Sequencing and phylogenetic analysis of *Herpes simplex virus type-2 gG* gene in Iran

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*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. Identification of glycoprotein G (gG-2) from HSV-2 as type-specific antigen have been helpful in development of reliable and accurate type-specific serological methods. For determination of the genetic relationship of HSV-2 glycoprotein G gene (gG) in Iran with those in other countries, DNA fragment of 1100 bp corresponding to gG from six HSV-2 strains have been isolated from human infected sera samples in Iran, it was amplified in PCR system and was sequenced for determining nucleotide sequence and compared with identified nucleotide sequence of this gene in other countries. The results indicated the presence of 0.3 to 9.5% variability in gG gene in six sequenced samples in Iran. A comparison made on gG gene in Iran with other countries show 0.3 to 14.3% variability in this gene. The greatest sequence similarity exists between sequences of gG in Iran and Sweden (EU018098 and EU018128) with a sequence similarity of 99.7% and the least relationship exists between sequences of gG gene of HSV-2 in Iran and USA (DQ236139) with a similarity of 85.7%.

**Key words:** *Herpes simplex virus type-2, gG gene, phylogenetic relationship, Iran.*

**INTRODUCTION**

*Herpes simplex virus type 2* (HSV-2) is the main cause of recurrent genital infection (Slomka, 1996). Most infections are asymptomatic. The virus establishes latent infection in the local ganglia and is reactivated and shed frequently. Antibodies to HSV infections become detectable in serum samples (Koelle and Wald, 2000). Because *herpes simplex virus type 1* (HSV-1), HSV-2 are genetically very similar, there is extensive antigenic cross-reactivity (Zandi et al., 2007). This cross-reactivity has resulted in difficulties in distinguishing between an infection with HSV-1 from that with HSV-2 by various serological tests. In addition, analysis of results of testing specimens containing antibodies for both viruses is difficult (Ashley et al., 1998). Identification of glycoprotein G (gG-1) from HSV-1 and glycoprotein G (gG-2) from HSV-2 as type-specific antigens has been helpful in development of reliable and accurate type-specific serological methods (Hashido et al., 1997).

Most polypeptides of HSV-1 and HSV-2 show higher degree of similarity (Dolan et al., 1998; Eing et al., 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).

Absence of a good vaccine against the HSV-2 infection has caused many problems in the world. This virus causes several diseases in human such as: eye infection, infection in epithelial and mucosal cells, genital herpes and non-infectious meningitis (Momtaz et al., 2010). During pregnancy, infection is associated with spontaneous abortion and congenital neonatal herpes (Bruisten et al., 2001). HSV-2 has also been associated with an increased risk of infection with HIV and increased disease severity (Bryson et al., 1993). HSV-1 and HSV-2
have approximately 83% nucleotide sequence similarity and as much as 85% amino acid sequence identity for some proteins. As a result, HSV-1 and HSV-2 show extensive serologic cross reactivity (Bruisten et al., 2001).

The aim of this research was to determine the genetic relationship of HSV-2 glycoprotein G gene (gG) in west south of Iran and then to compare the results with nucleotide sequences that have been registered for this gene in NCBI.

MATERIALS AND METHODS

After agreement with private and governmental clinical and pathological laboratories and clinical centers in both Isfahan and Chaharmahal Va Bakhtiari provinces in west south of Iran, 100 serum samples were collected from the suspected patients for Herpes simplex virus, with high IgG and IgM (IgG > 12 and IgM > 1.1). With the patients permission (82 samples from Isfahan province and 18 samples from Chaharmahal Va Bakhtiari province), the samples were then transferred to the Biotechnology Laboratory of Shahrekord Islamic Azad University of Iran kept in ice and stored at -20°C.

DNA extraction

DNA was extracted from serum specimens using Genomic DNA purification kit (Fermentas) according to manufacturer's recommendations.

PCR amplification of viral target gene

The open reading frame encoding truncated gG-2 gene was a 1.1 kilo base pair (Kbp) fragment that was amplified by nested polymerase chain reaction (nPCR) from isolated DNA from HSV-2 infected sera using the following set of primers. Their sequences were as those described by Jamalidoust et al. (2007): External forward primer: 5’ TTT GGT GGC CTG CGT TTC 3’; External reverse primer: 5’ GGG GAC CAG ACA AAC GAA C 3’; Internal forward primer: 5’ GGA TCC TTT ATT CGC ATG GCA CG 3’; Internal reverse primer: 5’ AGG CTG TGG GAA CCA GAA CAG GGG 3’.

