Dominance of Variant Human Herpesvirus 6 A Among Hemodialysis Patients, Ahvaz, Iran

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Introduction. Hemodialysis (HD) patients are a high-risk population for acquiring blood-borne viruses such as HHV-6. HHV-6 can remain latent in the host cells after primary infection; the reactivation of virus may result complications such as seizure, respiratory failure, hepatitis, and encephalitis. There is a limited report concerning HHV-6 infection in HD patients in Iran. Thus, this study was conducted to determine the frequency of HHV-6 among HD patients.
Methods. We determined HHV-6 DNA in sera samples of 84 patients undergoing HD. The DNA was extracted from the sera samples and the presence of HHV-6 DNA variants A and B was evaluated by nested PCR.
Results. 52/84 (61.9%) of HD patients were males and 32/84 (38.1%) females. The age ranges of patients were between 18 to 85 years and the mean age was 52 ± 1.52 (± SD) years. Out of 84 sera samples, HHV-6 DNA was detected in 10 (11.9%) participants, including 6/52 (11.5%) in males and 4/32 (12.5%) in females. HHV-6A was detected in 10/10 (100%) of positive cases. No HHV-6 B was found in HD patients. The distribution of HHV-6A DNA was not significant between genders ($P > .05$). Out of 84 HD patients, 55 (65.47%) cases were over 50 years, among them 10 (18.18%) cases were positive for HHV-6 A infection ($P < .05$).
Conclusion. The results showed that only HHV-6 DNA variant A was found in 11.9% of HD patients. Regarding the consequence of HHV-6 reactivation, to manage and improve treatment, the screening of HHV-6 DNA test should be implemented for HD patients.

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INTRODUCTION

Human Herpes Virus 6 (HHV-6), which belongs to the Roseolovirus genus of the ß-herpes virus subfamily, is a ubiquitous virus around the world.[1] The virus encompasses two different variants: HHV-6A and HHV-6B. Roseola infantum is a common childhood disease causes by HHV-6A and HHV-6B. Despite the fact that HHV-6A and HHV-6B infections are self-limited, complications such as seizure, otitis, respiratory and gastrointestinal symptoms, encephalitis and hepatitis have been frequently reported.[2] HHV-6A and HHV-6B are lymphotropic viruses and may also infect other cell types, including monocytes and macrophages, astrocytes, fibroblasts, and cells of endothelial or epithelial origins. HHV-6 infects various tissues

including brain, salivary glands, tonsils, lungs, kidneys, and liver.[3]

In immunocompetent hosts, HHV-6 reactivation is not usually associated with serious disease whereas, in immunosuppressed hosts, viral reactivation may lead to serious consequences, including hepatitis, idiopathic pneumonitis, bone-marrow suppression, encephalitis, fever and rash, graft versus host disease (GVHD), and delayed engraftment.[4] Both HHV-6A and HHV-6B are able to integrate their genome into the host chromosome. HHV-6 which is also associated with some malignant disorders, including Hodgkin's disease, Non-Hodgkin's lymphoma, acute lymphoblastic leukemia and carcinoma.[5] High mortality rate has been observed in organ transplant recipients when the patients are simultaneously co-infected with HHV-6, HHV-7, and human cytomegalovirus (HCMV) infection.[6] HHV-6 may involve in several autoimmune diseases such as Hashimoto thyroiditis, connective tissue diseases, multiple sclerosis (MS), diabetes and chronic fatigue syndrome.[7-10] The rout of transmission is via saliva, blood transfusion, and blood products.[11,12] Since HD patients are immunocompromised population, they may also acquire several chronic viral infections including HBV, HCV, HEV, HIV, CMV, EBV, and HHV-6 which give rise to high rates of morbidity and mortality in this group of patients.[13-18] HHV-6 reactivation may lead to post renal transplant rejection.[11,16,19,20] There are limited data on HHV-6 infection in patients undergoing HD in Iran. Therefore, the goal of this study was to investigate the frequency of HHV-6 variants A and B DNA among HD patients in Ahvaz city. Ahvaz is the capital of Khuzestan province, located at southwest region of Iran.

