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Original Article

Comparison of Passive Haemagglutination and Neutralization Tests to Demonstrate Genital Herpes Virus (HSV2) Antibodies in Pregnant Women

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Abstract

Background and Aims: Herpes simplex virus (HSV) is responsible for several significant human viral diseases, with severity ranging from subclinical to fatal infection. Herpes simplex virus type 2 (HSV2) infections are more commonly seen in association with the genitalia and surrounding areas, and can be transmitted to newborns during childbirth. Generalized infections in newborns are also predominantly HSV2. Therefore, due to the increasing HSV2 infections especially subclinical in women, the need to diagnose herpes simplex virus infections has increased.

Materials and Methods: In the present study, the passive haemagglutination (PHA) test was applied to determine the titer of Anti-HSV2 antibodies. Sheep red blood cells (SRBCs) were treated with tannic acid at concentration of 0.002% and sensitized with HSV2 that had been propagated in HeLa cells. The tannic acid treated and HSV2 sensitized SRBCs were added to U-shaped 96 wells microtiter plates which contained serial dilutions of patient's sera. Furthermore, serum neutralization test (SNT) was applied as a gold standard to determine the specificity and sensitivity of PHA test.

Results: The results of PHA were examined after one hour incubation at 37°C. The endpoint was the highest dilution of serum which gave positive agglutination and compaired with serum neutralization test (SNT as a gold standard to determine the specificity and sensitivity of PHA test. Peripheral blood samples were obtained from 100 pregnant women and evaluated by both SNT and PHA tests. The specificity and sensitivity of PHA test were 92.68%, and 100% respectively. The results indicated that PHA test was easy, rapid and inexpensive but also had acceptable sensitively and specificity.

Conclusion: PHA test can be used to determine the level of Anti-HSV2 antibodies and the rate of infection in pregnant women which could be an indication of infection and the risk of transmitting herpes to the newborn.

Keywords: Herpes Simplex virus type 2; passive haemagglutination test; serum neutralization test

Introduction

Inorder to demonstrate two distinct antigenic types of herpes simplex virus (1, 2), numerous methods have been developed to differentiate these two closely related viruses. These viruses are fast-growing, cytolytic which tend to establish latent infections in neurons (3).

Herpes simplex virus type 2 (HSV2) infections is considered as the major cause of genital herpes which can cause recurrent and painful genital ulcer (3). Although about 80 percent of

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primary genital infections are caused by HSV2 but increasing cases of genital herpes are found due to HSV1 (2).

Although primary infections are usually subclinical but occasionally occur as a sever disease especially in women. Small vesicular lesions caused by these viruses are seen in women on the vulva, vagina, cervix, urethra, or on the perinea and in men on the penis. Local symptoms of disease are include pain, itching, redness, inflammation, pus, urine imprisonment, inguinal lymphadenopathy, fever, and restless. Systemic symptoms are common in both sexes, especially in women. Spread of infection to the central nervous system may also occur in 10 percent of cases (4).

Although severity of the recurrent disease is less than the primary disease, but that is usually associated with pain and sexual dysfunction. HSV2 infection in the newborns may cause severe disease, which is usually acquired from infected mother (5). Newborn infants can become infected with herpes virus type 1 and 2: in the uterus, passing through the mother's birth canal, and immediately after childbirth having close contact with someone who has herpes mouth sores (5).

Symptoms of neonatal infection with HSV2 are including; growing disease with 80 percent mortality, Encephalitis with high mortality and local disease in mucosal surfaces in untreated individual(6).

It has been shown that the high levels of antibody against HSV2 in mother at delivery is an indication of possible transmission of the virus to the fetus (7).

In these experiment two applicable methods were applied to determine the level of antibodies against HSV2 in pregnant women and their sensitivities and specificities were compared.

Methods

Cell Line

HeLa cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 5% heat inactivated FCS, 100 IU/ml penicillin and 100 µg/ml streptomycin at 37ºC with an atmosphere of 5% CO2.

