Laboratory study

HERPES SIMPLEX VIRUS INFECTIONS

J. Marin, B. Drinovec and S. Koren

ABSTRACT

The laboratory diagnosis of herpes simplex viruses represents an important step in the follow-up of the infected person. When the suitable specimen is taken early in the course of the infection several diagnostic methods are available for the virus identification. In the present study 220 specimens were examined using the relatively rapid direct antigen immunodetection. Herpes simplex virus infection was found in 19.5 %. In some specimens electron microscopy and virus culture were used additionally. Positive findings were the most frequent in cases of ocular diseases, followed by cases with skin and mucous membrane lesions. The authors believe that the rapid techniques cannot fully replace conventional ones, yet in daily practice immunofluorescence is a powerful diagnostic aid.

KEY WORDS

herpes simplex virus, HSV-1, HSV-2, rapid viral diagnosis

INTRODUCTION

The genomes of herpes simplex virus (HSV) type 1 and 2 can be distinguished by restriction enzyme analysis of viral DNA or by using monoclonal antibodies against glycoprotein G which is type specific (1). The two types of viruses are extremely widespread in the human population. They grow rapidly in many types of cells and cause cytolytic infections. Pathological changes are due to necrosis of infected cells together with the inflammatory response. Lesions induced in the skin and mucous membranes by HSV-1 and HSV-2 are the same and resemble those of varicella zoster. As the two types are responsible for a spectrum of diseases the laboratory diagnosis plays an important role in the outcome of the infection.

We tried to test the rapid viral diagnosis techniques using mainly viral antigen immunodetection. There were at least two main reasons which stimulated our work. First, the early diagnosis helps to prevent hospital crossinfections and their spread to contacts, especially in cases presenting atypical symptoms. Second, the use of antiviral drugs both prophylactically and therapeutically depends on specific and early diagnosis (2). There are other advantages of rapid

Table 1. Clinical material for HSV detection as referred to the clinical manifestations

origin	specimen	clinical manifestation
anogenital region	cells scraped from the base of herpes-like lesions (cervix,vagina)	Vulvitis herpetica, Endometritis,Cervicitis ac., Cervicitis chr., Cervicitis recid.
ocular region	conjunctival swabs, corpus vitreum material	Conjunctivitis ac., Conjunctivitis chr., Conjunctivitis recid., Keratitis, Keratoconjunctivitis
skin and mucous membranes not from anogenital region	cells scraped from the base of herpes-like lesions vesicle fluid	herpetic vesicular lesions, stomatitis herpetica
other regions	CSF, tracheal aspirates, pharyngeal swabs, pleural fluid	immunodeficiencies, undefined febrile states, neurological diseases

diagnostic techniques. For example, these methods do not depend on virus multiplication and do not even require the presence of live virus. Hence, viral antigens may be detected at a central laboratory using specimens collected in localities remote from a virus laboratory.

MATERIAL AND METHODS

From January 1992 to May 1993, 220 clinical specimens from patients with different clinical manifestations and from different age groups (9 days to 88 years) were examined for HSV. The specimens were collected in the whole region of Slovenia. Clinical material was classified as presented in Table 1.

All the material taken was sent to the laboratory as quickly as possible. Smears, vesicle fluids and aspirates were immediately put into the transport medium. CSF requires no specific transport media and was collected into dry sterile container.

Electron microscopy (EM).

This is an important method for detecting viruses present in vesicular lesions of the skin and in CSF (3,4). A drop of a fluid specimen was collected, transferred to a grid, blotted and stained with 3 % phosphotungstic acid prior to microscopy. A negative report required the specimen to be examined with another diagnostic technique. A positive report could be issued within an hour of receipt of the specimen in the laboratory.

Direct examination by antigen immunodetection.

Practically all the material listed in Table 1 was suitable for the direct examination of the viral antigen. In the laboratory the specimens were shaken gently and centrifuged. The cells from the pellet were smeared on special glass slides, air dried and fixed in chilled acetone. The fixed slides could be kept for a longer period at -20°C. Carefully selected controls representing identical clinical material were included in each test. Monoclonal antibodies conjugated with fluorescein isothiocyanate and stained with Evans blue (Syva MicroTrak,

Sweden) were added to the cell smears and the slides were incubated in a moist chamber 30 min. at 37°C. After washing the slides several times in phosphate buffer saline the slides were nearly dried, covered with cover glass and microscoped under UV light. Monoclonal antibodies to HSV-1 and HSV-2 were available and so we were able to distinguish the two virus types. This method provides diagnosis within two hours of receipt of the specimen (5).

Virus culture.

