

# Gram Staining Towards Field Diagnosis of Herpes Simplex Keratitis

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*This report is of a young man with herpes simplex virus-induced keratitis diagnosed by Gram staining of corneal scraping. Acyclovir therapy resulted in rapid healing of the corneal lesions. Ordinary light microscopy on Gram-stained corneal scraping could offer valuable information about herpes simplex virus keratitis.*

**Key Words:** Acyclovir, Diagnosis, Herpes simplex virus, Gram stain, Keratitis

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## Introduction

Herpes simplex virus (HSV)-induced keratitis (HSK) is one of the most common causes of corneal blindness.<sup>1</sup> After extensive corneal scarring following HSK, corneal transplantation is the only treatment option, but even then the outcome is associated with complications such as epithelial healing, recurrent HSK, transplant rejection, and failure of the graft.<sup>2</sup> Early diagnosis and appropriate treatment can significantly reduce this morbidity. Antiviral therapy with acyclovir or famciclovir<sup>3</sup> hastens healing, although recurrences are frequently encountered. The diagnostic tools described in the literature for HSK (cell culture, polymerase chain reaction, immunological assays, and Giemsa stain)<sup>4,5</sup> are expensive, time consuming, dependent on expertise, and not freely available. However, HSV-induced intranuclear inclusions have been demonstrated by Gram staining of corneal scrapings in an adult man in the outpatient clinic of a private hospital in Delhi, India.

## Case Report

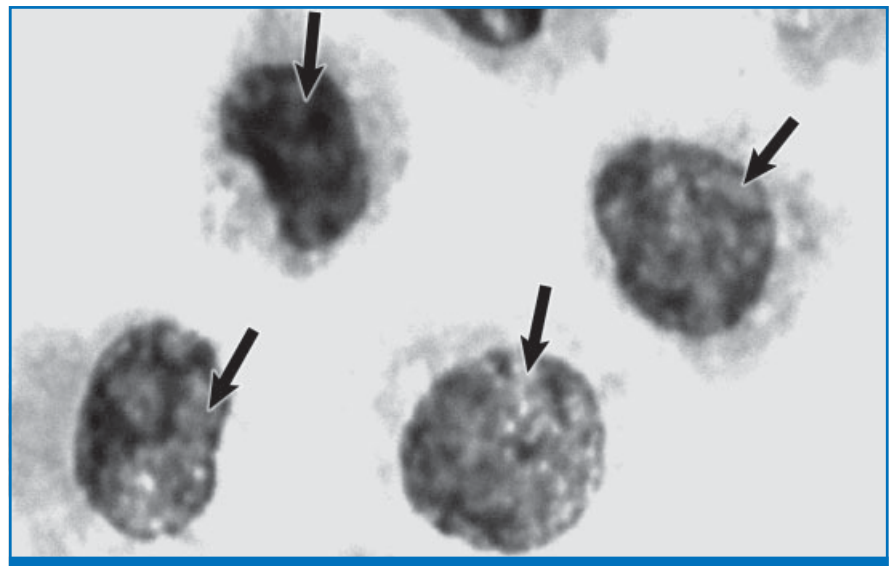
In November 2002, a young man presented at the outpatient department of Sant Parmanand Hospital, in New Delhi, India. The 100-bed hospital caters to a low- and middle-income population with ophthalmic and non-ophthalmic disorders. There is a

nominal one-off registration fee of Rs10/- (US\$0.02) per patient for clinical examination in the outpatient department.

A 21-year-old male presented with pain, redness, watering, and diminution of vision in his left eye of 3 days duration. There was no history of trauma, steroid use, or prior episode. At examination, there was lid oedema, conjunctival congestion (mainly circumcorneal), and a superficial dry looking irregular-shaped corneal infiltrate with diffuse edges over the central and para-central cornea with oedema around the infiltrate. There was no corneal thinning and the rest of the anterior segment details were within normal limits. Based on these clinical details, a provisional diagnosis of infectious keratitis was made. Corneal scrapings were sent for microbiological investigation, namely Gram stain and potassium hydroxide (KOH) 10% smear, to confirm the diagnosis.

Gram staining of the corneal scrapings showed many epithelial cells containing acidophilic, intranuclear inclusions with no inclusion or vacuolation in their cytoplasm (Figure 1). A few multinucleated giant cells with similar inclusions were also seen. KOH smear did not show any organism. The patient was negative for HIV.

**Figure 1.** Gram-stained corneal epithelial cells showing intranuclear inclusions.



The patient was given acyclovir 3% eye ointment 5 times daily along with homatropine 1% 3 times a day. Subsequent follow-up showed rapid relief of symptoms and quick recovery of signs. There was residual corneal scarring over the para-central cornea with good healing within 2 weeks of starting therapy.

### Discussion

Light microscopy of Gram-stained scrapings from the cornea should be attempted in routine clinical practice. Confirmation of findings in more patients would validate the utility of a simple staining procedure such as Gram's stain for a specific diagnosis of HSV keratitis. This would interest clinicians in countries with limited facilities for cell culture or molecular biology investigations. Demonstration of intranuclear inclusions in corneal cells (Figure 1) should be feasible in a clinician's office in any hospital or health care facility. This would assist with selecting the appropriate antiviral therapy, as was evident in the case described here, and a better clinical outcome. Simple but specific HSV diagnosis would be a valuable armamentarium for clinicians in developing countries with minimal laboratory facilities and would be of interest to clinicians in Asia, Africa, and Latin America where facilities for cell culture<sup>4</sup> or multi-step Giemsa staining<sup>5</sup> are not likely to be readily available.

Simplified diagnosis of ophthalmic complications of HSV would assist in the judicious management of patients with glaucoma receiving prostaglandin analogues or who have concurrent HIV infection. The ophthalmic manifestations of

HSV have been found among patients with glaucoma taking latanoprost and bimatoprost therapy. Three patients have developed HSV keratitis after they were administered latanoprost and latanoprost therapy was stopped.<sup>6</sup> Inactive HSV has been reactivated in a 66-year-old woman after initiating bimatoprost therapy. Reactivation resolved after discontinuation of bimatoprost and initiation of acyclovir, ofloxacin, and betaxolol<sup>7</sup>. Furthermore, confirmed HIV seropositivity has been higher in patients with recurrent HSV keratitis compared with matched patients with a first episode of HSV keratitis ( $p < 0.05$ ).<sup>8</sup>

A simplified diagnosis of ocular manifestations of HSV in children would assist in a rational prescription of acyclovir therapy, thereby reducing morbidity. This would also avoid non-specific antimicrobial therapy and ambiguity in the diagnosis of infectious keratitis. Oral acyclovir therapy given to 7 patients with HSV keratitis aged 6 weeks to 5 years at the University of Minnesota Hospitals and Clinics was effective with a resolution of HSV keratitis.<sup>9</sup> Three such patients have been managed with a prophylactic dosage of oral acyclovir. There have been no adverse reactions with good tolerance.

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