

Herpes Simplex Keratitis in South India: Clinico-Virological Correlation

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Purpose: A retrospective cross-section study to analyze the prevalence of herpes simplex virus-induced keratitis (HSK) among 3,000 patients attending a corneal clinic in South India between 1995 and 1997, and to evaluate laboratory techniques for detecting HSK.

Methods: The clinico-virological correlation was studied using herpes simplex virus (HSV) isolation on the Vero cell line, HSV-specific antigen detection by indirect immunofluorescence (IF) microscopy, and serum anti-HSV IgG quantitation, IgM estimation, and tear secretory IgA (sIgA) detection by ELISA.

Observations: HSK had a prevalence of 7.8% (234 patients) in this study. A virological correlation could be obtained in 44.4% of the cases that had epithelial manifestations and in 14.8% of the cases that had only stromal disease. In 161 cases where both culture and IF microscopy were used, IF detected 27 cases (26.8%) more than cell culture. The difference in sensitivity between cell culture and IF was found to be statistically significant (McNemar's test, $P < .05$). An elevation in IgG titer was seen in 17 (30.4%) cases. IgM was detected in only 2 cases of the 62 (3.2%) analyzed. Of the 138 cases analyzed, sIgA was positive in 28 (20.3%) cases. A proved diagnosis could be made in 58% of cases when the specimen was collected during the first week after disease onset, and in only 5% when the time interval increased to 4 weeks.

Conclusions: HSV antigen detection by indirect IF is a rapid and sensitive diagnostic tool for HSK. Tear secretory IgA (sIgA) is a specific marker for acute herpetic keratitis, and the detection of HSV-specific tear sIgA is a valuable adjunct to virus isolation and antigen detection in the laboratory diagnosis of HSK. For a successful diagnosis, the specimen should be collected as soon as possible after HSK onset. **Jpn J Ophthalmol 1999;43:303-307** © 1999 Japanese Ophthalmological Society

Key Words: Clinico-virological correlation, epithelial keratitis, herpes simplex keratitis, secretory IgA.

Introduction

Herpes simplex virus (HSV) is the most common infective cause of blindness in developed countries, with a reported incidence of between 5.9 and 20.7 episodes per 100,000 persons each year.^{1,2} The prevalence studies in Tunisia showed that HSV was isolated from 41% of patients whose corneal material

was cultured.³ A clinico-virological correlation study in Chile⁴ showed a 77% correlation using culture and polymerase chain reaction techniques in cases of epithelial keratitis, and a 20% correlation in stromal keratitis cases using polymerase chain reaction. HSV ocular infections include conjunctivitis, blepharitis, and epithelial infections like dendritic ulcer, geographic ulcer and punctate epithelial keratitis, stromal infections like disciform and necrotizing stromal keratitis, and iritis.⁵ There are many reports of HSV keratitis (HSK) occurring in atypical form.^{6,7} Documentation of HSK's clinical and laboratory features from the Indian subcontinent has not yet been re-

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ported. HSK being a treatable ocular infection, clear documentation of its clinical and virological features is relevant in day-to-day clinical practice to diagnose the infection early and for instituting appropriate treatment strategies to control the disease.^{8,9} Hence, a study was conducted to characterize HSV keratitis as observed in Chennai, South India.

Materials and Methods

Patients and Materials

Inclusion in this study was based on the clinical diagnosis of HSK. Corneal involvement was indicated by a characteristic punctate epithelial keratitis, a dendritic or a geographic ulcer. The patients with punctate epithelial keratitis visited us with the chief complaint of redness and tearing in the eye. Clinically, there was a presence of mild congestion in the eye with a few punctate dots on the cornea that were stained by 2% fluorescein. Corneal sensation was decreased, and few cases had a history of facial vesicles. Stromal keratitis included either disciform keratitis or necrotizing stromal keratitis.

The study was conducted from March 1995 to September 1997. During this period, 3,000 patients attended the cornea clinic at the Regional Institute of Ophthalmology in Chennai. Of this total, 234 patients with suspected HSK were studied. The remainder of the 3,000 cases were diagnosed as having nonherpetic infectious keratitis such as fungal keratitis (24.3%), bacterial keratitis (20.7%), *Acanthamoeba* keratitis (11.5%), and varicella-zoster keratitis (0.6%). Other cases were leucoma (16.6%), dry eye syndrome (7.9%), bullous keratopathy (4.6%), nummular keratitis (1.5%), corneal degeneration (1.1%), dystrophy (2.3%), traumatic corneal abrasions (0.7%), and acid/alkali burns (0.4%).

