A Study of Indirect Immunofluorescence Staining in the Diagnosis of Cutaneous Herpes Simplex Virus Infection

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For more practical laboratory diagnostic methods for cutaneous herpes simplex virus (HSV) infection, we examined indirect immunofluorescent (IIF) staining, and compared it with Tzanck smear and viral culture in 103 patients suspected of having HSV infections.

In the 60 specimens for Tzanck smear and viral culture, Tzanck smear was positive in 35(58.3%) cases, and the viral culture in 56(93.3%). In the 43 specimens for viral culture and IIF staining, viral culture was 40(93%) positives and IIF staining was 37(86%) positives. In the 25 specimens, in which the viral cultures were positive, IIF staining was positive in 22(88%) cases and Tzanck smear in 16(64%).

It is suggested that the IIF staining is a simple, accurate, and rapid method for the diagnosis of cutaneous HSV infection. (Ann Dermatol 4:(2) 68–71, 1992)

Key Words: Indirect immunofluorescent (IIF) staining, Herpes simplex virus

Herpes simplex virus (HSV) infection with its increasing trend throughout the world is one of the most common infectious diseases. It discomforts the infected individuals and causes social health problems due to its frequent relapsing characteristics[1].

Although clinical diagnosis is most often apparent, occasionally atypical presentations may lead to an inappropriate diagnosis. In addition, when the infection could be present in persons with immunodeficiency states, pregnant women or newborn infants, rapid and accurate diagnosis is of great importance for the immediate and intensive care. For the diagnosis of HSV infections, the Tzanck smear is commonly used for its advantages of simplicity, inexpensiveness and rapidity[2, 3], but it has limiting factors in clinical uses because of relatively low positive rates[4]. Similarly, the viral culture, which is known to be the most sensitive and accurate method[5, 6], has difficulties in clinical uses because it is expensive and a relatively time-consuming test. We, therefore, introduce indirect immunofluorescence (IIF) staining with monoclonal antibodies, which are specific to the HSV type 1 and type 2, in the diagnosis of HSV infections and compare its effectiveness with Tzanck smear and viral culture.

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PATIENTS AND METHODS

Patients: 103 patients with clinically typical HSV infection in the Dermatology department of Hanyang University Hospital from March of 1987 to June of 1990 were grouped A or B. In group A, sixty patients were sampled to compare the sensitivity to Tzanck smear and viral culture. Forty three patients in group B were sampled to compare the sensitivity to Tzanck smear, viral culture and IIF staining in the diagnosis of HSV infections.

Tzanck smear[7]: The specimens were obtained from the floor of vesicular or crusted lesions. The dried microscope slides were stained with Wright solution. If the multinucleated giant cells were seen on light microscope examination, the result was regarded as positive.

Viral culture[8, 9]: The specimens which were obtained in the same manner as the Tzanck smear were placed in transport media and stored in a deep freezer at −70°C before the culture. For the viral culture, Vero cells (African green monkey kidney cells) were used. After innoculation of the specimen onto the monolayer of Vero cells, the monolayer was examined at 24 hour intervals for the presence of typical cytopathic effects. By inverted microscopic examination, ground glass-like cytoplasms, in clumps or in crater forms, were regarded as positive.

IIF staining[10-12]: The specimen which was obtained in the same manner as the Tzanck smear was smeared onto a microscopic slide that had two circles 10mm in diameter. It was then fixed in acetone. For the positive control, kos strains (HSV-1) and YS4 strains (HSV-2) were used. And for the negative control, the vero cells were used. A drop of mouse monoclonal antibodies, which are specific to the HSV type 1 and type 2 (diluted 1:50 in distilled water) (Chemicon^R, USA), was added onto the slide and the slide was incubated for 30 minutes at 36°C. Then, fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (diluted 1:20 in phosphate-buffered saline) (Zymed^R) was added and incubated in the same manner. By fluorescence microscopic examination, the cytoplasmic bright apple green color fluorescence was considered a positive finding (Fig. 1).

