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Amplification and Cloning of Herpes Simplex Virus Type 2 Glycoprotein G from an Iranian Isolate

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Abstract: Herpes Simplex Virus type-2 (HSV-2) is the main cause of genital herpes infection. Its prevalence is increasing worldwide and varies widely with generally higher rate in developing than developed countries and urban than rural areas. HSV-2 Iranian isolate was propagated in HeLa cell line. The viral genome was extracted by phenol-chloroform and used as template in nested polymerase chain reactions (n-PCR) to amplify gG-2 gene. The amplified gene was cloned into a cloning vector (pTZ57R/T) and transformed into competent *E. coli* DH5a. The recombinant vector encoding the gene of interest was confirmed by restriction enzyme analysis and sequencing.

Key words: HSV-2, Glycoprotein G-2, PCR, cloning

INTRODUCTION

Herpes simplex virus type 1 (HSV-1) and HSV-2 are well known as similar subtypes of HSV and closely related enveloped human viruses. The nucleotide sequence of their DNA is sufficiently similar and viable intertypic recombination's result from mixed infection with genetically marked viruses (Ashley and Wald, 1999; Minko *et al.*, 2002). HSV-2 is the main cause of recurrent genital infection (Slomka, 1996). Most primary infections are asymptomatic and people silently shed virus and after primary infection, the virus establishes latent infection in the local ganglia. It is reactivated frequently and antibody titers against HSV become detectable in serum samples (Koelle and Wald, 2000).

Many of the HSV-2 reactivations are asymptomatic and clinically not recognized (Brown *et al.*, 1995). Most HSV infections are transmitted in the absence of lesions (Bryson *et al.*, 1993). Severe generalized infections are seen particularly in neonates and immunocompromised (human immunodeficiently virus infected) patients (Oladepo *et al.*, 2000). In general, epidemiological studies indicate that adult population in the developed countries have found a relatively low seroprevalence of HSV-2, but HSV-2 based antibody in some developing countries have revealed higher rates of infection in adults (Slomka, 1996). Risk factor for acquiring HSV-2 infections is related to sexual life style, gender, race and socioeconomic status. Identification of HSV-2 infected individual is important to reduce the risk of transmission at risk groups.

Laboratory diagnosis of HSV infections is based on direct detection of the virus and/or on serological assessments (Ashley, 1993). Virus detection is useful in the diagnosis of clinical cases, but it fails to detect latent infections. The serological surveys are helpful to confirm clinical case and to identify latently infected individuals and it can be used to identify a symptomatically and subclinically HSV-2 infected ones (Cowan, 2000; Hogrefe *et al.*, 2002). Most polypeptides of HSV-1 and HSV-2 show higher degree of similarity (Eing *et al.*, 2001; Dolan *et al.*, 1998) which caused many cross reactive reactions in serological assays (Wald and Ashley, 2002). The most reliable typing of HSV antibodies is based on glycoprotein G (gG). Glycoprotein G-1 and gG-2 have similar sequences at their amino termini but, the N-terminal part of the cell- associated gG-2 is unique for type 2 and contains most of the type specific epitopes (Ashley, 2001; Grabowska *et al.*, 1999; Levi *et al.*, 1999).

Since applying of full length cloned gG-2 resulted inefficient expression of target gene, in this study truncated form of gG-2 was amplified from isolated DNA of HSV-2 infected cell culture using designed primers and sequenced after cloning into pTZ57R/T.

MATERIALS AND METHODS

Cells and viruses: Vero and HeLa cells were cultured in DMEM medium supplemented with 5% fetal bovine serum (Gibco), 0.2% sodium bicarbonate (sigma) and 100 IU mL⁻¹ penicillin.