All patients included in the study were residents of Chennai, South India. The nature of the study and the possible outcome were explained, and a written consent was obtained from each participant.

Corneal scrapings were collected from those patients who showed epithelial manifestations. From those with stromal disease, only tears and blood were collected, leaving the epithelium intact. Scrapings were obtained from stromal patients who had epithelial manifestations like ruptured bullae. Paired serum samples could be collected in only 56 cases at an interval of 3–4 weeks. Tear samples sufficient for analysis could be collected in 138 cases. Scrapings were collected in Hanks' balanced salt solution (Hi-Media, Bombay, India) containing antibiotics and

3% fetal bovine serum (Sigma, St. Louis, MO, USA). Specimens were transported on ice and processed immediately. Whenever there was a delay they were stored at -70°C until processed. The tear samples were mixed with an equal volume of glycerol and were stored at -20°C until processed.

Laboratory Studies

The laboratory investigations included HSV isolation and detection of HSV-specific antigens from scrapings, quantitation of anti-HSV IgG in paired serum samples, IgM estimation, and detection of tear secretory IgA (sIgA). Isolation of HSV was done on the Vero cell line. The cultures were detected by the characteristic cytopathic effect of HSV, and were identified by virus-specific direct immunofluorescence (IF) microscopy (MicroTrak HSV-1/HSV-2 culture identification/typing test; Syva, San Jose, CA, USA). The corneal scrapings were subjected to an "in-house" indirect IF test for HSV antigens using polyclonal rabbit anti-HSV-1 antibodies (McIntyre strain; DAKO, Glostrup, Denmark) and anti-rabbit IgG FITC as primary and secondary antibodies, respectively. The criterion of positivity was the detection of one or more cells with bright apple green fluorescence in the cytoplasm and/or nucleus. The details of culture and indirect IF procedures were reported previously.¹⁰ Serum anti-HSV IgG assay and tear sIgA tests were carried out by "in-house" ELISA based on the procedure of Fox et al¹¹ with modifications. Briefly, 96-well plates (Corning Inc., Corning, NY, USA) were coated with infected Vero cell lysate and control vero antigen. Serum and tear samples (diluted 1:2 and 1:400, respectively) were incubated in antigen-coated wells, and antigen-specific IgG and sIgA were detected by conjugates, anti-human IgG horseradish peroxidase (HRP), anti-human secretory component HRP (DAKO), and enzyme substrate 3,3', 5,5'-tetramethyl benzidine. Virus-specific IgG concentration was calculated from a standard curve of absorbance at 450 nm plotted against purified human IgG standards. This was constructed by incubation of anti-human IgG HRP conjugate in wells coated with dilutions of purified human IgG. After the initial runs, the range was set at 2.5 $\mu\text{g}/\text{mL}$ –160 $\mu\text{g}/\text{mL}$. Values in tears (sIgA) were expressed in absorbance units, as no purified secretory component was available to construct a standard curve. Values were considered positive if they fell outside two standard deviations of the background level as measured in the corresponding sera.¹¹ A specificity analysis was done earlier using tear sam-

ples collected from healthy eyes undergoing preoperative evaluation for cataract extraction (n = 20) and from 10 cases of eyes treated in the cornea clinic for redness and tearing due to various nonherpetic causes like dry eye (n = 2), nummular keratitis (n = 6), and aphakic bullous keratitis (n = 2).

Results

Of the 234 patients clinically suspected of having HSK, 159 were men and 75 were women (M/F = 2.1:1). The mean age was 29 years and the range was 9 months to 65 years. One hundred and thirty-seven cases presented with the first episode of the disease and 97 with recurrent infection. One hundred and fifty-three had epithelial keratitis and 81 had stromal infiltration. Eight of the 81 stromal keratitis cases (9.9%) had some epithelial manifestation like ruptured bullae. One hundred and four cases presented with dendritic ulcer, 26 had geographic ulcer, and 23, punctate epithelial keratitis. Disciform and necrotizing stromal keratitis were seen in 66 and 15 cases, respectively (Table 1). Forty-eight of the first episode patients and 32 recurrent cases had laboratory evidence of HSV infection (either HSV isolation/antigen detection/sIgA positivity/elevation of IgG titer). Of these, 68 had epithelial keratitis and 12, stromal infiltration. An analysis of the clinical symptoms in these laboratory proven cases showed dendritic ulcer in 54, geographic ulcer in 8, and punctate epithelial keratitis in 6 cases. Six patients each presented disciform and necrotizing stromal keratitis. Ocular infection was unilateral in 78 cases and bilateral in 2.

