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# A Comparative Study Between Viral Isolation and Indirect Immunofluorescence In The Diagnosis of Herpes Simplex Virus

MOHAMED S. EL-ANSARY, MD,
ABDULWAHAB S. AL-FOUZAN, MD, PhD

*From the Department of Dermatology, Al-Sabah Hospital, Kuwait.*

## SUMMARY

Fifty patients with oral ulcers were studied clinically and investigated for the detections of Herpes Simplex Virus (HSV) through virus isolation from their lesions (vesicles and ulcers) and detection of the presence of antiviral antibodies (both IgM and IgG) in their sera using the indirect immunofluorescene (IIF) technique.

The results of this study proved that virus isolation is the most reliable method for diagnosis, though the use of antibody serological tests could be a useful adjunct to virus isolation in situations where a rapid laboratory diagnosis is needed.

Oral Herpes Simplex virus infection can be viewed, in the main, as a trivial disorder causing patients minor physical discomfort[1].

The prevalence of HSV may be high in innocent infections, as high as 1/3 of the population[2]. However, HSV infection and its complications with troublesome recurrences may make the problem worse[3]. The apparent increase in HSV infection over recent years may be partly due to increased publicity about the disease, the current antiviral treatment, the inclusion of both primary and recurrent cases in clinic follow up and the increased use of viral cultures for diagnosis [4].

The aim of this work is to share in the study of the detection of HSV through virus isolation and detection of antiviral antibodies using IIF technique, as well as the evaluation of the diagnosis by the above mentioned methods.

## Materials and Methods

Fifty patients, attending the outpatient clinic of Dermatology department at Al Sabah Hospital - Kuwait, with positive history of oral vesicles or ulcers or were contacts of clinically positive patients. The lesions were situated on or in the oral cavity including the lips, tongue and palate.

Each candidate in this study was subjected to the following:

1) Full history was taken regarding history of exposure, duration of the disease, rate of recurrence (if any). These patients did not receive any local or systemic antiviral therapy.
2) Sampling from the floor of the ulcer, ruptured vesicles or the vesicles aspirate were subjected to virus isolation on tissue culture using fibrobasts of African green

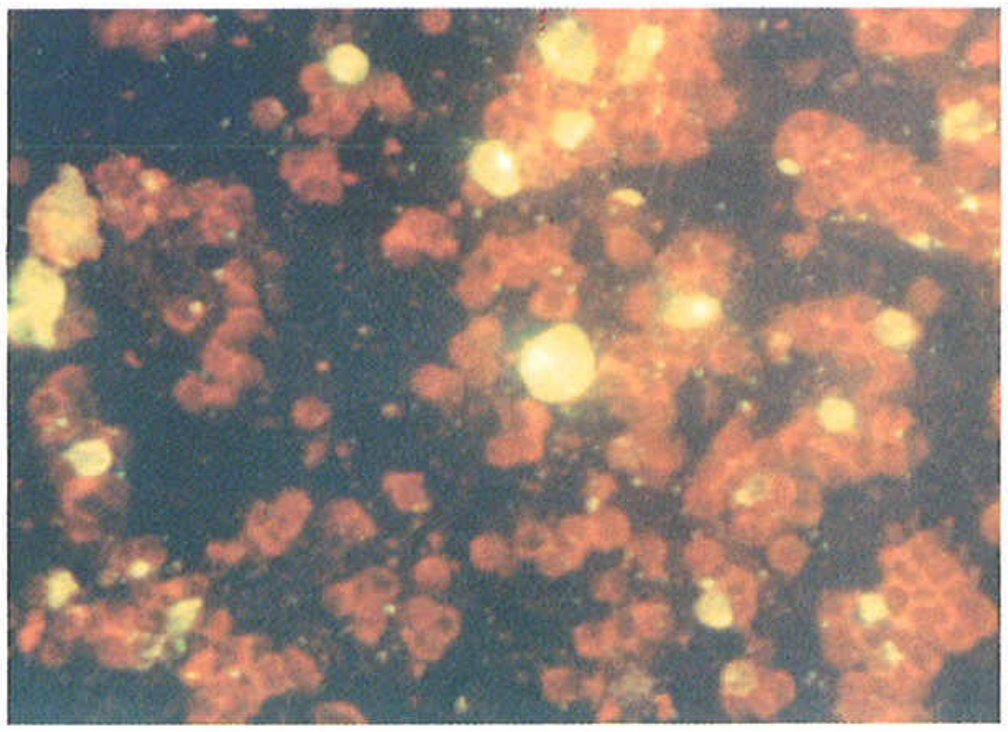

*Fig.1 Indirect immunofluorescence showing cells infected with HSV (yellow), and non-infected cells (green).*

monkey kidney (Vero cells). This was done on two steps first by infection of the tissue culture and then observation of cytopathogenic effect for seven days period.

