Herpes Simplex Virus and Pterygium in Taiwan

Yeong-Fong Chen, MD, Ching-Hsi Hsiao, MD,* Kah-Wai Ngan, MD,*† Ling Yeung, MD,* Hsin-Yuan Tan, MD,* Kai-Hsiang Yang, MD,* Samuel Chao-Ming Huang, MD,* and Ken-Kuo Lin, MD**

Purpose: To evaluate the presence of herpes simplex virus (HSV) in pterygia to study the possible association between HSV and pterygia in Taiwan, a tropical country with a high prevalence of pterygium.

Methods: Sixty-five pterygia, 10 normal conjunctiva, 8 conjunctival nevi, and 2 malignant conjunctival melanomas were obtained. Clinical histories were recorded for each patient. HSV detection was accomplished by polymerase chain reaction amplification of viral sequences. HSV-positive specimens underwent subsequent DNA in situ hybridization. Results were statistically analyzed.

Results: By using polymerase chain reaction, HSV was detected in 3 (5%) pterygia, and no conjunctival control displayed HSV. All 3 HSV-positive pterygia studies were DNA in situ hybridization negative. There was no statistically significant correlation between pterygium and the presence of HSV.

Conclusions: HSV is not associated with pterygium formation in Taiwan; the pathogenesis of pterygia is still incompletely understood.

Key Words: pterygium, herpes simplex virus

(*Cornea* 2008;27:311–313)

Pterygium is a wing-shaped fold of fibrovascular tissue arising from conjunctival tissue and invading the superficial cornea. It is a common worldwide eye disease, with higher prevalence in tropical countries such as Taiwan. However, its etiology and pathogenesis remain inconclusive. Proposed hypotheses include environmental exposure such as ultraviolet (UV) light, wind and dust,[1] chronic inflammation,[1] immunologic reactions,[2] genetic factors,[3–5] and viral infection.[6–12] Nevertheless, the pathogenesis of pterygium formation is multifactorial. Detorakis et al[13] has proposed a "2-hit" process of the presence of an oncogenic agent and preexisting UV-related genetic damage.

Several studies have proposed the potential contribution of viral infection in pterygium formation,[6–12,14,15] potentially tumorigenic viruses, herpes simplex virus (HSV), and human papillomavirus (HPV), to name the most prominent. Most published reports have studied the relationship between HPV and pterygium; the involvement of HPV in pterygia varies from 0% to 100% in different geographic regions.[8–11,14] Few studies of the association between HSV and pterygium have been reported.[6,7] In this study, we investigated the presence of HSV in pterygia in Taiwan to see if HSV plays a role in pterygium formation in this country with a high prevalence of pterygia.

Received for publication May 22, 2007; revision received September 18, 2007; accepted September 23, 2007.

From the *Department of Ophthalmology and the †Department of Pathology, Chang Gung Memorial Hospital, College of Medicine, Chang Gung University, Taipei, Taiwan.

Reprints: Yeong-Fong Chen, Department of Ophthalmology, Chang Gung Memorial Hospital, No. 199, Tung Hwa North Road, Taipei 105, Taiwan (e-mail: a10429@adm.cgmh.org.tw).

MATERIALS AND METHODS

Patients and Specimens

This study included 62 consecutive patients (65 eyes) with pterygium who were treated by surgical excision at Chang Gung Memorial Hospital, Taiwan. Conjunctival specimens were from 8 with conjunctival nevus and 2 with malignant conjunctival melanomas, and 10 normal conjunctival samples from 10 patients undergoing cataract surgery were analyzed as negative controls. Medical and ocular histories were recorded for each patient. The clinical diagnoses of pterygium, normal conjunctiva, conjunctival nevus, and malignant conjunctival melanoma were confirmed histopathologically by 1 pathologist (K.W.N.) in all cases.

DNA Extraction

Three 5-μm paraffin-embedded tissue sections were deparaffinized through xylenes and rehydrated through serial alcohol. Sections were finely minced with disposable razor blades and digested in a solution of proteinase K (1 mg/mL) in 50 mM Tris buffer (pH 7.8) for 16–20 hours at 55°C. Proteinase K was denatured by heating samples at 95°C for 10 minutes. Samples were further purified by using a DNAeasy kit (Qiagen, Venlo, The Netherlands). Finally, 50 μL of DNA solution was eluted and 1 μL of the aliquot was used for polymerase chain reaction (PCR) amplification.