Herpes simplex virus type 2 (HSV-2) Iranian isolates (Tehran-Iran) that were confirmed by RFLP (Tafreshi *et al.*, 2005) and other reliable tests such as HSV monoclonal, were propagated in HeLa and Vero cell lines until 80-90% of cytopathic effect (CPE) was observed. The infected cells were scraped from the bottles, rinsed with Phosphate Buffered Saline (PBS) and subjected to freeze-thawing three times, then centrifuged at 3000 rpm for 10 min and supernatant was aliquoted and kept at -80°C and used for further studies. The virus was tittered using HeLa mono layer cells on 96-well microplate and it was reported as TCID₅₀ (Lennett *et al.*, 1995).

Bacterial strains and plasmids: The *E. coli* DH5a was used as a host during the cloning experiments and for propagation of plasmids. The cloning vector pTZ57R/T (fermentase), was used for cloning truncated HSV-2 gG gene.

Viral genome extraction from cell culture: Forty milliliter of harvested supernatant were incubated with 40 µL Dnase for 1 h at 37°C. Poly Ethylene Glycol 6000 (8 V/W), 10% NaCl 5 M was added. The supernatant after was incubated over night at 4°C and centrifuged for 1 h at 4000 rpm. One milliliter of lyses buffer (Tris HCl 10 mM, EDTA 12 mM, NaCl 10 mM) and proteinase K (250 µg) was added to prepared pellet and incubated at 56°C for 2 h. Finally, viral genome was extracted with phenol-chloroform and was precipitated with cold ethanol.

The extracted genome was used as a template for amplifying desired gene in PCR amplifying system.

PCR amplification of viral target gene: The open reading frame encoding truncated gG-2 gene was a 1.1 K. bp fragment that amplified by nPCR from isolated DNA from HSV-2 infected HeLa cells using the following set of primers:

External forward primers: 5' TTT GGT GGC CTG CGT TTC 3'

External reverse primers: 5' GGC GAC CAGACA AAC GAA C 3'

Internal forward primers: 5' GGA TCC TTT ATT CGC ATG GCA CG 3'

Internal reverse primers: 5' AGG CTT TGG GAA CCA GAA CAG GGG 3'

The internal forward and reverse primers contained *Bam*H I and *Hind* III sites, respectively for subcloning.

The PCR mixture contained 10 mM MgCl₂, 100 pMol of each primers, 20 ng chromosomal DNA and Taq DNA

polymerase (Cinagene-Iran). Reactions were carried out using an ependorf thermocycler based on following program:

First round: 95°C for 5 min (1 cycle), 95°C for 1 min, 65°C for 1 min, 72°C for 1:30 min (30 cycle) and 72°C for 10 min.

Second round: 95°C for 5 min (1 cycle), 95°C for 1 min, 37°C for 1 min, 72°C for 1:30 min (4 cycle), 95°C for 1 min, 55°C for 1 min, 72°C for 1:30 min (25 cycle) and 72°C for 10 min.

PCR products were analyzed by agarose gel electrophoresis followed by ethidium bromide staining.

Cloning of PCR product: The PCR products were cloned into pTZ57R/T vector using the procedures described by the supplier (fermentase). The ligated products were then transformed into the *E. coli* DH5a. The transformants were selected on LB-Amp (50 mg mL⁻¹) plate supplemented with IPTG and X-Gal. The recombinant vector was confirmed using restriction enzyme analysis and was then subjected to Sanger xynucleoside method on an Automated DNA Sequencer (MWG Co., Germany) using universal M13 pUC forward and reverses primers.

RESULTS

A clinical isolate of HSV-2 was propagated in HeLa cells and the virus was harvested after 48 h while maximum cytopathic effect was observed and its titer determined using HeLa cell monolayers on 96-well microplate, calculated through karber formula and reported as 10^{-4.5} TCID₅₀/100 µL.

The PCR was performed using designed specific primers to obtain the gG2 gene. The PCR product was evaluated through electrophoresis on 1% agarose gel (Fig. 1).

