol* 1995;**76**:1057–62.
19 Smits HL, Tieben LM, Tjong AHS, *et al.* Detection and typing of human papillomaviruses present in fixed and stained archival cervical smears by a consensus polymerase chain reaction and direct sequence analysis allow the identification of a broad spectrum of human papillomavirus types. *J Gen Virol* 1992;**73(Pt 12)**:3263–8.
20 Manos MM, Ting Y, Wright D, *et al.* Use of polymerase chain reaction amplification for the detection of genital human papillomaviruses. *Cancer Cells* 1989;**7**:209–14.
21 Lindeberg H, Krogdahl A. Laryngeal dysplasia and the human papillomavirus. *Clin Otolaryngol* 1997;**22**:382–6.
22 McDonnell JM, McDonnell PJ, Mounts P, *et al.* Demonstration of papillomavirus capsid antigen in human conjunctival neoplasia. *Arch Ophthalmol* 1986;**104**:1801–5.
23 Horn PL, Lowell DM, LiVolsi VA, *et al*. Reproducibility of the cytologic diagnosis of human papillomavirus infection. *Acta Cytol* 1985;**29**:692–4.
24 Bonnez W. In: Richman D, Whitley R, Hayden F, eds. *Clinical virology*. New York: Churchill Livingstone, 1997:569–611.
25 Lorincz AT, Reid R, Jenson AB, *et al.* Human papillomavirus infection of the cervix: relative risk associations of 15 common anogenital types. *Obstet Gynecol* 1992;**79**:328–37.
26 Zimmerman LE, Sobin LH. *Histological typing of tumours of the eye and its adnexa*. Geneva: WHO, 1980:23–7.
27 Eike A, Buchwald C, Rolighed J, *et al*. Human papillomavirus (HPV) is rarely present in normal oral and nasal mucosa. *Clin Otolaryngol* 1995;**20**:171–3.
28 Buchwald C, Franzmann MB, Jacobsen GK, *et al*. Human papillomavirus and normal nasal mucosa: detection of human papillomavirus DNA in normal nasal mucosa biopsies by polymerase chain reaction and in situ hybridization. *Laryngoscope* 1994;**104**:755–7.
29 Melkert PW, Hopman E, van den Brule AJ, *et al.* Prevalence of HPV in cytomorphologically normal cervical smears, as determined by the polymerase chain reaction, is age-dependent. *Int J Cancer* 1993;**53**:919–23.
30 Alani RM, Munger K. Human papillomaviruses and associated malignancies. *J Clin Oncol* 1998;**16**:330–7.
31 Waddell KM, Lewallen S, Lucas SB, *et al.* Carcinoma of the conjunctiva and HIV infection in Uganda and Malawi [see comments]. *Br J Ophthalmol* 1996;**80**:503–8.
32 McDonnell JM, Wagner D, Ng ST, *et al.* Human papillomavirus type 16 DNA in ocular and cervical swabs of women with genital tract condylomata. *Am J Ophthalmol* 1991;**112**: 61–6.
33 Tabrizi SN, McCurrach FE, Drewe RH, *et al.* Human papillomavirus in corneal and conjunctival carcinoma. *Aust NZ J Ophthalmol* 1997;**25**:211–5.
34 Buchwald C, Skoedt V, Tos M. An expansive papilloma of the nasolachrymal drainage system harbouring human papilloma virus. *Rhinology* 1996;**34**:184–5.
35 Madreperla SA, Green WR, Daniel R, *et al.* Human papillomavirus in primary epithelial tumors of the lacrimal sac. *Ophthalmology* 1993;**100**:569–73.
36 Fierlbeck G, Rassner G, Thiel HJ, *et al.* [Virus-induced papilloma of the conjunctiva. Detection of HPV 6a DNA.] *Z Hautkr* 1990;**65**:497–9.
37 Michel JL, Guiguen Y, Leger F, *et al.* [Human papillomavirus 6/11 in conjunctival papilloma.] *Ann Dermatol Venereol* 1996;**123**:90–2.
38 Mincione GP, Taddei GL, Wolovsky M, *et al*. Detection of human papillomavirus (HPV) DNA type 6/11 in a conjunctival papilloma by in situ hybridization with biotinylated probes. *Pathologica* 1992;**84**:483–8.