PCR

Nested PCR was performed to amplify the HSV DNA by using 2 paired sets of primers, which targeted both HSV-1 and HSV-2 (Table 1). The first and second primers were to amplify 327- and 200-bp DNA fragments, respectively. The reactions were performed by using a programmable thermal controller (PTC-100; MJ Research Co., Watertown, MA). The conditions for the nested PCR were set as follows: cycles (35 cycles for the first-round PCR with outer primers and 25 cycles for the second-round PCR with inner primers) of

TABLE 1. Primer Sequences

Primer	Sequence
Tk1	5′-ATCGTCTACGTACCCGAGCCGATGACTTAC-3′
Tk2	5′-GGCCTGGGGGGTCATGCTGCCCATAAGTA-3′
TkRN2	5′-GCGAACATCTACACCACACAACAC-3′
TkLN	5′-ATGGCGGTCGAAGATGAGGGTGA-3′

denaturing at 94°C for 1 minute, annealing at 74°C (in the first-round PCR) and at 58°C (in the second-round PCR) for 1 minute, and extension at 72°C for 1 minute. The DNA extracted from HSV cultures was used as positive controls. Negative control samples containing water were always processed in a manner parallel to the patient samples.

DNA In Situ Hybridization

HSV PCR–positive pterygia were studied with DNA in situ hybridization (ISH) with digoxigenin-labeled HSV-1 and HSV-2 probes from a commercial kit (DAKO, Carpinteria, CA) according to the manufacturer's instructions. In brief, sections of 5-μm paraffin-embedded block were floated onto slides and baked onto slides at 55°C. Sections were dewaxed with xylene; washed with 100% ethanol, 90% ethanol, and 75% ethanol; and rehydrated. The specimens were immersed in proteinase K solution (10 μg/mL) at 37°C for 10 minutes and washed with distilled water. A digoxigenin-labeled HSV probe was denatured in hybridization buffer at 90°C for 10 minutes and then at −20°C for 3 minutes. After slides were air-dried, tissues were added, and the slides were coverslipped at 37°C overnight. Sections were washed with 2× SSC (1 × SCC is 0.15 M NaCl plus 0.015 M sodium citrate) and mouse antidigoxigenin antibody, biotinylated goat anti–mouse, and then streptavidin–biotin complex for 30 minutes, and finally with 3.3-diaminobensidine staining and hematoxylin counterstain and subjected to light microscopy. Positive control sections were included in the experiment.

Statistical analysis was performed by using SPSS software (SPSS, Chicago, IL). The Fisher exact test was used for analyzing the presence of HSV in the control and pterygium groups and in the primary and recurrent pterygium groups. Statistical significance was defined as $P \leq 0.05$.

RESULTS

Demographics

The 62 patients with pterygium were made up of 31 men and 31 women, with a mean age of 58.7 years. Three patients had bilateral pterygia. Pterygium was located nasally in 64 eyes and temporally in 1 eye. Thirty-five pterygia were primary lesions, and 30 were recurrent. In 35 eyes with primary pterygium, pterygium excision was performed by the bare sclera method plus topical mitomycin C (0.2 mg/mL) twice a day for 1 week. In 30 eyes with recurrent pterygium, pterygium excision and amniotic membrane transplantation were performed. The mean follow-up time was 13.5 months. Three cases had recurrence during follow-up. The mean age of the negative control group was 57.1 years. No patients had an ocular history consistent with HSV infection.

PCR Results

In 65 eyes with pterygia, HSV was detected in 3 primary pterygia. None of the virus-harbored samples had recurrence during follow-up. All conjunctival controls were negative for the presence of HSV DNA. There was no statistically significant correlation between pterygium and the presence of HSV (2-sided exact significance: 0.243), and presence of HSV was not significantly different for primary and recurrent pterygia (2-sided exact significance: 1.0).

DNA ISH Results

The 3 pterygia that were HSV positive by using PCR studies were all DNA ISH negative.

DISCUSSION

Pterygium development is strongly correlated with UV exposure.[1] Because the lesion has never been noted in animals,[16] other risk factors than UV radiation must be needed for pterygium pathogenesis to occur. HSV infection is prevalent; up to 90% of individuals have antibodies to HSV by 10 years of age.[17] Primary HSV infection is followed by latent viral infection in the ganglia.[17] According to the "skin trigger theory," individuals with latent HSV infection in the ganglia actively shed infectious virions to peripheral sites with or without clinical symptoms[17]; Kaufman et al[18] reported that the percentage of asymptomatic subjects who intermittently shed HSV-1 DNA in tears or saliva was higher than the percentage of subjects with positive neutralization antibodies to HSV. The repeated exposure of the cells to the virus could induce certain molecular changes in those cells and provide an opportunity for HSV to interact with environmental factors such as UV light.

HSV has been reported to enhance the oncogenic potential of chemical carcinogens by promoting their activation of certain cellular protooncogenes and inactivating the p53 tumor suppressor gene.[19–21] Dushku and Reid[22] found increased nuclear p53 expression without apoptosis in the limbal epithelium of pterygia, limbal tumors, and most pingueculae; their further study documented no correlation between p53 overexpression and HPV.[14] To the best of our knowledge, no studies have been conducted to investigate if HSV plays a role in p53 overexpression in pterygium.