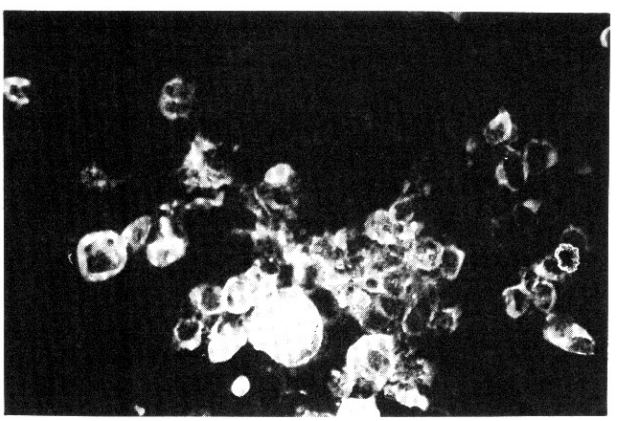

Fig. 1. Positive immunofluorescence staining with monoclonal antibodies (×200).

RESULTS

Group A: In the 60 specimens for Tzanck smear and viral culture, Tzanck smear showed 35(58.3%) positives, and viral culture showed 56(93.4%) positives ($p<0.05$) (Table 1). Of the 56 viral culture positive specimens, Tzanck smear revealed 34(60.7%) positives and 22(39.3%) negatives, while, of the 35 Tzanck smear positives, viral culture revealed 34(97.1%) positives and 1(2.9%) negatives.

Group B: In the 43 specimens for viral culture and IIF staining, viral culture showed 40(93%) positives, and IIF staining showed 37(86%) positives ($p>0.05$) (Table 2). In the 25 specimens, in which viral cultures were positive, IIF staining accounted for 22(88%) positives and Tzanck smear 16(64%) positives showing statistical differences in the sensitivity of Tzanck smear and IIF staining ($p<0.05$) (Table 3).

DISCUSSION

In our study, the viral culture was shown to be a more sensitive method than Tzanck smear in the diagnosis of HSV infections. Similar results were seen in previous reports by Solomon et al[4] and Kim et al[9].

There are immunological[5, 13, 14] and nonimmunological methods in the differentiation of HSV-1 and HSV-2. Of the immunological methods, immunofluorescence assay using monoclonal antibody is preferred recently, because it is relatively accurate, simple and rapid, with little cross-reactivity[5, 8-12]. Although we cannot directly estimate the differences in diagnostic sensitivity between direct immunofluorescence (DIF) and IIF staining. Moseley et al[6] suggest that IIF is more sensitive than DIF, because IIF uses 'type-specific' antisera. We, therefore, adopted the IIF staining with monoclonal antibody and FITC-conjugated goat anti-mouse IgG.

Comparing the sensitivity between viral culture and IIF staining, our results showed no statistical differences (Table 2), as was previously reported by Goldstein et al[11] and Balachadran et al[12].

In our study, there were 2(5.4%) IIF false posi-

Table 1. Comparison of the results of Tzanck smear and culture in clinical herpes simplex virus infections
(Mar. 1987—Dec. 1989)
(n=60)

HSV Tzanck smear/culture	Number*	%
Tzanck smear positive	35	58.3#
culture positive	56	93.3#
Tzanck smear positive & culture positive	34	56.7
Tzanck smear positive & culture positive	22	36.7
Tzanck smear positive & culture negative	1	1.7
Tzanck smear negative & culture negative	3	5.0

* No. of specimens tested as either positive or negative
# $p<0.05$

Table 2. Comparison of the two techniques by culture results and indirect immunofluorescence staining in herpes simplex virus infections
(Dec. 1989—Jun. 1990)
(n=43)

HSV culture/IIF staining	Number	%
Culture positive	40	93#
IIF positive	37	86#
Culture positive & IIF positive	35	81.4
Culture positive & IIF negative	5	11.6
Culture negative & IIF positive	2	4.7
Culture negative & IIF negative	1	2.3

$p>0.05$

Table 3. Comparison of the sensitivity by Tzanck smear and indirect immunofluorescence staining in virus culture positives
(Dec. 1989—Jun. 1990)
(n=25)

HSV Tzanck smear/IIF staining	Number	%
Tzanck smear positive	16	64#
IIF positive	22	88#
Tzanck smear positive & IIF positive	16	64
Tzanck smear positive & IIF negative	0	0
Tzanck smear negative & IIF positive	6	24
Tzanck smear negative & IIF negative	3	12

# $p<0.05$

tives and 5(12.5%) IIF false negatives comparing with the results of the viral culture. The false negatives in the IIF staining is accounted for by small amounts of antigen[15], transient inactivation of virus, types of lesions[4, 15], and loss of infectivity of virus during transport of specimen, etc.[6, 11]. On the other hand, the false positives in the IIF staining can be explained by the fact that the sampling for the IIF staining was performed before that of the viral culture. And both false negatives and positives could be reduced by sampling the full blown lesion and practicing skillful procedures[6, 12, 16].