HSV-1 and HSV-2 are relatively easy to cultivate and isolate. Culture of HSV is a very sensitive tool, if only the clinical material is obtained early in the course of the infection. Vesicle fluid is excellent starting material. As for the other specimens virus containing cells should be scrabbed as vigorously as possible. If the specimen cannot be processed within 4 hours from the collection it must be stored in liquid nitrogen or kept at -70°C. There is a wide choice of cells suitable for the virus culture. We mainly used Vero and FL amnion cells grown in MEM medium supplemented with 10 % fetal calf serum. Specimens were treated with antibiotics and added to the cell monolayers. After inoculation the cells were grown in an incubator at 37°C. The eventual appearance of cytopathogenic effect (CPE) was monitored daily.

ELISA detection of HSV antigen (Enzygnost-HSV Ag, Behring).

This method was mainly used as the confirmatory test in positive virus cultures and in few cases as the sole diagnostic procedure. Medium taken from positive virus culture or homogenized clinical material was used as the source of antigen. The whole procedure could be done within 5 hours.

RESULTS

All specimens (220 in number) were examined by the direct antigen immunodetection. Positive results were seen as a strong cytoplasmic fluorescence (Fig. 1). Negative results showed no fluorescence (Fig. 2). HSV-1 or HSV-2 were detected in 43 cases (19.5 %). Regarding the clinical picture of patients positive results were dispersed as shown in Table 2.

Table 2. Positive results in patients with different clinical manifestations of HSV infection

clinical manifestation	No of positive cases	%
anogenital disease	7	16.3
ocular disease	26	60.5
skin and mucous membrane lesions	8	18.6
other symptoms	2	4.6
total	43	100.0

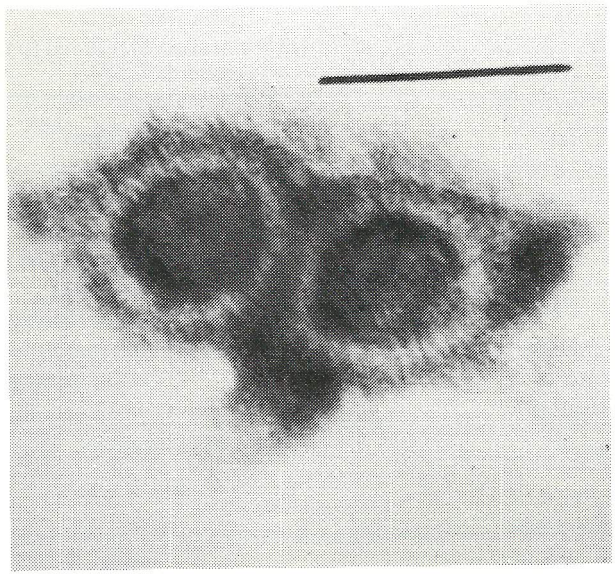

Fig. 3. HSV particles as seen under EM (vesicle fluid).

Out of 7 positive anogenital herpes cases HSV-2 was diagnosed in 6 of them, in 1 case of cervicitis HSV-1 was determined (28-year-old female patient). HSV-2 was found also in 2 conjunctival swabs (one from a 29-year-old patient with keratitis, and one from a 38-year-old patient with conjunctivitis). 2 HSV-1 positive cases of ocular disease were mixed infections with Adenovirus (both cases with keratoconjunctivitis, 68 and 79- years-old patients) and another 4 with Echovirus (one case with chronic conjunctivitis, three cases with acute conjunctivitis, 38 to 49 years old patients).

Some specimens were examined with EM. These were vesicle fluids (Fig. 3) and CSF; in these specimens there were only few virus infected cells. This is the reason why the direct antigen detection method was not suitable in such cases.

12 specimens (CSF, corpus vitreum material, pleural fluid, 9 conjuctival swabs) were inoculated in Vero cells for the virus culture. Direct antigen examination elucidated 2 positive cases (pleural fluid of an immunocompromised patient and one conjuctival swab). Using the virus culture we isolated and diagnosed HSV-1 in a corpus vitreum material which was negative in the direct test. When conjunctival swabs were inoculated typical CPE was seen within one day (Fig. 4); in the other two specimens CPE were observed only after 5 to 7 days after the inoculation.

DISCUSSION

Following the recommendations of the WHO Scientific group for Rapid laboratory techniques for the diagnosis of viral infections (2) we succeeded in introducing the rapid test for HSV-1 and HSV-2 diagnosis in our laboratory. It is recognized that the rapid techniques cannot fully replace conventional ones (4); we believe that in HSV detection virus culture remains the "gold standard". Yet when there is a call for the rapid diagnosis of HSV, immunofluorescence is a convenient and reliable method. In daily practice specific diagnosis is the key to the specific chemotherapy, particularly important in patients with herpes encephalitis, neonatal herpes or virus dissemination as a complication of immunosupression (6,7).

Regardless of the method used the number of HSV-1 and HSV-2 identified in our study was relatively small. HSV positive results were most frequent in ocular disease cases - 60.5 %, followed by positive findings in skin and mucous membrane lesions - 18.6 %; in anogenital herpes the virus was identified in 16.3 %. We were looking for several parameters which could correlate (age, sex, clinical manifestation) but we remained without final conclusions.

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