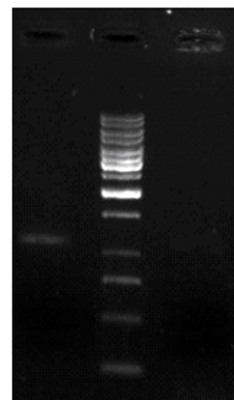


Fig. 1: Gel electrophoresis of PCR product, From left to right: PCR product of HSVgG-2, 1000 bp gene ruler, negative control

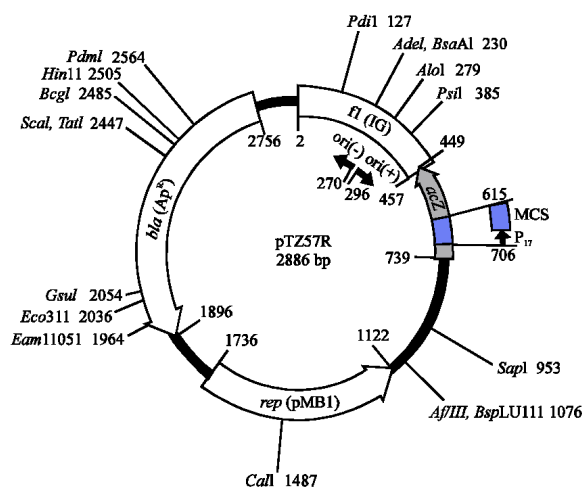


Fig. 2: A schematic diagram of the cloning vector which was used for the insertion of desired truncated pG-2

Fig. 3: Restriction enzyme analysis of pTZ57R/T-gG-2; From left to right: 1000 bp gene ruler, *Bam*HI and *Hind*III digested plasmid, linear *Bam*HI digested plasmid, respectively.

The recombinant pTZ57R/T was confirmed by restriction enzyme analysis using *Bam*HI and *Hind* III (Fig. 3) and sequencing. Analysis of sequencing was accomplished by chromas software (version 1.45-Australia) and revealed about more than 98% homology with sequences that are presented in GenBank. The nucleotide sequence data was deposited in GenBank database under the accession number: DQ864975.

Antibodies to HSV-2 are rarely detected before puberty; antibody prevalence correlated with sexual activity, age at first intercourse, number of lifetime sexual partners and history of other Sexually Transmitted Disease (STDs). The relative prevalence of HSV-2 infection varies significantly with socioeconomic and educational status. HSV antibody approaches 60% in sexually active people in lower socioeconomic classes in the united state, whereas that in middle to high socioeconomic group average 25% (Ashley, 2001).

Herpes simplex virus types 1 and 2 have approximately 83% nucleotide sequence similarity and as much as 85% amino acid sequence identity for some proteins. As the result, HSV-1 and HSV-2 show extensive serologic cross reactivity (Bruisten *et al.*, 2001). The discovery of glycoprotein G (gG) in mid-1980s seems to have resolved this difficulty, because it is antigenetically distinct between HSV-1 and HSV-2 (Ashley and Wald, 1999). The number of nucleotides in HSV-1 gG and HSV-2 gG encoding genes has been determined to be 730 and 2097, respectively (Slomka, 1996). The molecular weight of HSV-2 gG is over 104 KDa and two forms of this protein is observed on infected cell membrane (34 and 104 KDa), while this phenomena has not been reported for HSV-1 gG (Minko *et al.*, 2002). Glycoprotein G has 238 and 699 amino acids in HSV-1 and HSV-2, respectively.

At present, Food and Drug Administration (FDA) has recognized the use of recombinant gG-2 based techniques for HSV-2 serologic detection (Brown *et al.*, 1995). In addition, an article reviewing various diagnostic and serological techniques for HSV-1/2 identification has emphasized on the importance of using diagnosing and differentiating gG-2 from gG-1 specific antibodies (Palu *et al.*, 2001; Wald and Ashley, 2002).

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