

Detection of Herpes Simplex Virus and Human Papilloma Virus in Ophthalmic Pterygium

Efstathios T. Detorakis, M.D., George Sourvinos, Ph.D., and
Demetrios A. Spandidos, Ph.D., D.Sc., F.R.C.Path., F.R.S.H.

Purpose. To evaluate the presence of herpes simplex virus (HSV) and human papilloma virus (HPV) in pterygia and phenotypically normal conjunctiva and the possible relation between viral presence and clinical information. **Methods.** Fifty pterygia and respective conjunctival specimens were obtained. A personal and family history was recorded for each patient. HSV and HPV detection and typing were accomplished by polymerase chain reaction amplification of viral sequences. Results were statistically analyzed. **Results.** HSV (type 1) was detected in 11 (22%), HPV (type 18) in 12 (24%), and both HSV-1 and HPV-18 in 3 (6%) of pterygia. No conjunctival specimen displayed HSV, whereas HPV was detected in four (8%). Postoperative recurrence and history of conjunctivitis were significantly more common in patients with simultaneous detection of HSV and HPV. **Conclusion.** The fact that HSV was not detected in conjunctival specimens implies a more specific correlation with pterygium, as compared with HPV. The detection of potentially oncogenic viruses, such as HSV and HPV, supports the concept that pterygium can be considered a neoplastic condition. The correlation of postoperative recurrence and a history of conjunctivitis with the simultaneous detection of HPV and HSV, implies a possible viral cooperation affecting the clinical profile of pterygium.

Key Words: Pterygium—Polymerase chain reaction—Herpes simplex virus—Papilloma virus.

PURPOSE

Pterygium is a lesion of the corneoscleral limbus. Although benign in nature, it can threaten vision in many ways and a surgical excision is commonly indicated.¹ Despite numerous theories thus far, the exact pathogenesis remains unknown, although a strong correlation with environmental exposure (e.g., to sunlight, dust, and wind) has been accepted.² Other factors proposed to be implicated are inheritance, possibly in an autosomal dominant form,³ immunologic reactions, possibly as a type I mechanism involving deposition of IgE in the stroma,⁴ tear film disturbances, although such disturbances could be either a cause or result of pterygium,⁵ light focusing by transcameral pathways,² irradiation,⁶ and chronic local inflammation.² Additionally, genetic alterations, such as loss of heterozygosity, have been found in pterygium, especially at

chromosomal areas 17q, 9p, and 9q, suggesting the possible involvement of tumor suppressor genes in the pathogenesis of this condition.^{7,8}

Previous studies have reported the occasional presence of herpes simplex virus (HSV)⁹ and human papilloma virus (HPV)¹⁰ in ophthalmic pterygia. HPV has also been isolated from neoplastic conditions of cornea and conjunctiva.¹¹ Additionally, there are reports that HSV is involved in tumorigenesis.^{12,13} Taking into account that pterygium has been proposed to be a neoplastic condition,⁷ the presence of potentially tumorigenic viruses implies a possible involvement of viral infection in the pathogenesis of this condition. The current study aims at evaluating the presence of HSV and HPV in pterygia and adjacent phenotypically normal conjunctiva as well as the possible correlation of results with clinical information.

METHODS

The samples were obtained from patients successively treated at the Ophthalmological Clinic of the University Hospital of Heraklion, Crete, Greece. Medical and ophthalmologic histories were recorded for each patient, a slit-lamp examination was performed, and pterygia were photographed before surgery. The lesions were excised in toto, under local anesthesia, using the bare sclera technique.¹ In each case, a specimen of adjacent clinically normal conjunctival tissue (from 12 o'clock of corneoconjunctival limbus), was obtained. Immediately after surgery, tissue specimens (either pterygia or conjunctiva) were stored at -70°C . DNA extraction was subsequently performed under a standard protocol using organic detergents.¹⁴

Fifty pterygia were obtained, 24 (48%) from men. All patients were from rural areas of Crete. The average age (mean \pm SE) was 65 ± 1.96 years (range, 30–92 years) and 69 ± 2.95 years (range, 30–92 years) in men and 61 ± 2.43 years (range, 39–84 years) in women. In 26 cases (52%), pterygium did not extend beyond 1 mm on the corneal surface, whereas in 24 cases (48%), it was more advanced.² In 48 cases (96%), the location of the pterygium was nasal and in 2 cases (4%), it was temporal to the cornea. Bilateral presence of pterygium was observed in 18 patients (36%). In 37 cases (74%), pterygium was operated for the first time; in 6 (12%), for the second time; in 6 (12%), for the third time; and in 1 case (2%), for the fourth time. Pterygium was reported to exist on the average for 11.89 ± 1.37 years (range, 0.5–35 years) before the operation. In 26 patients (52%), other ophthalmologic conditions, apart from pterygium, such as cataract, glaucoma, and ophthalmic

Submitted December 4, 1999. Revision received June 8, 2000. Accepted June 16, 2000.