Finally the infected cells were tested using the immunofluorescence kit for the detection of HSV accoridng to the manufacturer's instructions[5] and the results were interpreted by using an Olympus fluorescence microscope (Fig.1)

3) 5ml of blood samples were obtained and were put in dry sterile siliconized tubes in 37c
for two hours, then centrifuged at 2000 r.p.m. for ten minutes.
The obtained serum was subjected to IIF test kit for the detection of antibodies to HSV (IgM & IgG) according to manufacturer's instructions[5].
Another sample of blood was withdrawn two weeks later for IgG titration in case of negative virus isolation, negative IgM and positive IgG.

**Results:**

Thirty five out of 50 patients (70%) with oral lesions were males and the rest 15 patients (30%) were females (table 1). Their ages ranged from 15-50 years with mean age 30.2 years. The duration of the present attack varied from two to 10 days. Thirty eight patients were married and the rest were singles. Nineteen patients were found positive for HSV (38%), females constituted six out of 15 patients (40%) and 13 male patiens out of 35 (31%) (table 1).

**TABLE 1** - POSITIVE CASES OF HSV IN ORAL LESIONS IN RELATION TO SEX

| Sex | No. of patients | Positive cases | Percentage |
|---|---|---|---|
| Males | 35 | 13 | 31% |
| Females | 15 | 6 | 40% |
| Total | 50 | 19 | 38% |

The mean duration of having symptoms was 6.26 days. Sixteen out of the 19 positive cases were in their lst attack and only three positive cases were in their recurrent attack. Positive cases of oral HSV in relation to age are listed in (table 2). Among the positive cases, 14 patients were married and five patients were singles.

**TABLE 2** - POSITIVE CASES OF HSV IN ORAL LESIONS IN RELATION TO AGE

| Age group | No. of patients | Positive cases | Percentage |
|---|---|---|---|
| 15-20y | 6 | 3 | 50% |
| 21-30y | 21 | 9 | 44.7% |
| 31-40y | 19 | 4 | 21% |
| 41-50y | 4 | 1 | 25% |
| Total | 50 | 19 | 38% |

When the patients of this group were subjected to laboratory investigations (table 3), the virus could be isolated in four patients without detection of IgM reaction, while two patients had positive IgM only in their sera. The virus could be isolated together with positive IgM in 12 patients and only one patient had a rising IgG level when another sample of blood was tested two weeks later. So a total of 19 patients out of 50 were proven to have HSV infection by different methods of

**TABLE 3.** TOTAL NUMBER OF ORAL HSV +VE CASES ACCORDING TO DIFFERENT METHODS OF INVESTIGATIONS OF ORAL LESIONS.

| Type of test | Number of cases |
|---|---|
| +ve virus isolation / +ve IgM | 12 |
| +ve virus isolation only | 4 |
| +ve IgM only | 2 |
| IgG rising titre only | 1 |
| Total | 19 |

investigations.

## Discussion:

Herpetic lesions around the mouth were first described about 100AD. The cytopathologic method, by the end of the 19th century, could establish the viral aetiology of the disease[6].

Oral herpes is a common disease, through the total number of people suffering from oral herpes are yet unknown. However, half a million new cases of oral herpes are reported each year in the United States[7].

Follow up studies in infants, children, teenagers up to adulthood showed that HSV could be detected in very high percentage that approaches 100% by late adulthood. This is related to primary HSV infection[8].

The prevalence of HSV infection may also be due to the social habit of kissing among men when they meet. Also, women have the same attitude and have the same background of expressing social feeling through kissing the same sex. Unlike the habit in European countries where men shake hands and kiss women. Also, such kissing may propagate the HSV infection either in Arab or European countries.

Out of the fifty patients, 19 (38%) were considered to be positive for HSV infection. The virus could be isolated in 16 out of 19 positive cases (84.2%), while raised IgM levels in the sera were detected in 14 out of 19 patients (73.7%). A rising titre of IgG was detected in only one patient (0.5%).

The results show that no single method could be considered as a perfect method for diagnosing HSV infection.

Virus isolation was found to be the most reliable method for diagnosis, (84.2%). This figure was obtained from patients with oral lesions, and similar results were reported (86%) by Schmidt[9], (80%) by Moseley[10] and again (82%) by Schmidt[11].

The discrepancies between one another method could be due to the time of sampling techniques, inactivation of the infectious virus during transport, non infections viral antigen production in cells inoculated with patient samples or truly methodology false positivity[9].

The use of IIF for detection of IgM in sera of the studied groups showed positivity results of 73.7%, the results showed less reliable figures than that obtained by virus isolation.

However two patients showed positive results for IgM while the virus could not be isolated from their lesions.

The least reliable results were obtained by the use of IIF for the detection of IgG, as this class of immunoglobulins is well known to have a late, slower and more persistent elevation in response to infection compared to that of IgM.

Hence, raised titre of IgG was considered as evidence of HSV infection, only one patient showed this change.

So, a conclusion can be made that HSV has a high rate specially among middle age group, and whereas virus isolation is the most reliable method for confirming the diagnosis of HSV infection in patients presenting with oral lesions, the IIF method still be useful as rapid and easier test to perform for diagnosis and sometimes in detecting conditions in which HSV could not be isolated.

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