MATERIALS AND METHODS

Sample Collection

This cross-sectional study was conducted on 84 sera samples of HD patients, including 32 females and 52 males who referred to Golestan Hospital, Ahvaz, Iran. Written consent was taken from each participant. The Ethics Committee of the Ahvaz Jundishapur University of Medical Sciences approved this study.

Preparation of Blood Samples

Five ml of blood was taken from each HD patients. Their sera were separated by centrifugation at 3000 × g for 10 minutes and stored at -20°C before use.

DNA Extraction

DNA was extracted from sera samples by High Pure Viral Nucleic Acid kit (Roche, Diagnostics, Germany) according to the manufacturer's instructions. The kit was applied for purification of viral nucleic acids from serum, plasma, or whole blood samples. The kit product number was 11858874001, lot number; 26674100, expiration date November 2018. The extracted DNA was store -20°C prior to HHV-6 nested PCR.

Amplification of HHV-6 DNA

All samples were subjected to nested PCR for detection of HHV-6 DNA using following primers derived from the immediate-early gene locus of HHV-6A and HHV-6B.[21] The sequences of the outer primers were 5′-TTCTCCAGATGTGCCAGGGAAATCC-3′ and 5′-CATCATTGTTATCGCTTTCACTCTC-3′.[21] The sequences of the inner primers were 5′-AGTGACAGATCTGGGCGGCCCTAATAACTT-3′ and 5′-AGGTGCTGAGTGATCAG TTTCATAACCAAA-3′.[21] The reaction mixture containing, 25mM of Mgcl2, PCR buffer 10X (Roche, Germany), 200 mM dNTP, 10 pmol of primers and. Taq DNA polymerase 1U (Roche, Germany). Thermo cycler was programmed with the following conditions: The first round followed as thermal condition 94°C for 5 min following 35 cycles of 94°C for 1 min, 57°C for 1 min and 72°C for 1 min and final extension at 72 °C for 10 min. The second round was carried out using inner primers with the same conditions mentioned in the first round.

Gel Electrophoresis

The resulting amplified products were subjected to 2% agarose gel stained with SYBR Safe DNA gel stain. The bands were visualized using UV trans illuminator (Figure 1). The expected sizes of the PCR products for the HHV-6A were 195bp and for HHV-6B were 423 bp. To verify the results the PCR, product of each positive sample was sequenced.

Sequencing Analysis

Out of 10 samples positive for HHV-6 DNA, randomly 4 samples were selected and sequenced

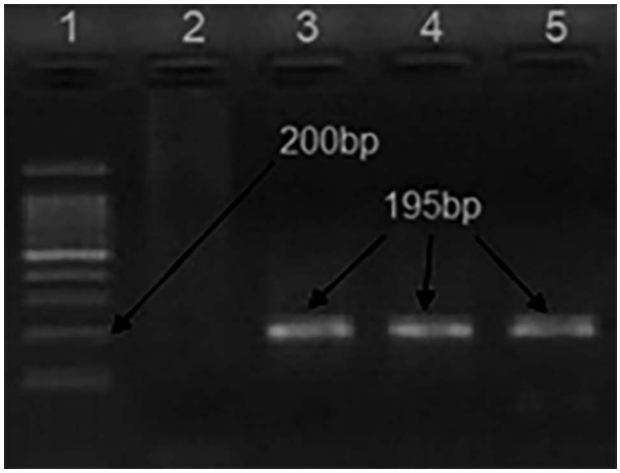

Figure 1. Photograph of Gel Electrophoresis. HHV6, variant A, (Lane 1: molecular ladder, Lane 2: negative control, Lane 3: positive control, Lane 4, 5: positive samples)

(Applied BI Bioneer Company, South Korea). The sequences of all four samples were deposited in GEN Bank. The DNA sequences of four isolates HHV-6 were aligned with the sequences of HHV-6 references retrieved from Gene Bank using online Blast (https://blast.ncbi.nlm.nih.gov).