From the Department of Ophthalmology (E.T.D.), and the Laboratory of Virology (G.S., D.A.S.), Medical School, University of Crete, Heraklion, Crete, Greece.

Address correspondence to Dr. D.A. Spandidos, Laboratory of Virology, Medical School, University of Crete, Heraklion, Crete, Greece.

TABLE 1. Incidence of HPV and HSV in examined pterygia and respective conjunctival specimens

Virus	Positive pterygium specimens (n, %)	Positive conjunctival specimens (n, %)
HPV	12 (24%)	4 (8%)
HSV	11 (22%)	0

allergic reactions, were diagnosed. Clinical signs of either active HPV or HSV infection were not found in any case. Additionally, no patient reported an ophthalmologic history compatible with either HSV or HPV infection. Eleven patients (22%) reported the use of eye drops (including steroid and nonsteroidal antiinflammatory therapy, decongestants, and artificial tears) for the alleviation of symptoms related to pterygium. A positive family history of pterygium (occurrence of pterygium among first degree relatives) was reported in 11 patients (22%). A history of conjunctivitis, either a chronic irritation or frequent episodes of inflammation, was reported by seven (14%) patients.

A 25- μ L polymerase chain reaction (PCR) was performed, containing 200 ng of genomic DNA, 1 μ M of each primer, 250 μ M of deoxynucleoside triphosphates, 2.5 μ L of 10X buffer (670 mM of Tris HCl, pH 8.5; 166 mM of ammonium sulphate; 67 mM of magnesium chloride; 1.7 mg/mL of bovine serum albumin; 100 μ M of β -mercaptoethanol; and 1% (wt/vol) Triton X-100) and 1 unit of Taq DNA polymerase. HSV presence was initially evaluated with general primers for the amplification of a 476-base pair-long sequence from the DNA polymerase genes of HSV-1 and HSV-2.¹⁵ Subsequently, HSV-positive samples were further classified into HSV-1-positive and HSV-2-positive with the use of type-specific primers, amplifying a 93-base pair fragment of HSV-2 glycoprotein C gene and a 110-base pair fragment of HSV-1 thymidine kinase gene.¹⁶ General primers GP5 and GP6 (from HPV L1 gene, with a 150-base pair PCR product) were used for the detection of HPV.¹⁷ Further typing of HPV was accomplished with strain-specific sets of primers (amplifying sequences within the E6 gene).¹⁸ DNA samples extracted from cell cultures infected with HSV and HPV were used as positive controls.

The reactions were initially denatured for 3 minutes at 94°C. Thirty-five cycles were used in all cases. In each cycle, denaturation was performed at 94°C (50 seconds for HPV and 60 seconds for HSV). Annealing was performed at 52°C for 45 seconds (HPV) and at 64°C for 40 seconds (HSV). Extension was performed at 72°C for 50 seconds in both cases. In a 2% agarose gel-containing ethidium bromide, 10 μ L of the PCR product was electrophoresed. In each case, negative control samples were also electrophoresed, allowing direct comparison with pterygial, conjunctival, and positive control specimens. Gels were subsequently examined under ultraviolet light. In the case of HSV typing, PCR products were electrophoresed in a 10% polyacrylamide gel and were silver stained. The analyzes were performed twice and the results were highly reproducible. Statistical analysis of the results was performed with the package SPSS 8.0 for Windows (SPSS, Chicago, IL, U.S.A.). Statistical significance was set at $p < 0.05$.

RESULTS

The frequency of viral detection in the examined samples of pterygium, as well as normal conjunctiva, is shown in Table 1. In

50% (two of four) of HPV-positive conjunctival specimens, respective pterygium specimens were not HPV-positive. HSV-1 was detected in all HSV-positive samples, whereas HPV-18 was detected in all HPV-positive samples. In three cases, both HPV and HSV DNA were detected in the examined pterygial specimens.