For replication of mentioned gene segment, we used Mastercycler gradient PCR machine (Eppendorf, Mastercycler® 5330, Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany). The cycle conditions for the first step amplification consisted of initial denaturation at 95°C for 5 min followed by 30 cycles of 95°C for 60 s, 65°C for 60 s, 72°C for 90 s and a final extension of 72°C for 10 min. Nested PCR amplification was carried out with 5 µl of the primary PCR product as template. Both first and second round amplification reactions contained 1.5 mM MgCl2, 200 µM deoxyribonucleotide triphosphate, 1 µM of each primer, 1 U of Taq DNA polymerase in a final volume of 50 µl. The thermal conditions for the second round amplification consisted of 35 cycles of 95°C for 60 s, 56°C for 60 s, 72°C for 90 s and a final extension of 72°C for 10 min. Twenty microlitres of final PCR and nPCR products were run on a 1.5% agarose gel containing ethidium bromide in 1X TBE buffer together with 1 bp DNA ladder (Fermentas). PCR products were purified with high pure PCR product purification kit (Roche Applied Science) according to the manufacturer's recommendations. Single DNA strands were sequenced with ABI 3730 XL device and Sanger sequencing method (Macrogen, Korea).

Sequence analysis

The nucleotide sequences were edited using Edit View v.1.0.1 (Applied Bioscience, Australia) and the 7 sequences registered in GenBank (accession numbers: EU018098, EU018128, HM011373, DQ236137, DQ864975, HM011430 and DQ236139) were aligned separately using the Clustal W v1.81 in order to obtain a consensus sequence. Subsequently, the sequences were analysed using the BioEdit package v.7.0.4.1 to compare the nucleotide sequences.

The nucleotide sequence of the Iranian HSV-2 gG gene was compared with the corresponding sequences from other regions of the world. An unrooted dendrogram was constructed using the Njplot software and statistical support for the dendrogram was obtained by bootstrapping using 1000 replicates.

RESULTS AND DISCUSSION

Out of 100 samples, 26 (26%) had 1100 bp segment of HSV-2 gG gene in nested-PCR assay. The nucleotide sequences of the 1100 bp fragment of the HSV-2 gG gene from 6 Iranian isolates were compared with the sequences of the gG gene from the known reference sequences obtained from the GenBank nucleotide sequence database (7 sequences corresponding to gG). The nucleotide sequences had a variability of 0.3 to 9.5% for gG gene (Table 1) and variations consisted only of nucleotide sub-situation. Frame shift, deletion, insertion and nonsense mutations were not observed.

A comparison made on the sequences of gG between Iran and other countries showed 0.3 to 14.3% variability in HSV-2 gG. The greatest sequence similarity exists between Iranian gG sequence and EU018098-Sweden, EU018128-Sweden, with a sequence similarity of 99.7% and the least relationship between Iranian gG sequence and DQ236139-USA with a similarity of 85.7%.

Classification of Iranian HSV-2 isolates using sequence alignment analysis and construction of the phylogenetic tree of gG gene revealed that they fell into two closely related clusters that had 0.3 to 14.3% variability for gG gene (Figure 1).

The discovery of glycoprotein G (gG) in mid-1980s seems to have resolved this difficulty, because it is anti-glycoprotein G (gG) in 1980s. The molecular weight of HSV-2 gG is over 104 KD and two forms of this protein is observed on infected cell membrane (34 and 104 KD), while this phenomenon has not been reported for HSV-1 gG (Ikoma et al., 2002). Glycoprotein G has 238 and 699 amino acids in HSV-1 and HSV-2, respectively.

The present study was conducted for the first time in Iran to detect gG gene in HSV-2 strains isolated from infected patients in both Isfahan and Chaharmahal Va Bakhtiari provinces in west south of Iran to determine nucleotide sequences of this gene and to compare obtained sequences with those from other countries.
Table 1. Sequence identity matrix of partial gG gene of Iranian HSV-2 virus isolates in comparison with 7 known reference sequences.

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<th>Seq-</th>
<th>EU018098-Sweden</th>
<th>EU018128-Sweden</th>
<th>Sample 1</th>
<th>Sample 6</th>
<th>Sample 5</th>
<th>HM01137-Germany</th>
<th>Sample 3</th>
<th>DQ236137</th>
<th>DQ864975-Iran</th>
<th>Sample 4</th>
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<td>0.991</td>
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Figure 1. Dendrogram based on sequence alignment analysis of 6 Iranian isolates and 7 of the reference isolates for gG gene of HSV-2 from other regions of the world.
In this research, the fragments 1100 bp (corresponding to gG) isolated from six HSV-2 strains in Iran were amplified and sequenced in PCR system.

As shown in Figure 1, our samples, other Iranian isolates and the Sweden samples were placed in the same branch and other European samples which had more affinity with our samples in this research were placed in a near branch next to the Iranian isolates and the least relationship existed with the USA samples. This research showed high homology in sequence of gG gene of HSV-2. Then, with these results from the research samples and other species of Iran and other countries, a good vaccine can be made against the HSV-2 species in the world.

REFERENCES


