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SHORT COMMUNICATION

Conjunctival papilloma and human papillomavirus: Identification of HPV types by PCR

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PURPOSE. *To report the identification of human papillomavirus types in four cases of conjunctival papillomas and to review the literature regarding human conjunctival papillomavirus (HPV).*
METHODS. *Specimens from conjunctival papillomas of four patients were analyzed for the presence of HPV by polymerase chain reaction and subsequent filter hybridization. HPV types 6, 11, 16, 18, 31, and 33 were investigated. Histologic sections were analyzed for the presence of koilocytosis.*
RESULTS. *Histologic examination confirmed HPV infection in all cases. HPV type 11 was detected in all specimens.*
CONCLUSIONS. *HPV is frequently implicated in the pathogenesis of proliferative squamous lesions. HPV type 11 was the most frequently found in benign conjunctival lesion in this study. (Eur J Ophthalmol 2006; 16: 473-7)*

KEY WORDS. *Human papillomavirus, Conjunctival papilloma, PCR*

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INTRODUCTION

Conjunctival papilloma is a benign and common lesion of the stratified squamous epithelium of the tarsal and bulbar conjunctiva (1). The incidence is highest among patients aged between 20 and 39 years. The etiology of conjunctival papillomas is not established, but human papillomavirus (HPV) infection is thought to be an important factor (2).

The HPV family includes double stranded DNA viruses with specific tropism for keratinocytes. Human papillomaviruses essentially induce skin and mucosal epithelial lesions and have been implicated in the pathogenesis of epithelial neoplasms of the genital (uterine cervix), urogenital, and respiratory tracts (3).

Several studies have shown that most papillary lesions of the conjunctiva and lacrimal sac may be related to HPV types 6 (4) and 11 (2) while squamous dysplasia or carcinoma seem to be more related to HPV types 16 and 18 (5).

Various authors have demonstrated that koilocytosis, the morphologic hallmark of HPV infection, has a low sensitivity as diagnostic indicator of infection (6). Thus, at present, the most used test in these cases is polymerase chain reaction (PCR), an *in vitro* DNA amplification technique which greatly enhances the detection of defined DNA sequences (5, 7).

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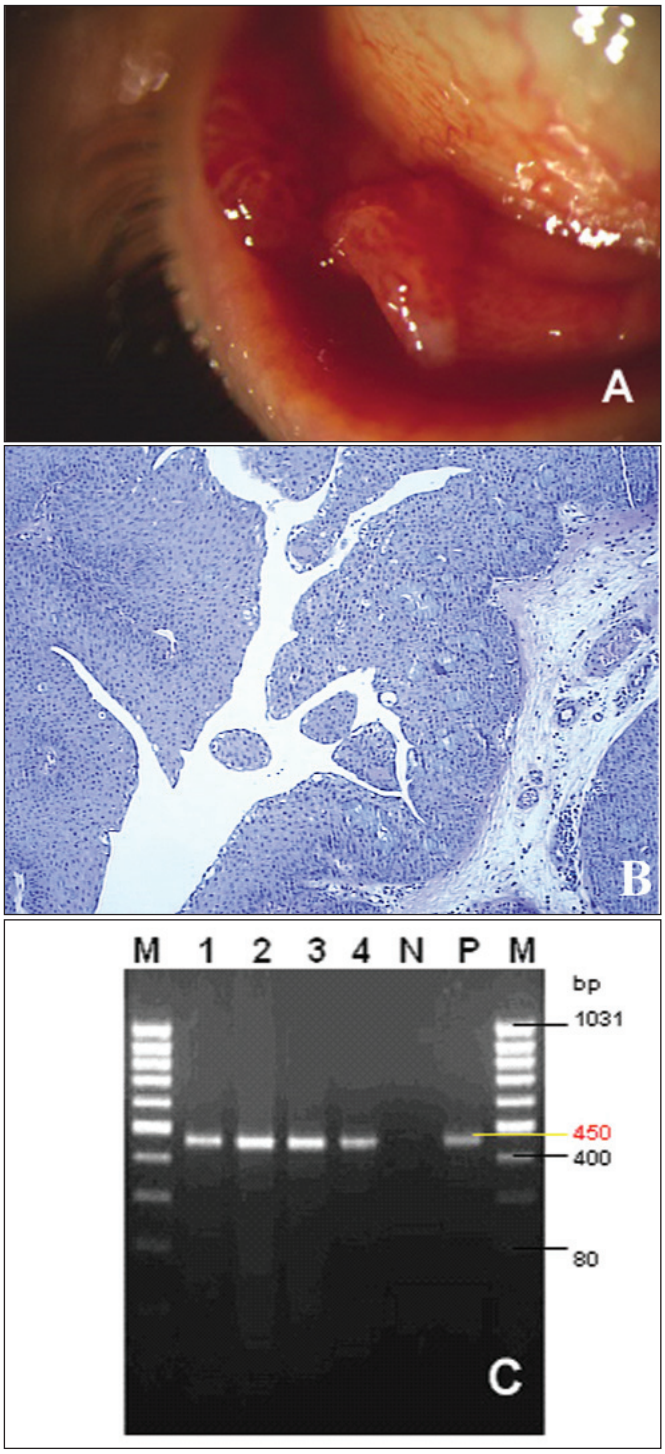