Virus propagation

Herpes simplex virus (HSV) was isolated from patients and identified as HSV1 or HSV2 using immunofluorescence and monoclonal antibodies (8). HeLa cells were infected at a multiplicity of infection (moi) of 1 pfu/cell and incubated for one hour, Unabsorbed virus suspension was discarded and the cells were washed two times by sterile phosphate buffer saline (pH=7). Serum free DMEM containing 100 IU/ml penicillin and 100 µg/ml streptomycin was added to the cells. After appearance of cytopathic effects (CPE) in almost 70% of the cells, virus was harvested by freezing and thawing. Cell debris was removed by centrifugation at 8000g for 15 min at 4ºC and the supernatant was used as whole antigen for later use (9).

Study population

One hundred pregnant women aging 16-40 years old were selected from Imam Tehran hospital. Blood sampling was performed in sterile tubes from the umbilical cord of women during childbirth and in the delivery room by the hospital staffs. Tubes containing cord blood were transferred to the laboratory placed on dry ice under sterile conditions.

Virus neutralization test (VNT)

Collected serum samples were used for detection of HSV-2 neutralizing antibodies. Briefly, two fold dilutions of heat inactivated of each serum ranging from 1/2 to 1/128 were prepared in serum-free DMEM. Each dilution was mixed with 100 TCID50 HSV-2 and kept for one hour at 37°C in a total volume of 200 µl. Duplicate 100 µl samples were added to HeLa cells in 96-well micro titer plates. DMEM containing 2% heat inactivated newborn-calf serum was added to all inoculated cells including positive and negative controls and were incubated at 37°C. The neutralization titer of each serum was considered as: the reciprocal titer of the highest dilution preventing CPE in inoculated cells (9).

Passive haemagglutination test

Passive haemagglutination test was used to assay antibody titer using blocking methods. Three micolitter of sheep blood cells washed

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with PBS three times. Cells at a concentration of 2.5% (v/v) were treated with 0.002% (w/v) tannic acid in PBS pH 7.2 for 10 min at 37°C. The cells were washed and resuspended at a concentration of 2.5% (v/v) in an optimal dilution of antigen in PBS pH 6.4 which had been determined previously by a chequerboard titration. Subsequently 100 µ/ml purified HSV-2 were added and incubated in room temperature. After 30 min at 37°C the cells were washed and stored at a concentration of 2.5% (v/v) in PBS pH 7.2 containing 1% (v/v) heat-inactivated rabbit serum. Serum samples that had been inactivated in 56°C for 30 min, was added to wells in different dilutions. Normal rabbit serum and antiserum against HSV-2 that already had been diluted fourfold in PBS were considered as negative and positive control respectively (10).

Statistical analysis

The sensitivity and specificity of PHA test were calculated by using the following formulae: Sensitivity = [(number of true positives) / (number of true positives + number of false negatives)] × 100; Specificity = [(number of true negative) / (number of true negative + number of false positive)] × 100 (11).

Results

HSV-2 specific neutralizing antibodies

Micro neutralization test was performed using 100 TCID50 of the wild-type HSV2. Virus neutralization titer was the reciprocal of the highest dilution of the serum that completely inhibited CPE in the inoculated cells (figure 1, 2). Data in Table 1 shows the HSV-2 specific neutralizing antibody titer ≤ 16. In 82% of the tested sera, neutralizing antibody against HSV-2 was not detected, whereas 18% had neutralizing antibody against HSV-2.

Comparison of sensitivity and specificity of passive Haemagglutination test versus neutralization test

Presence or absence of antibody against HSV-2 was examined by neutralization test in 100 serum samples. Then their titers of antibody were also determined using indirect Haemagglutination test (Figure 3). Results have showed in Table 2. Sensitivity and specificity of PHA test were calculated 100 and 92.68 respectively.

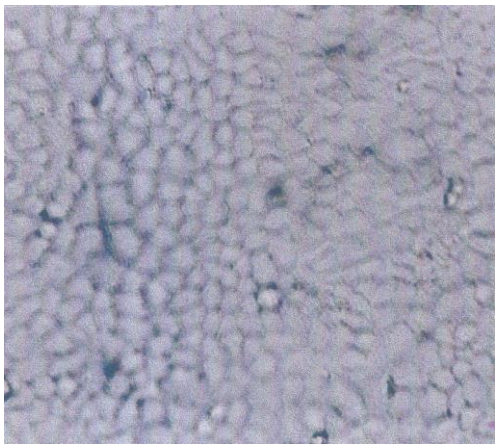

Fig. 1. Monolayer culture of uninfected HeLa cells.