In the 25 specimens, in which Tzanck smear, viral culture and IIF staining were all done, we ascertained that IIF staining and viral culture were more sensitive than Tzanck smear as Solomon et al's[4] and Moseley et al's[6] reports.

In conclusion, the results of Tzanck smear showed relatively high correlation to the results of IIF staining and viral culture. However, the results of IIF staining showed a significant agreement with the results of viral culture.

It is suggested that the IIF staining with monoclonal antibody is a simple, inexpensive, accurate, and relatively rapid method for the diagnosis of cutaneous HSV infections.

REFERENCES

1. Corey L, Spear PG: *Infections with herpes simplex viruses. N Engl J Med 314:686-691, 1986.*
2. Solomon AR: *The Tzanck smear: Viable and valuable in the diagnosis of herpes simplex, zoster, and varicella. International J Dermatol 25:169-170, 1986.*
3. Barr RJ, Herten J, Graham JH: *Rapid method for Tzanck preparation. JAMA 237:1119-1120, 1977.*
4. Solomon AR, Rasmussen JE, Varani J et al: *The Tzanck smear in the diagnosis of cutaneous herpes simplex. JAMA 251:633-635, 1984.*
5. Hsiung GD, Landry ML, Mayo DR et al: *Laboratory diagnosis of herpes simplex virus type 1 and 2 infections. Clin Dermatol 2(2):67-82, 1984.*
6. Moseley RC, Corey L, Benjamin D et al: *Comparison of viral isolation, direct immunofluorescence, and indirect immunoperoxidase technique for detection of genital herpes simplex virus infection. J Clin Microbiol 13:913-918, 1981.*
7. Naib ZM: *Exfoliative cytology in the rapid diagnosis of herpes simplex virus infection. In the human herpesviruses. Nahmias AJ, Dowdle W, Schinazi R(eds), Elsevier North Holland, New York, 1981, pp381-386.*
8. Bird BR, Forrester FT: *Basic laboratory techniques in cell culture. Centers for Disease Control, Atlanta, Ga, 1981, pp148-150.*
9. Kim YT, Kim JH, Cho SH et al: *Studies on isolation of herpes simplex virus: Isolation rate of HSV from various sites and types of mucocutaneous lesions. Korean J Inf Dis 19:97-106, 1987.*
10. Peterson E, Schmidt OW, Goldstein LC et al: *Typing of clinical herpes simplex virus isolates with mouse monoclonal antibodies to herpes simplex virus type 1 and type 2: comparison with type-specific rabbit antisera and restriction endonuclease analysis of viral DNA. J Clin Microbiol 17:92-96, 1983.*
11. Goldstein LC, Corey L, McDougall JK et al: *Monoclonal antibodies to herpes simplex viruses: Use in antigenic typing and rapid diagnosis. J Infect Dis 147:829-837, 1983.*
12. Balachadran N, Frame B, Chernesky M et al: *Identification and typing of herpes simplex viruses with monoclonal antibodies. J Clin Microbiol 16:205-208, 1982.*
13. Nahmias AJ, Chiang WT. del Buono I et al: *Typing of herpes virus hominis strains by a direct immunofluorescent technique. Proc Soc Exp Biol Med 132:386-390, 1969.*
14. Benjamin DR: *Rapid typing of herpes simplex virus strains using the indirect immunoperoxidase method. Appl Microbiol 28:568-571, 1974.*
15. Schmidt NJ, Dennis J, Devlin V et al: *Comparison of direct immunofluorescence and direct immunoperoxidase procedure for detection of herpes simplex virus antigen in lesion specimens. J Clin Microbiol 18:445-448, 1983.*
16. Nahmias A. del Buono I. Pipkin J et al: *Rapid identification and typing of herpes simplex virus type 1 and 2 by a direct immunofluorescence technique. Appl Microbiol 22:455-458, 1971.*