Fig. 1 - *Picture* **1A** *shows the pink, sessile lesion in the nasal conjunctiva of patient 3. Histological analisys of the papilloma shows the typical presence of acanthotic epithelium with many blood vessels going into the individual fronds (hematoxylin/eosin magnification 2X,* **1B***). The PCR shows the presence of HPV type 11 in all patients (lines from 1 to 4), as demonstrated by the evidence of a DNA fragment of 450 KB (M= size marker; N= negative control; P= positive control; Figure* **1C***)*

Published studies have reported the incidence of HPV types in Danish (6), Japanese (7), and American (8) patients with conjunctival papilloma. There are at present no published data regarding Italian patients.

The aim of this study was to identify HPV types, by PCR, in four cases of human conjunctival papilloma and to review the literature.

METHODS

The clinical data of four patients who were referred to our department in 2001 with diagnosis of conjunctival papillomas (Fig. 1A) were recorded (Tab. I).

All patients underwent surgical excision of lesions and the samples were processed according to the subsequently described procedure.

All conjunctival lesions were excised under local anesthesia followed by cryotherapy treatment in the conjunctival margins.

No recurrence of the lesion was observed after 3 years of postoperative follow-up.

All excised samples were divided into two fragments. One fragment from each specimen was formalin-fixed and embedded in paraffin. Sections were cut from each paraffin block and mounted on slides. One slide from each sample was stained with hematoxylin/eosin, and a second slide was stained according to the PAS method. Slides were examined to evaluate the presence of koilocytosis.

The second fragment of the excised sample was frozen and stored at –80°C until HPV DNA detection. High-molecular weight DNA isolation from tissue specimens was carried out by standard procedures (9). Briefly, DNA was extracted by sodium dodecyl sulfate/proteinase K treatment, followed by repeated phenol and chloroform extractions, and then precipitated with ethanol. DNA concentration was determined spectrophotometrically and the quality of DNA samples was assessed by agarose gel electrophoresis.

Genomic DNA (0.5 µg) was analyzed with an assay that allows the detection of a broad spectrum of HPV genotypes by consensus primer-mediated PCR. MY11 and MY09 primers, selected from a highly conserved region (L1) within the HPV genome, were used as described by Resnick et al (10). An aliquot of the amplified DNA was analyzed by 1.5% agarose gel electrophoresis and ethidium bromide staining to visualize fragments of the expect-

ed size (450 bp). To identify HPV types, PCR products were hybridized with six probes, recording the six most common viral types reputed to cause human conjunctival papilloma. The amplification products were subjected to DNA enzyme immunoassay (Gen-Eti-K-DEIA-HPV, Sorin, Saluggia, Italy) for HPV typing (genotypes 6, 11, 16, 18, and 31), according to the manufacturer's instructions. Type-specific PCR was used to detect HPV-33 DNA, as described by Van den Brule et al (11).

Negative controls included reaction mixtures lacking any DNA template and reaction mixtures containing human DNA without HPV target sequences. Procedures to prevent specimen contamination and PCR carryover were rigorously observed at every step in this analysis.

RESULTS

The histopathologic evaluation of the four samples showed the typical features of human conjunctival papilloma: fibrovascular core; acanthotic epithelium, tending to slight or moderate keratinization; and typical koilocytotic cells in the superficial epithelial layers (8) (Fig. 1B).

To identify HPV types, PCR products were hybridized with six probes, recording the six most common viral types reputed to cause human conjunctival papilloma. The HPV type 11 probe, specifically hybridized to the viral sequence, was identified in all analyzed specimens (Fig. 1C). There were no other identifiable bands (of the other viral probes) even after prolonged exposure.

DISCUSSION

HPV is a family of double-stranded DNA viruses with a genome organized into coding region, eight early (E1–E8) and two late (L1, L2) open reading frames, noncoding regions, and upstream regulatory region.