One hundred and sixty-one corneal scrapings (153 epithelial cases and 8 stromal keratitis cases that had epithelial manifestations) were subjected to culture and antigen detection. HSV was isolated from cor-

neal scrapings of 36 cases (22.4%). In the analysis of the virus isolation in terms of clinical presentation, the maximum isolation was seen in cases with dendritic ulcer (n = 30), followed by geographic ulcer (n = 4). One isolate was obtained from a patient with punctate epithelial keratitis and another from a patient with necrotizing stromal keratitis and ruptured bullae. The time required to develop the characteristic cytopathic effect varied from 1 to 7 days. Forty-four percent of the HSV-positive specimens showed this characteristic effect by day 3, and in 55.6% of the cases it took as long as 4–7 days. Immunofluorescence microscopy detected HSV-specific antigen in 64 (39.8%) cases. The difference in sensitivity between cell culture and IF was found to be statistically significant (McNemar's test, $P < .05$).

HSV-specific IgG was measured by ELISA in paired serum samples obtained from 56 HSK cases. An elevation in IgG titer was seen in 17 (30.4%) cases. This includes 10 cases where HSV was proven in the laboratory and 7 cases where no laboratory evidence of HSV infection was found. The HSV IgM test was done only in 62 proven positive cases. IgM was detected in only 2 (3.2%) of the 62 cases analyzed.

The results of the sIgA analysis were as follows. Of the 138 cases analyzed, sIgA was positive in 28 (20.3%) cases; sIgA was not detected in any of the controls. The breakdown of the positive cases showed 19 to be epithelial, and 9 were stromal. Of the 19 epithelial cases, 16 showed either virus isolation or antigen positivity. Among the stromal keratitis cases that were positive for sIgA, four cases showed an elevation in IgG titer. The results of the laboratory investigations are shown in Table 2.

An analysis of positive laboratory diagnosis with the time of specimen collection after disease onset revealed that a proven diagnosis could be made in 58% of cases when the specimen was collected during the first week, and in only 5% when the time interval increased to 4 weeks. A virological correlation could be obtained in 44.4% (95% CI: 36.7–52.4) of the cases that had epithelial manifestation and 14.8%

Table 1. Details of Clinical Presentations of Cases Included in Study (n = 234)

Type of Lesion	No.	%
(a) Epithelial (n = 153)		
Dendritic	104	68
Geographic	26	11.1
Punctate Epithelial keratitis	23	9.8
(b) Stromal (n = 81)		
Disciform	66	28.2
Necrotizing keratitis	15	6.4
(c) Stromal with epithelial manifestation (n = 8)		
Necrotizing stromal with ruptured bullae*	8	3.4

*Added to stromal cases when analyzing total cases.

Table 2. Overall Positivity of Various Diagnostic Markers

Diagnostic Tests	Total No. Tested	Positive (%)
Cell culture	161	36 (22.4)
Antigen detection	161	64 (39.8)
Serum IgG	56	17 (30.4)
Serum IgM	62	2 (3.2)
Tear sIgA	138	28 (20.3)

(95% CI: 8.3-23.8) of the cases that had only stromal manifestation. In 161 cases where both culture and IF were used, IF detected 27 cases (26.8%) more than cell culture.

A retrospective analysis was also made on the recurrence of HSK in our clinical population. Although clinical records were available in 40 recurrent cases, for the remainder we had to rely solely on the testimony of patients. The analyzed data show the number of recurrent herpetic attacks varied from thrice a year to one attack in 5 years, the average being 1 attack in 1.43 years. The major factor often associated with recurrence was fever, as 38% of the cases reported fever before a recurrent attack. Direct corneal trauma resulting from injury was reported before recurrence in four cases. Two cases had a history of excessive exposure to sun (occupational). Upper respiratory tract infection was seen in only one case. In another case, multiple factors like trauma and fever were reported. No associated factor could be traced in the remainder of the cases. There was no evidence of seasonal variability.