The history of conjunctivitis was significantly correlated with the simultaneous presence of HSV and HPV (Pearson bivariate correlation coefficient = +0.41; $p = 0.04$). Postoperative recurrence was more common in patients with a simultaneous detection of HSV and HPV, although the correlation was not statistically significant (Pearson bivariate correlation coefficient = +0.28; $p = 0.06$). The correlation between the isolated detection of either HSV or HPV and the clinical parameters examined was not statistically significant. Representative cases of HSV-positive and HPV-positive samples are shown in Figures 1 and 2, respectively.

DISCUSSION

Ophthalmic infection by HSV-1 is reported to be the most common cause of blindness due to infection in the United States.¹⁹ After the initial active stage, HSV infection can become latent, as confirmed by both viral culture and DNA hybridization techniques.^{20,21} Neurons are the most frequent site of latent infection. According to the "skin trigger theory," HSV released from ganglion cells continuously reaches peripheral sites, where it can be reactivated in situ in response to various stimuli.²² It has been shown that electrical stimulation of trigeminal ganglion in rabbits with latent HSV infection enables isolation of the virus from tears in the absence of clinical ocular infection.²³ Apart from the release of HSV from ganglion cells, it has been proposed that the virus can also remain latent in non-neural sites, such as the cornea.²¹ Moreover, HSV-1 has been isolated from chronic eyelid and conjunctival lesions in animal models²⁴ and can cause chronic blepharitis and conjunctivitis (as well as keratitis) in humans, although the pathogenesis of these inflammatory ocular and dermal lesions is not well understood.²⁴

The detection of HSV in pterygia examined in the current study, complies with the results of a previous work.⁹ In both studies, HSV was not detected in any phenotypically normal conjunctival specimen, suggesting that HSV could have a specific role in pterygium pathogenesis. Nevertheless, a causal relation between HSV

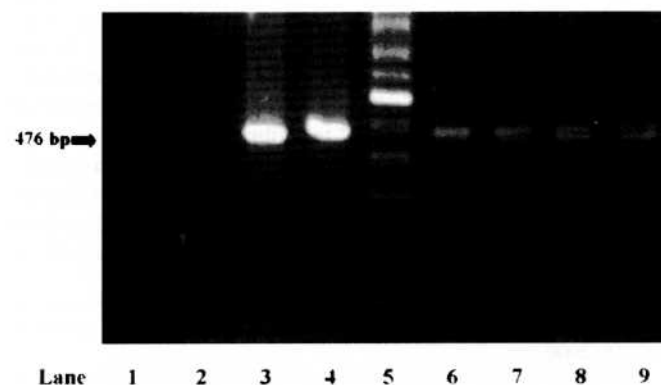


FIG. 1. Agarose 2% gel with electrophoresed PCR products (476 base pairs sequence of HSV DNA polymerase gene). Lanes 1 and 2 are blanks; lanes 3 and 4, positive controls; lane 5, DNA marker; and lanes 6–9, positive specimens.

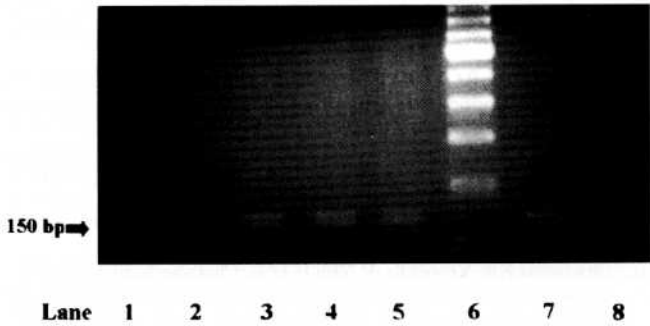


FIG. 2. Agarose 2% gel with electrophoresed PCR products (150 base pairs sequence of HPV L1 gene). Lane 1 is blank; lanes 2–5, positive specimens; lane 6, DNA marker; and lanes 7 and 8, positive controls.

infection and the development of the pterygium cannot be proved on the basis of present findings. The detection of HSV could be coincidental, perhaps as a result of a skin-trigger-type mechanism due to the irritating effect of pterygium. The detection of HSV-1 is in agreement with the fact that ocular herpetic infections, particularly those concerning the outer eye, such as herpetic keratitis and conjunctivitis, are most frequently caused by the this type of HSV.²⁵