Sequence and Phylogenetic Tree Analysis

The partial nucleotide sequences of U90 (196 nt) of the isolated HHV-6 from Ahvaz were aligned with the different HHV-6 variants isolated from different regions of the world using ClustralW method. This method is a reliable, practical and efficient tool for multiple sequence alignment and is used in the phylogenetic tree.[22] The tree topology was built according to the maximum-likelihood under the tamura-Nei substitution model with the site heterogeneity gamma and invariant sites using MEGA 6. The accuracy of tree was assessed by 1000 bootstrap replicates. The scale bars represent the frequency of nucleotide substitutions (Figure 2).

Statistical Analysis

Data were statistically defined in terms of median and range or mean, standard deviation (± SD), and frequencies. The data were analyzed with SPSS 16 package program (SPSS Inc., Chicago, IL,

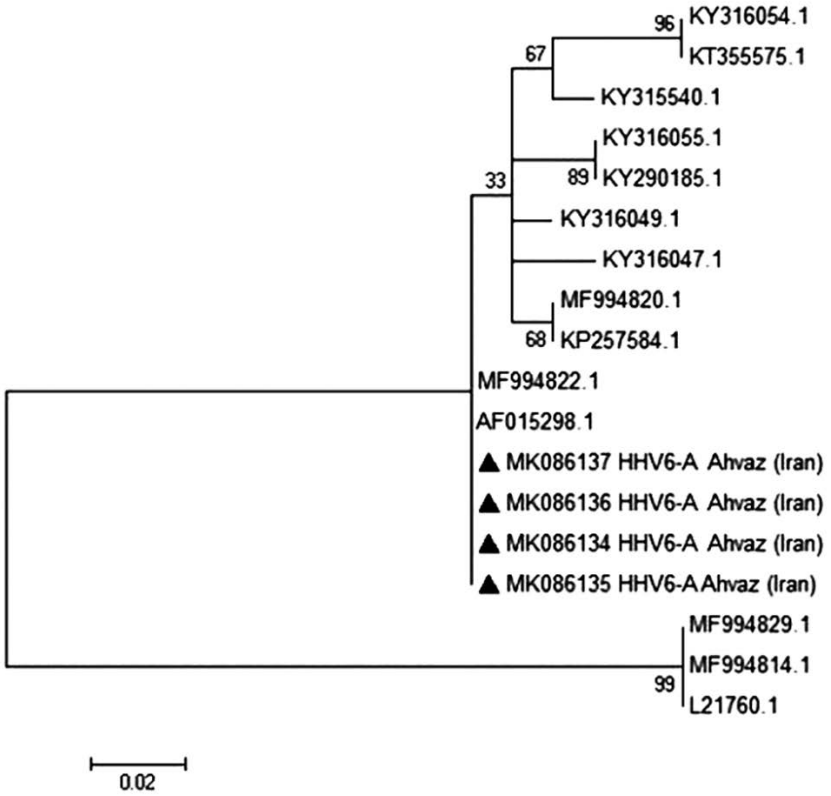

Figure 2. A Phylogenic tree was constructed with maximum- likelihood method using the partial sequences of U90 region of HHV-6A, reference sequences were retrieved from GenBank with their Accession Numbers .The results of phylogenetic tree show the isolated Iranian HHV-6A with triangle black with accession numbers (MK086134 to MK086137) are cluster with HHV-6A accession number MF994822.1 isolated from USA . The accuracy was assessed by 1000 bootstrap replicates (Scale bar = 0.02).

USA) and Fisher's exact test was used to analyze the significance of differences in the HHV-6 DNA detection between the two genders. A P value < .05 was considered statistically significant.

RESULTS

Of eighty-four HD patients 52 (61.9%) were males and 32 (38.1%) were females. The patients'age range varied from 18 to 85 years with mean age of 52 ± 1.52 (± SD) years. The mean age of males and females were 51 ± 1.57 and 53 ± 1.47, respectively.