Up to now, more than 80 types of HPV have been isolated and they are classified on the basis of sequence variation from the known types in specific regions of the viral genome (12).

All papillomaviruses are intranuclear organisms with specific tropism for cutaneous and mucosal keratinocytes. Infection probably occurs as a result of basal cell exposure to HPV particles after minor traumas to the epithelium. Three possible forms of HPV infection can be defined: latent, subclinical, and clinical. The clinical signif-

TABLE I - CLINICAL DATA OF FOUR PATIENTS WITH DIAGNOSIS OF CONJUNCTIVAL PAPILLOMA

Patient	Sex	Age, yr	Symptoms	Duration of symptoms	VA	Anterior segment examination	Genital–anal condyloma
1	F	32	OD: foreign body sensation, ocular hyperemia, photophobia	12 mo	OD: 20/80 OS: 20/20	OD: red, sessile, avascular lesion in the superior tarsal conjunctiva	Y
2	F	18	OO: foreign body sensation, ocular hyperemia, burning (OD>OS)	8 mo	OD: 20/20 OS: 20/20	OO: pink, pedunculated, avascular lesion in the inferior tarsal conjunctiva (OD>OS)	Y
3	F	12	OS: redness, burning	8 mo	OD: 20/20 OS: 20/20	OS: pink, sessile lesion in the nasal conjunctiva, adjacent the caruncle	N
4	M	25	OD: photophobia, redness, tearing	18 mo	OD: 20/20 OS: 20/20	OD: pink, sessile, inferior conjunctival lesion	Y

Patient 1 presented with complicated type 1 diabetes mellitus, systemic arterial hypertension, celiac disease, and psoriasis, and nuclear cataract OD.
VA = Visual acuity; OD = Right eye; OS = Left eye; OO = Both eyes

icance of HPV-associated lesions is determined mostly by their location, the infecting viral type, and the factors related to the host (e.g., immune suppression, ultraviolet exposure) (13-15).

At present, HPV types rarely found in invasive cancers are characterized as low risk HPVs. In contrast, high risk types are frequently found in invasive cancers. The remaining HPV types that are infrequently associated with invasive cancer are defined as intermediate risk HPVs (16).

Previous studies have shown that HPV types 6 and 11 are associated with benign lesions of the ocular surface (2, 4, 8, 13), whereas HPV types 16 and 18 are high risk HPVs related to squamous dysplasia or carcinoma (5, 6, 14). Carcinoma rarely develops from a conjunctival papilloma, but simultaneous conjunctival infection by low and high risk HPVs is known to occur (6, 17).

There is a considerable molecular heterogeneity not only among HPV isolates from different ethnic populations but also among individuals from the same population (18), as supported by two different studies on the Japanese population. In fact, in a case series of Japanese patients (7), PCR analysis of four of nine benign lesions revealed the presence of HPV type 6 DNA only, while HPV was not found in the other five specimens. However, Saegusa et al detected HPV-16 in 12 papillomas of Japanese patients (19).

Our data confirm that in our patients, as in other European patients (6, 20), HPV type 11 is the most detected in human conjunctival papillomas. Indeed, Sjo et al (6) demonstrated HPV 6/11 in 40 of 47 investigated papillomas in Denmark, while in 1989 Mantyjarvi et al identified HPV type 11 in a single papilloma of a Finnish patient (20). Eng et al in a study conducted on the Chinese population noted that HPV-6 or HPV-11 is present in a substantial percentage of conjunctival papillomas (64%) (21).

The manner of transmission has not been clarified. Some authors consider that it may occur as a result of fetal passage through an infected birth canal or by ocular contact with contaminated hands or objects (6, 22).

In three of our patients, the history of anal and/or genital condyloma seems to confirm autoinoculation as a probable transmission mode. The age of the third patient suggests infant-acquired conjunctival infection, originating from the mother, probably during delivery.

Therapeutic success is difficult to assess because of spontaneous regression and recurrence of the lesion. The standard therapy includes surgical excision of the lesion followed by cryotherapy or application of an inhibitor of DNA synthesis (5-fluorouracil). In our study, we excised all papillomas with subsequent application of cryotherapy without recurrence after 3 years of postoperative follow-up. However, we cannot rule out the possibility of viral persistence or latency.

In conclusion, we confirm that HPVs are frequently implicated in the pathogenesis of human conjunctival papilloma and that HPV-11 is the most detected type in these benign lesions.

The authors have no proprietary interest.

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