Over 70 types of HPV, belonging to the *Papovaviridae* family, have been described thus far.²⁶ It has been proved that HPV possesses oncogenic potential and contributes to the development of various preneoplastic and neoplastic conditions.²⁶ DNA of many types of HPV, particularly of types 16 and 18, has been detected in papillomas, dysplasia, and cancers observed on the eyelids, lacrimal outflow tract, conjunctiva, and cornea.^{27,28} The detection of polyclonal HPV antigen from a set of pterygia, as well as normal conjunctival specimens, has been reported in the past.¹⁰ In the current study, based on a different method (PCR amplification of HPV DNA sequences), the presence of HPV in pterygial tissue, as well as normal conjunctiva, is confirmed. The fact that HPV-18 was detected in all cases is in agreement with reports that this type of HPV is commonly implicated in ophthalmic dysplasias and neoplastic lesions.²⁸ Pterygium has been considered a neoplastic condition because, apart from the tendency to grow in size, postoperative recurrences are common and sometimes more aggressive than the primary lesion, requiring more sophisticated surgery.¹ Furthermore, fibroblasts from pterygia have been shown to behave as neoplastic cells *in vitro*,²⁹ and random histologic examination of excised lesions has revealed neoplastic features.³⁰ The detection of HPV in pterygium further supports the neoplastic nature of this condition.

Apart from HPV, there are strong indications that HSV is also involved in a multistage process of oncogenic transformation.^{12,13} The virus has been reported to display co-carcinogenicity *in vivo* by enhancing the oncogenic capacity of chemical carcinogens, through promotion of the chemical carcinogen-induced activation of certain cellular proto-oncogenes and inactivation of *P53*.³¹ Unlike other DNA tumor viruses, the transformed cells do not consistently retain or express a given set of viral genes. HSV-mediated transformation can be obtained using fragments of viral DNA that did not wholly encode viral proteins.¹³ The failure to detect viral DNA in all transformed cells led to the "hit-and-run" hypothesis of HSV transformation.¹² HSV can cause both point mutations and

gene rearrangements, as well as induce gene amplification, particularly of sequences harboring an origin of replication, such as SV40 or papillomaviruses.¹³ It has been suggested that HPV and HSV fragments can coexist and perhaps cooperate during multistep transformation.^{32,33} The interaction between these viruses could possibly affect the clinical profile of pterygium, as indicated by the fact that the postoperative recurrence and history of conjunctivitis were more common in patients with simultaneous detection of HSV and HPV.

REFERENCES

- Gans L. Surgical treatment of pterygium. In: Belin MW, ed. *Focal points. Clinical modules for ophthalmologists*. San Francisco: American Academy of Ophthalmology, 1996:1–12.
- Coroneo MT. Pterygium as an indicator of ultraviolet insulation: a hypothesis. *Br J Ophthalmol* 1993;77:734–9.
- Hilgers JHC. Pterygium: its incidence, heredity and etiology. *Am J Ophthalmol* 1960;50:635–44.
- Pinkerton OD, Hokama Y, Shigemura L. Immunologic basis for the pathogenesis of pterygium. *Am J Ophthalmol* 1984;98:225–8.
- Kadayifcilar SC, Orhan M, Ircek M. Tear functions in patients with pterygium. *Acta Ophthalmol* 1998;76:176–9.
- Finger PT, Curtin BJ, Packer S, et al. Scleral hyperplasia induced by heat. *Am J Ophthalmol* 1986;102:25–32.
- Spandidos DA, Sourvinos G, Kiaris H, et al. Microsatellite instability and loss of heterozygosity in human pterygia. *Br J Ophthalmol* 1997; 81:493–6.
- Detorakis ET, Sourvinos G, Tsampralakis J, et al. Evaluation of loss of heterozygosity and microsatellite instability in human pterygium: clinical correlations. *Br J Ophthalmol* 1998;82:1324–8.
- Spandidos DA, Xinarianos G, Ergazaki M, et al. The presence of herpesvirus in pterygium. *Int J Oncol* 1994;5:749–52.
- Varinli S, Varinli I, Koksali Erkesi M, et al. Human papillomavirus in pterygium. *Cent Afr J Med* 1994;40:24–6.
- Donnell JM, Mayr AJ, Martin WJ. DNA of human papillomavirus type 16 in dysplastic and malignant lesions of the conjunctiva and cornea. *N Engl J Med* 1989;320:1442–6.
- Cox M, Maitland N, Scully C. Human herpes simplex-1 and papillomavirus type 16 homologous DNA sequences in normal, potentially malignant and malignant oral mucosa. *Eur J Cancer B Oral Oncol* 1993;29B:215–9.
- Galloway DA, McDougall JK. Alterations in the cellular phenotype induced by herpes simplex viruses. *J Med Virol* 1990;31:36–42.
- McPherson MJ, Quirke P, Taylor GR. *PCR. A practical approach*. Oxford: Oxford University Press, 1991.
- Rogers BB, Josephson SL, Mak SK. Detection of herpes simplex virus using the polymerase chain reaction followed by endonuclease cleavage. *Am J Pathol* 1991;139:1–6.
- Nicoll JA, Love S, Burton PA, et al. Autopsy findings in two cases of neonatal herpes simplex virus infection: detection of virus by immunohistochemistry, *in situ* hybridization and the polymerase chain reaction. *Histopathology* 1994;24:257–64.
- Snijders PJF, van den Brule AJC, Schrijnemakers HFJ, et al. The use of general primers in the polymerase chain reaction permits the detection of a broad spectrum of human papillomavirus genotypes. *J Gen Virol* 1990;71:173–81.
- Arends MJ, Donaldson YK, Duvall E, et al. HPV in full thickness cervical biopsies: high prevalence in CIN2 and CIN3 detected by a sensitive PCR method. *J Pathol* 1991;165:301–9.
- Pavan-Langston D. Ocular viral diseases. In: Merigan TC, Whitley RJ, Galasso GJ, eds. *Antiviral agents and viral diseases of man*, 4th ed. Philadelphia: Lippincott, 1997:207–45.
- Stevens JG, Newburn AB, Cook ML. Latent herpes simplex virus from trigeminal ganglia of rabbits with recurrent eye infection. *Nat New Biol* 1972;235:216–7.

