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Full Length Research Paper

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 meningitidis (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*

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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 Jamalidoost et al. (2007): External forward primer: 5′ TTT GGT GGC CTG CGT TTC 3′; External reverse primer: 5′ GGC 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 CTT 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 $MgCl_2$, 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 kp 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 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 KD and two forms of this protein is observed on infected cell membrane (34 and 104 KD), while this phenomena 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. In

Table 1. Sequence identity matrix of partial *gG* gene of Iranian *HSV-2* virus isolates in comparison with 7 known reference sequences.

Seq->	EU018098 Sweden	EU018128 Sweden	Sample 1	Sample 6	Sample 5	HM01137 Germany	Sample 3	DQ236137 USA	DQ864975 Iran	Sample 4	HM011430 Germany	DQ236139 USA	Sample 2
EU018098-Sweden	ID	0.997	0.996	0.997	0.957	0.994	0.91	0.992	0.906	0.901	0.899	0.858	0.9
EU018128-Sweden	0.997	ID	0.997	0.996	0.958	0.993	0.908	0.991	0.91	0.902	0.899	0.858	0.901
Sample- 1	0.996	0.997	ID	0.997	0.959	0.994	0.911	0.992	0.969	0.905	0.899	0.858	0.923
Sample-6	0.997	0.996	0.997	ID	0.958	0.995	0.912	0.993	0.97	0.906	0.9	0.859	0.924
Sample-5	0.957	0.958	0.959	0.958	ID	0.955	0.914	0.955	0.963	0.916	0.896	0.857	0.92
HM011373-Germany	0.994	0.993	0.994	0.995	0.955	ID	0.908	0.994	0.971	0.918	0.903	0.861	0.925
Sample-3	0.91	0.908	0.911	0.912	0.914	0.908	ID	0.915	0.917	0.956	0.905	0.972	0.918
DQ236137-USA	0.992	0.991	0.992	0.993	0.955	0.994	0.915	ID	0.969	0.925	0.904	0.862	0.923
DQ864975 Iran	0.906	0.91	0.969	0.97	0.963	0.971	0.917	0.969	ID	0.931	0.921	0.882	0.914
Sample-4	0.901	0.902	0.905	0.907	0.916	0.918	0.922	0.925	0.931	ID	0.932	0.969	0.936
HM011430-Germany	0.899	0.899	0.899	0.9	0.896	0.903	0.905	0.904	0.921	0.932	ID	0.936	0.92
DQ236139-USA	0.858	0.858	0.858	0.859	0.857	0.861	0.983	0.862	0.882	0.969	0.936	ID	0.917
Sample -2	0.9	0.901	0.923	0.924	0.92	0.925	0.918	0.923	0.914	0.936	0.92	0.971	ID

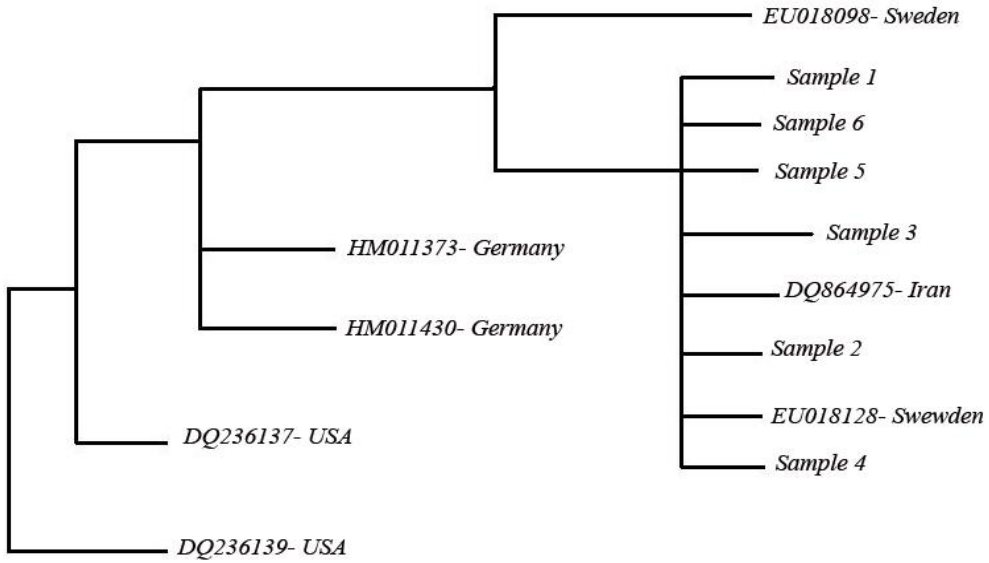

Figure 1. Dendrogram based on sequence alignment analysis of 6 Iranian isolates and 7 of the reference isolates for *gG* gene of *HSV-2* from other regions of the world.

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.

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