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LABORATORY STUDY

PCR for varicella zoster virus genome negative in corneal epithelial cells of patients with Thygeson's superficial punctate keratitis

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Abstract

Purpose **To find out if varicella zoster virus is the causative agent of Thygeson's superficial punctuate keratitis.**
Methods **Epithelial cells were harvested from the punctuate epithelial lesions 9 patients with Thygeson's superficial punctate keratitis. After DNA extraction polymerase chain reaction was carried out with varicella zoster virus primers.**
Results **All samples were negative with regard to varicella zoster virus genome.**
Conclusions **This result suggests that varicella zoster virus is most probably not the causative agent of Thygeson's superficial punctate keratitis.**
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Keywords: keratitis; varicella zoster virus; cyclosporin A

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Introduction

Thygeson's superficial punctate keratitis is a chronic corneal inflammation first described in 1950.[1–3] Its aetiology is still unclear. Virological research of Braley and Alexander[4] gave questionable results in 1953, whereas Lemp *et al*[5] were able to isolate varicella zoster virus in one patient in 1974. Here, we present a series of nine patients with Thygeson's superficial punctate keratitis with moleculargenetic analysis of corneal epithelial cells with regard to varicella zoster genome.

Patients

Mean age of five female and four male patients was 28.4 (6–52) years. Both eyes were involved in seven patients, only one eye in two patients. Thygeson's superficial punctate keratitis was diagnosed if multiple, punctate, whitish epithelial lesions with little subepithelial oedema (Figure 1), slight symptomatology, a prolonged course with remissions/exacerbations and a response to immunomodulative therapy (topical steroids or cyclosporin A[6]) were present. Mean duration of symptoms was 55.9 (2–156) months. Analysis followed the tenets of the Declaration of Helsinki, and written informed consent was obtained to harvest epithelial cells from punctate epithelial lesions. If both eyes were involved, the eye with the more pronounced epithelial lesions was included in the analysis. Cells were softly taken from the corneal epithelial lesions using tiny brushes (Accellon™ Multi, Medscan Medica AB, Malmö, Sweden). Thereafter, the brushes were taken into suspension with BSS® and immediately frozen at −80°C.

DNA extraction was made with the QIAmp DNA Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. Two rounds of PCR were carried out using 5 μl of the extracted DNA in a total of 100 μl. *Taq* DNA polymerase (2 U) was used according to the manufacturer's instructions (Promega, Germany). In the first round primers VZV1 (5′-ATG TCC GTA CAA CAT CAA CT-3′) and VZV2 (5′-CGA TTT TCC AAG AGA GAC GC-3′) and in the second round primers VZV3 (5′-ACA TCC ACC GGA AGC CCA TGA-3′) and VZV4 (5′-CGG TCG

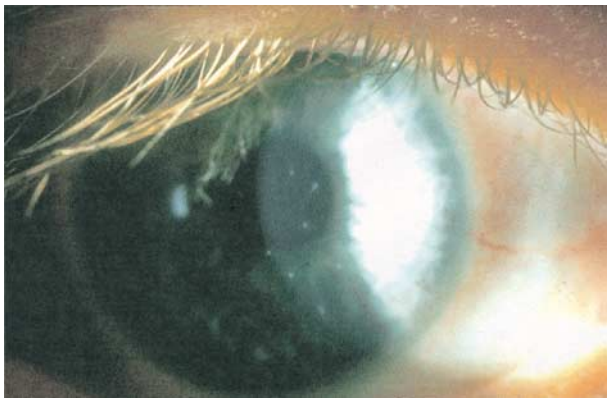

Figure 1 Multiple, punctate, whitish epithelial lesions with little subepithelial oedema in Thygeson's superficial punctate keratitis.

ATC GAA TTA CGG GCC-3′) were used. These sequences were derived from the sequence of VZV ORF4 (Genebank accession number AY034034). In both rounds of PCR, amplification was done for 34 cycles, each consisting of 1 min at 94°C, 1 min at 40°C, and 2 min at 68°C after an initial denaturation at 94°C for 4 min and followed by a final elongation step at 68°C for 5 min. The products of the second PCR (159 bp) were visualized by electrophoresis in 1% agarose gels and staining with ethidium bromide. All samples were negative with regard to varicella zoster virus genome.

Comment

Diagnosis of Thygeson's superficial punctate keratitis is made if multiple, punctate, whitish lesions occur in the corneal epithelium.[1–6] Similar, but not identical, corneal epithelial lesions were described in varicella zoster virus keratitis.[7] The tendency to recur over years suggests that an unknown virus, possibly varicella zoster virus, is causative. Lemp *et al*[5] were able to isolate varicella zoster virus from the corneal surface of a 10-year-old boy with Thygeson's superficial punctate keratitis. In their paper, they discuss, however, that the presence of the virus may be a fortuitous event not related to Thygeson's superficial punctate keratitis.[5] Using modern moleculargenetic methods, varicella zoster virus genome could not be detected in any patient of the present analysis. This result suggests that varicella zoster virus is most probably not the causative agent of Thygeson's superficial punctate keratitis.

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