21. Sabbaga EMH, Dunkel EC, Pavan-Langston D, et al. Detection of HSV nucleic acid sequences in the cornea during acute and latent ocular disease. *Exp Eye Res* 1988;47:545-53.
22. Hill TJ, Blyth WA. An alternative theory of herpes simplex recurrence and a possible role for prostaglandins. *Lancet* 1976;1:397-9.
23. Green MT, Rosborough JP, Dunkel EC. In vivo reactivation of herpes simplex virus in rabbit trigeminal ganglia: Electrode model. *Infect Immun* 1981;34:69-74.
24. Maggs DJ, Chang E, Nasisse MP, et al. Persistence of herpes simplex virus type 1 DNA in chronic conjunctival and eyelid lesions of mice. *J Virol* 1998;72:9166-72.
25. O'Day DM, Jones BR. Herpes simplex keratitis. In: Tasman W, Jaeger EA, eds. *Duane's clinical ophthalmology*. Philadelphia: J. B. Lippincott Company, 1994:1-27.
26. Zur Hausen H. Papillomavirus infections: a major cause of human cancers. *Biochim Biophys Acta* 1996;1288:55-78.
27. McDonnell JM, McDonnell PJ, Stout WC, et al. Human papillomavirus DNA in a recurrent squamous carcinoma of the eyelid. *Arch Ophthalmol* 1989;107:1631-4.
28. Lauer SA, Malter JS, Meier JR. Human papillomavirus type 18 in conjunctival intraepithelial neoplasia. *Am J Ophthalmol* 1990;110:23-7.
29. Chen JK, Tsai RJ, Linn SS. Fibroblasts isolated from human pterygia exhibit transformed cell characteristics. *In Vitro Cell Dev Biol Anim* 1994;30A:243-8.
30. Degrassi M, Piantanida A, Nucci P. Unexpected histologic findings in pterygium. *Optom Vis Sci* 1993;70:1058-60.
31. Park NH, Li SL, Xie JF, et al. In vitro and animal studies of the role of viruses in oral carcinogenesis. *Eur J Cancer B Oral Oncol* 1992;28B:145-52.
32. Dhanwada KR, Veerisetty V, Zhu F, et al. Characterization of primary human fibroblasts transformed by human papilloma virus type 16 and herpes simplex virus type 2 DNA sequences. *J Gen Virol* 1992;73:791-9.
33. Yamakawa Y, Forslund O, Chua KL, et al. Detection of the BC 24 transforming fragment of the herpes simplex virus type 2 (HSV-2) DNA in cervical carcinoma tissue by polymerase chain reaction (PCR). *APMIS* 1994;102:401-6.