Discussion

It is generally believed that corneal infections associated with HSV may represent as much as 10% of all corneal ailments treated in the corneal clinics in South India. Our study shows that HSK had a prevalence of 7.8% (95% CI: 6.9-8.8) among patients attending corneal clinics in South India. The true prevalence may have been higher because many patients with milder forms of disease generally visit private practitioners. Hence these conditions may remain unrecognized and patients may be treated needlessly with antibiotics or be exposed to hazards of unwise use of corticosteroids.^{8,9,12} Analysis by age at the first episode of HSV corneal infection showed that 68 of the 137 cases (49.6%) were seen in adolescents and young adults. Children under 5 years of age constituted only 10% of the cases. This result is in accordance with the previous reports.^{1,13} Whereas the classic incidence study by Liesegang et al¹ observed an increase in the percentage of young adults over the 1950-1982 period in Rochester (MN, USA), the prevalence study by Darougar et al¹³ reported only 7% positivity in children under 5 years of age in London. There are reports of a decrease in antibodies in young adults in developed countries,¹⁴ indicating that infections in childhood are less common. Whether the higher incidence of first episode disease among young adults in South India should be consid-

ered as an epidemiological feature of HSK in South India, or if many of the primary ocular infections among Indian children may be mild and go unnoticed, will require further evaluation.

Recurrence of HSK was higher in men than in women, the male to female ratio being 1.44 to 1, in accordance with the previous reports of Liesegang et al¹ and Laibson and Leopald.¹⁵ The reasons for the greater recurrence seen in men remain unexplained.

Analysis of recurrent keratitis in previous reports suggests that major causative factors include sunlight, trauma, heat, abnormal body temperature, menstruation, other infectious diseases, and emotional stress.¹⁶⁻¹⁸ There is no scientific evidence to prove their causative role in the recurrence of HSK, but our study observed fever before recurrence in a majority of the cases.

Laboratory investigations showed total positivity in 80 of 234 (34.2%) cases. In view of the preponderance of scraping materials from epithelial keratitis cases and the fact that a greater number of tests could be performed on these specimens, the virological correlation between epithelial keratitis cases and stromal infections was probably affected. The lower virus isolation rate for cell culture in comparison to indirect IF may reflect the requirement of viable viral particles to confirm the characteristic cytopathic effect. Studies of animal models^{19,20} clearly show that partially treated eyes give negative results for HSV isolation even though they remain HSV antigen positive. The other reasons for the lower HSV isolation rate in tissue culture could be (1) presence of antiviral substances like interferons in tears; (2) presence of preformed anti-HSV antibodies that may act alone or synergistically with interferons to inhibit viral replication; and (3) the decreased sensitivity of isolation methods. Besides the above reasons, the fact that many cases were already treated in local hospitals before being referred to the Regional Institute of Ophthalmology at Chennai has to be considered an additional explanation for the lower virus positivity rate.

In view of the exposure of the population to HSV early in life, the serum anti-HSV IgG has not been considered a useful diagnostic parameter.²¹ Many researchers from developed countries have reported that the circulating antibodies against HSV remain relatively constant, although fluctuations do occur.^{22,23} However, in true primary HSV infections, an increase in titer is likely. Among the paired samples of 11 first episode cases that showed an elevation of IgG in this analysis, 5 belonged to 5-year-old children. Consideration of the age of these children might lead one to

the assumption that this could be a true primary HSV infection. However, the results of IgM analysis showed that IgM was positive in only two of these five children, thereby indicating that only these two cases may be true primary HSV infections. These results confirm the limited value of routine serological markers in the laboratory diagnosis of HSK.

The tear sIgA was positive in 28 of the 138 (20.3%) cases analyzed; sIgA was not detected in any of the controls. This suggests that the presence of sIgA is indicative of herpetic infection. The correlation between sIgA positivity and any of the other classic tests (virus isolation, IF microscopy, elevation in IgG titer) was 71.4%. The detection of HSV-specific tear sIgA is a valuable adjunct to virus isolation and antigen detection in the laboratory diagnosis of HSK and, for a successful test, the specimen should be collected as early as possible.

It is noteworthy that even after using all the tests, we could diagnose only 34.2% of the clinically suspected cases as HSK. This probably indicates that more sensitive diagnostic techniques like polymerase chain reaction should be used in the diagnosis of HSK, to detect a maximum number of cases and thereby contribute to a better eye care/patient management. A study along these lines is in progress at our center.

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