

Evaluation of antibodies against glycoprotein D (gD) and glycoprotein G (gG) in HSV-1 infected individuals' serum samples

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Abstract. – Background and Objectives: Glycoproteins D (gD) and G (gG) of herpes simplex virus type 1 (HSV-1) are virus envelope glycoproteins that are able to induce HSV-1 antibody production in infected persons. Therefore, those proteins could be in interest to develop the serodiagnostic test(s) for HSV antibody detection. The aim of present study was the comparison of anti-gD and anti-gG antibodies in HSV-1 infected individuals' serum samples.

Materials and Methods: In this study, recombinant gD and gG were prepared and used for western blot test to detect the antibodies against HSV-1.

Results: Our data showed the total gD antibody titer was higher than gG antibody titer in the HSV-1 infected patient's sera but the gG antibody titer was high significantly.

Conclusions: According to our results, gD and gG can be used for designing the diagnostic laboratory tests to evaluate total antibody against HSV-1 and HSV-2.

Key Words:

Herpes simplex virus type 1, Glycoprotein D, Glycoprotein G, Western blotting.

(gD) and glycoprotein G (gG) that exhibit a very important role during the virus replication cycle^{2,3}. Glycoprotein D is expressed on HSV infected cell's surface and is essential for the viral infectivity^{4,5}. Indeed, gD is a common-type antigen and stimulates the high production of neutralizing antibodies especially between HSV-1 and HSV-2⁶⁻⁸. It serves as a receptor binding protein for entry to the target mammalian cells and is important in the integration of the viral envelope and plasma membrane of the cells^{9,10}.

Glycoprotein D is an important inducer for humoral and cellular immune responses against the virus infection and it is a very conserved glycoprotein with more than 98 percent homology between HSV strains^{3,8,9}. Glycoprotein G is required for viral entry into polarized cells¹¹. It has a role in virus release and cell to cell virus spread. Indeed, gG introduced as a candidate to develop the type specific serodiagnostic kits for HSV-1 and HSV-2¹². Both gD and gG are HSV surface glycoproteins and they are able to stimulate production of specific antibodies in infected individuals.

The aim of this study was to determine the level of anti-gD and anti-gG total antibodies in HSV-1 infected sera.

Introduction

Herpes simplex virus type 1 (HSV-1) is an enveloped spherical virus with an icosahedral capsid that is considered as an important infectious agent in human illnesses. Viral envelope contains at least 10 glycoproteins and 2 non-glycosylated¹. Two important envelope glycoproteins are glycoprotein D

Materials and Methods

Virus and Cell Lines

In this study one clinical isolate of HSV-1 was used. This virus was isolated from labial vesicles

of a patient and identified as HSV-1, using monoclonal antibody as described previously¹³. HeLa cell line as a proper cell line for HSV-1 propagation was grown in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Carlsbad, CA, USA) supplemented with 5% fetal bovine serum (FBS; Gibco, Carlsbad, CA, USA) 100 IU/ml penicillin and 100 µg/ml streptomycin at 37°C in the presence of 5% CO₂¹⁴.

We have used a recombinant baculovirus containing HSV-1 gD gene in order to produce the recombinant gD. The Sf9 insect cell line was used in order to propagation of recombinant baculovirus. The cells were grown in Grace's medium supplemented with 10% FBS, 3.33 g/l lactalbumin hydrolysate, 3.33 g/l yeast extract and 50 µg/ml gentamycin at 26 °C in a non-humidified incubator.

HSV-1 Propagation

HeLa cells (From Virology Lab of Tarbiat Modarres University) were infected by HSV-1 at a multiplicity of infection (moi) of 0.1 plaque forming unit/cell (PFU/cell), and viruses harvested from the growth medium when more than 75% of the cells showed the virus cytopathic effects (CPE). The titer of propagated HSV-1 was determined using Tissue Culture Infectious Dose 50 (TCID₅₀) method as described previously^{15,16}.

Virus Neutralization Test (VNT)

VNT was used for titration of HSV-1 neutralizing antibodies in serum samples as described previously¹³.

gD and gG Preparation

Sf9 cells were infected by recombinant baculovirus containing HSV-1 gD gene at a multiplicity of infection (moi) of 7 and the cells were incubated for 5 days at 26 °C in a non-humidified incubator. Then, the cells were scraped, 1M phenylmethylsulfonyl fluoride (PMSF) was added and the proteins were harvested using sonicator (Branson, Danbury, USA). The recombinant gG was prepared in E.coli and a DEAE-Sepharose CL-6B column (Fluka, Buchs, Switzerland) was used for its purification as described previously¹². The non-purified gD and purified gG proteins were used in SDS-PAGE and western blotting.