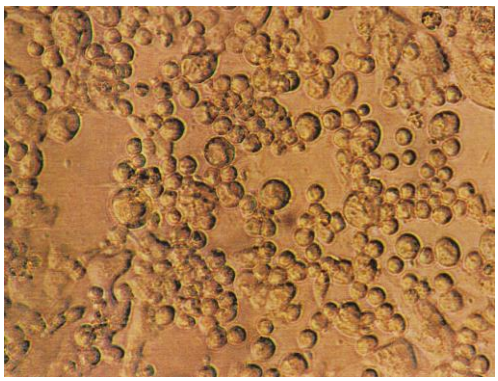

Fig. 2. Infected HSV-2 HeLa cells at a multiplicity of infection (moi) of 1.

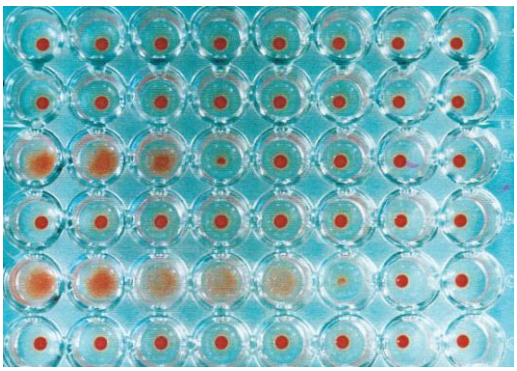

Fig. 3. An example of the results of passive haemagglutionation test.

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Table 1. Virus neutralization antibodies against HSV-2 in tested sera (control together).

Virus neutralization antibody titer*	$<2^{-4}$ or no antibody	$\geq 2^{-4}$	$\geq 2^{-5}$	$\geq 2^{-6}$	$\geq 2^{-7}$
Percentage of positive sera **	82	18	26	10	6

* Virus neutralization titer was expressed as the reciprocal of the highest dilution that completely inhibited virus CPE in inoculated cells

** Two fold dilutions of 100 serum samples were tested against 100 TCID50 of the HSV-2.

Table 2. The number of positive and negative sera in indicated dilution by VNT and passive haemagglutionation test.

PHA \ **SNT**	**Positive**	**Negative**	**Total**
Positive	18	16	24
Negative	0	76	76
Total	18	82	100

Discussion

Herpesviridae comprises large family of DNA viruses which are distributed worldwide. In this family, herpes simplex virus type 1 (HSV1) and 2 (HSV2) cause wide spectrom of infections and often have no clinical symptoms (12). Genital herpes that cause high mortality rate, about 80%, affects newborns during passage from the infected delivery canal. Studies have shown that neutralization antibodies were produced against the virus envelope proteins, gpD and gpB, are able to prevent the infection successfully and also they are responsible to limit the virus in the sensory nerve terminals. Different methods are used for HSV antibody detection including ELISA, IF, CF and virus neutralization test (VNT). Virus neutralization test is more specific than other serological tests (13), but this method requires cell culture and skilled personel and takes long time to perform.

In this study sensitivity and specificity of the passive haemagglutination test was compared with SNT, which was considered as gold standard.

In this study, 100 serum samples with reciprocal virus neutralization antibody titers of 16, 32, 64, and 128 were tested by passive haemagglutination test using sheep red blood cells (SRBCs). Similarly positive and negative sera were used as controls.

Scott *et al* showed that sheep erythrocytes treated with tannic acid and then sensitized with HSV can be hemagglutinated in presence of immune sera (14). Felton and Scott also presented experimental data to indicate that this reaction is essentially a specific antigen-antibody reaction (15). Bennett and Friedman used similar techniques for detection and measurement of antibody against adenovirus (16).

This experiment showed that haemagglutination test has high degree of sensitivity and specificity for detection of HSV antibody in serum samples. Applying haemagglutination test and serum neutralization test can be used to identify antibodies against HSV1, HSV2, measuring antibody titer and immune status in human population. Simplicity, and rapid performance of this test on a large number of serum samples are the advantages of haemagglutination test compared with the neutralization assay.

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