By using PCR techniques, we identified HSV in 3 pterygia in our study. However, subsequent DNA ISH studies did not confirm its presence. The PCR technique is more sensitive than ISH[23,24]; the 3 HSV PCR–positive but ISH-negative specimens could have been contaminated or perhaps incidental, nonsignificant infections with a small number of virions. However, HSV seems an unlikely risk factor in the development of pterygia in Taiwan because of the few HSV-positive samples in our study. Spandidos et al[6] have shown HSV in 45% of pterygia. Detorakis et al[7] detected HSV in 22% of pterygia but none in apparently normal conjunctival samples. Our results showed a low prevalence and statistical insignificance of HSV within pterygia and no occurrences within normal conjunctiva. The former does not agree with previous studies. The difference in prevalence might indicate that detection of HSV could be coincidental in other studies or that techniques used in this study were not adequate to show such an association. This difference may caused by other factors related to geographic regions or race, as reported in the presence of HPV in pterygia.[8–11,14] However, previous studies did not use ISH to show the localization of HSV in the HSV-positive pterygia.

In conclusion, our study indicates that the presence of HSV is not associated with pterygium formation in Taiwan, a tropical country with a high prevalence of pterygium. The pathogenesis of pterygia is still incompletely understood.

REFERENCES

1. Coroneo MT. Pterygium as an indicator of ultraviolet insulation: a hypothesis. *Br J Ophthalmol*. 1993;77:734–739.
2. Pinkerton OD, Hokama Y, Shingemura L. Immunologic basis for the pathogenesis of pterygium. *Am J Ophthalmol*. 1984;98:225–228.
3. Hilgers JHC. Pterygium: its incidence, heredity and etiology. *Am J Ophthalmol*. 1960;50:635–644.
4. Spandidos DA, Sourvinos G, Kiaris H, et al. Microsatellite instability and loss of heterozygosity in human pterygia. *Br J Ophthalmol*. 1997;81:493–496.
5. Detorakis ET, Sourvinos G, Tsamparlakis J, et al. Evaluation of loss of heterozygosity and microsatellite instability in human pterygium: clinical correlations. *Br J Ophthalmol*. 1998;82:1324–1328.
6. Spandidos DA, Xinarianos G, Ergazaki M, et al. The presence of herpesvirus in pterygium. *Int J Oncol*. 1994;5:749–752.
7. Detorakis ET, Sourvinos G, Spandidos DA. Detection of herpes simplex virus and human papilloma virus in ophthalmic pterygium. *Cornea*. 2001;20:164–167.
8. Varinli S, Varinli I, Koksal Erkisi M, et al. Human papillomavirus in pterygium. *Cent Afr J Med*. 1994;40:24–26.
9. Gallagher MJ, Giannoudis A, Herrington CS, et al. Human papillomavirus in pterygium. *Br J Ophthalmol*. 2001;85:782–784.
10. Piras F, Moore PS, Ugalde J. Detection of human papillomavirus DNA in pterygia from different geographical regions. *Br J Ophthalmol*. 2003;87:864–866.
11. Chen KH, Hsu WM, Cheng CC, et al. Lack of human papillomarvirus in pterygium of Chinese patients from Taiwan. *Br J Ophthalmol*. 2003;87:1046–1048.
12. Sjö NC, Buchwald C, Prause JU, et al. Human papillomavirus and pterygium. Is the virus a risk factor? *Br J Ophthalmol*. 2007;91:1016–1018.
13. Detorakis ET, Drakonaki EE, Spandidos DA. Molecular genetic alterations and viral presence in ophthalmic pterygium. *Int J Mol Med*. 2000;6:35–41.
14. Dushku N, Hatcher SLS, Albert DM, et al. P53 expression and relation to human papillomavirus infection in pingueculae, pterygia, and limbal tumors. *Arch Ophthalmol*. 1999;117:1593–1599.
15. Reid TW, Dushku N. Does human papillomavirus cause pterygium? *Br J Ophthalmol*. 2003;87:806–808.
16. Threlfall TJ, English DR. Sun exposure and pterygium of the eye: a dose-response curve. *Am J Ophthalmol*. 1999;128:280–287.
17. 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:145–152.
18. Kaufman HE, Azcuy AM, Varnell ED, et al. HSV-1 DNA in tears and saliva of normal adults. *Invest Ophthalmol Vis Sci*. 2005;46:241–247.
19. Cunningham AL, Diefenbach RJ, Miranda-Saksena M, et al. The cycle of human herpes simplex virus infection: virus transport and immune control. *J Infect Dis*. 2006;194:S11–S18.
20. 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:215–219.
21. Thomson BJ. Viruses and apoptosis. *Int J Exp Pathol*. 2001;82:65–76.
22. Dushku N, Reid TW. p53 expression in altered limbal basal cells of pingueculae, pterygium, and limbal tumors. *Curr Eye Res*. 1997;16:1179–1192.
23. Sebbelov AM, Svendsen C, Jensen H, et al. Prevalence of HPV in premalignant and malignant cervical lesions in Greenland and Denmark: PCR and in situ hybridization analysis on archival material. *Res Virol*. 1994;145:83–92.
24. Chardonnet Y, Lizard G, Chignol MC, et al. Analytical methods for evaluation on whole cells of human papillomavirus infection. *Bull Cancer*. 1995;82:107–113.