SDS-PAGE and Western Blotting

The prepared glycoproteins were mixed with electrophoresis sample buffer (50 mM Tris pH 8.6, 10% glycerol, 2% W/V sodium dodecyl sulfate (SDS) (Sigma, St.Louis, MO, USA), and 0.1% bromophenol blue, (no reduced method) and boiled for 10 min. Sodium dodecyl sulfate-polyacryl amide gel electrophoresis (SDS-PAGE) performed in 12% polyacrylamide. Then, the recombinant proteins were transferred on nitrocellulose paper and blocking was done using 5% gelatin solution. The serum samples with 1:32 anti-HSV-1 titer in virus neutralization test (VNT) were used for the western blotting and the dilutions of 1:200, 1:400, 1: 800 and 1:1600 were used in the western blotting method. Antibodies against HSV1 glycoproteins were detected by reacting the blots with alkaline phosphate-conjugated anti human antibody and visualizing by diaminobenzidine (DAB) as substrate^{17,18}.

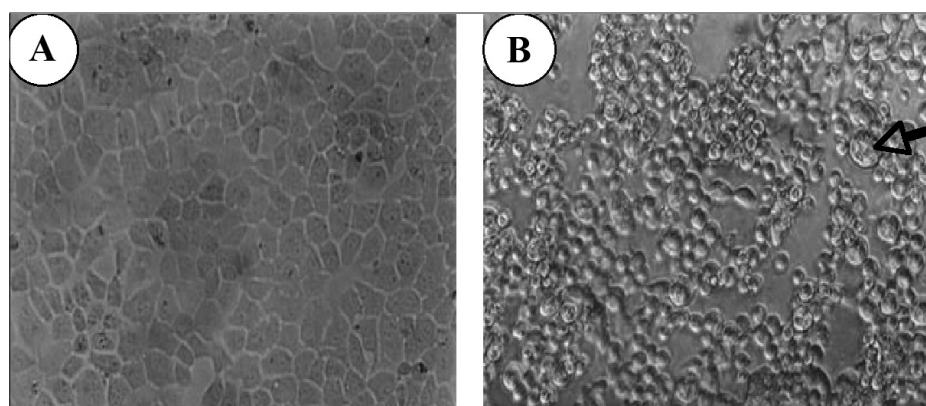


Figure 1. **A**, HeLa monolayer cells. The cells are adherent with epithelial appearance. **B**, Cytopathic effects (CPE) of HSV-1 on the HeLa cells. The CPE includes rounding and ballooning of infected cells and the multinucleated giant cells formation (arrow).

Results

Figure 1A shows HeLa monolayer cells. These cells are immortal and adherent cells with epithelial appearance. The HSV-1 cytopathic effects on HeLa cells are shown in figure 1B. CPEs of HSV-1 on HeLa cells are included rounding and ballooning of the infected cells and also multinucleated giant cells formation.

CPEs were detected in infected cells and the ratio of the CPE positive wells to CPE negative wells recorded. The titer of propagated HSV-1 was $10^{5.5}$ TCID₅₀/ml. The propagated viruses were used in VNT and antibody titer of HSV-1 was measured in serum of patients.

Fifteen serum samples out of 100 were detected as positive for HSV-1 antibody regarding their antibody titer which were equal or above 1:32 using VNT. The VNT determined positive sera were evaluated using western blotting method. The results from western blotting test using gD recombinant protein showed that all VNT detected positive samples were positive even using 1:3200 diluted sera.

Results from western blotting using recombinant gG showed that all VNT detected positive samples were positive but the most diluted sera which gave us positive results was 1:400.

mining and comparing the total antibody responses against two glycoproteins gD and gG, 1:200 to 1:1600 serial dilutions of positive sera with anti HSV-1 antibody titer of 1:32 in VNT, were used in Western blotting using recombinant gD and gG.

Previous studies showed gD could induce the high titer of neutralizing antibodies but the main problem for application of gD for serodiagnosis is the high similarity between gD-1 from HSV-1 and gD-2 from HSV-2^{6,8,19}. Therefore, gD could be good candidate to develop a serodiagnostic kit for total antibody against HSV-1 and HSV-2. The other important point is the gG can induce less total antibodies compare to gD but the titer of antibody is high enough for using this glycoprotein in diagnostic tests, especially if the differentiation between HSV-1 and HSV-2 is required.

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Discussion

Herpes simplex virus type 1 causes a wide range of human diseases, including systemic infections in infants and immunocompromised people and localized infections such as infectious lesions in the lips and mouth, mucosal membranes, skin, cornea, reproductive organs and central nervous system. HSV-1 is the most common cause of sporadic encephalitis in United States. Neonatal HSV-1 infections that may be occur in the uterus, at birth or after birth in the untreated cases have high mortality. HSV can be caused disseminated infections and lethal diseases in immunocompromised patients¹.

It is important to note the most of surface HSV-1 glycoproteins induces the cellular and humoral immune responses. Although gD is the most efficient HSV-1 glycoprotein for induction of neutralizing antibodies against the virus, it is not clear about the efficacy of these glycoproteins including gD and gG for the induction of total antibodies against the virus. For deter-

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