10/84 (11.9%) participants, including 6/52 (11.5%) males and 4/32 (12.5%) females, were positive for HHV-6 DNA. The sequences of four positive HHV6A samples were registered in GenBank with accession numbers, MK086134 to MK086137. The blast results of 4 sequencing samples showed 100% nucleotide identity with HHV-6A strains isolated from Uganda (accession numbers X83413.2, AF015298.1) and 97% nucleotide Identity with HHV-6A isolated from USA (accession no: MF994822.1 and KJ123690.1). No HHV-6 variant B was found in HD patients. The frequency of HHV-6A among genders was not significant ($P > .05$). Out of 84 HD patients, 55 (65.47%) cases were > 50 years old, among them 10 (18.18%) cases were positive for HHV-6 A infection ($P < .05$). All HD patients had multiple blood transfusions history.

DISCUSSION

Viral infections are considered as the major causes of morbidity and mortality in post organ transplant recipients. The consequences of viral infections have impact on host immune response and may result in acute and chronic allograft rejection. The rejections of kidney, liver and lung transplants were reported among post organ transplant recipients after HHV-6 infection.[23,24] There is sufficient and compelling evidence that HHV-6 reactivation may associate with worse consequences, which possibly result in encephalitis and multiple sclerosis.[9,25,26]

The frequency of HHV-6 A or B in HD patients is limited in Iran. Our study clearly indicated that 10/84 (11.9%) patients; including 6/52 (11.5%) males and 4/32 (12.5%) females, were only positive for HHV-6 DNA variant A. In the present survey, the frequency of HHV-6 variant A among genders was not significant ($P > .05$). Javid *et al.* (Iran) reported that the frequency of HHV-6 DNA was 8/149 (5.37%) among HD patients[19] which was consistent with our results. While Tarhan *et al.* (Turkey) have detected a high frequency of HHV-6 DNA variant B 7/25 (28%) among HD patients.[11] Lempinen *et al.* (Finland) described HHV-6-B viral antigens was detected in 28% of patients with chronic kidney disease undergoing dialysis.[27] Csoma *et al.* (Hungary) investigated HHV-6 DNA in 200 renal transplant recipients; they described the distribution of HHV-6 variant A (8/9) to be significantly higher than variant B (1/9) among organ recipients. The result was in agreement with our findings.[25] Kiani *et al.* delineated (Ahvaz) that the frequency of HHV6-A and HHV6-B among patients with Hodgkin lymphoma was 45.45% and 9.09%, respectively; and the distributions of HHV6-A and HHV6-B in patients with Non-Hodgkin lymphoma were 36.36% and 0%, respectively.[5] Thus, these results indicate that HHV-6A is predominant in this region. Yalcin *et al.* (Turkey) have only detected HHV-6 variants B (35%) in 16 patients with renal transplantation.[28]

Immunosuppressive drugs are used in transplant patients to avoid graft rejection and significantly decrease the rejection rates in these patients. The reactivation of latent HHV-6 was observed in post-transplant recipients receiving immunosuppressive drugs and resulted in serious threat to the immunosuppressed graft recipient.[29,30] The antiviral drugs, such as ganciclovir, foscarnet, or cidofovir are most effective against HHV-6 reactivation and reduce the degree of immunosuppression.[31,32] Notably, HHV-6 drug resistance has been reported during treatment of CMV infection.[33]

Our study has some limitations, which should be pointed out. To understand whether HD patients are in asymptomatic or latency phase, the detection of UL-94 gene needs to confirm the latency phase. Secondly, the diagnosis of HHV-6 DNA was carried out by nested PCR, which is slightly less sensitive than real-time PCR methods.

CONCLUSION

In conclusion, in the present study, a relatively high prevalence of HHV-6 variant A (11.9%), a predominant rate in this region, was detected among HD patients. No HHV-6 variant B was observed. The distributions of HHV-6 variant A among the males and females were not significant. The detection of HHV-6 UL-94 gene needs to evaluate status of viral latency. To improve HHV-6 treatment, the screening of HHV-6 DNA should

be implemented for HD patients. Furthermore, to assess asymptomatic or reactivation of virus the application of Real time PCR will be more efficient.

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CONFLICT OF INTEREST

All authors declare no conflict of interest.

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