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Prevalence of Human Herpes Virus 6 (HHV-6) in Patients with Hemodialysis

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Abstract

HHV-6 is the causative agent of exanthema subitum in children. The main modes of transmission of HHV-6 are body secretions such as infected saliva but it can be transmitted with blood and blood products that are infected with the virus. Therefore, it is thought that the immunocompromised hemodialysis patients who had multiple blood transfusions due to various reasons, and who collectively use devices such as hemodialysis machines are at risk for HHV-6 infection. The present study aimed to determine the incidence of HHV-6 infection in hemodialysis patients. Twenty-five healthy individuals that were matched with 25 hemodialysis patients in terms of gender and age were included in the study. The IgM and IgG sero-prevalence in the patient and control groups were investigated with the indirect fluorescence antibody method and HHV-6 DNA prevalence and the determination of variant types (variant A and variant B) were investigated with the PCR-RFLP molecular method. HHV-6 IgG and HHV-6 IgM antibody positivity ratios in the patient and control groups were 76% and 20% respectively. Out of 50 serum samples of hemodialysis patients and healthy individuals, HHV-6 DNA was positive in seven of 25 samples of hemodialysis patients (28%) and it was positive in eight of 25 samples of the control group (32%). Variant analysis was performed with the PCR-RFLP method in HHV-6 DNA positive hemodialysis patients and control patients. At the end of the analysis, while variant A was not detected in the patient and control groups, variant B was detected in a total of 15 individuals, including seven patients in the hemodialysis patient group and eight patients in the control group. In conclusion, the researchers believe that there is a need for controlled studies including more samples to determine the clinical importance of HHV-6 DNA and HHV-6 variants in hemodialysis patients.

Keywords: Human Herpes virus- 6 (HHV-6); Variant A; Variant B; Prevalence; Hemodialysis patients

Introduction

Human Herpes Virus 6 (HHV-6) is a virus which especially causes infection in $CD4^+$ T lymphocytes and ranks among the herpes group that is widely seen all over the world. It is reported that more than half of the adults in developed countries and 80-100% of the population in developing countries is infected with this virus. HHV-6 infection is generally asymptomatic in healthy individuals. While it may cause infectious mononucleosis-like syndrome and chronic fatigue syndrome in adults, it is the causative agent of sixth disease (exanthema subitum) in children [1,2]. As it can stay in the latent phase, reactivation may cause severe diseases with primary or secondary infections in immunocompromised patients. The virus especially causes infection in $CD4^+$ T lymphocytes and stays in the latent phase in the lymphoid tissue. The main modes of transmission of HHV-6 are body secretions such as infected saliva but it can be transmitted with blood and blood products that are infected with the virus. Therefore, it is thought that immunocompromised hemodialysis patients who have multiple blood transfusions due to various reasons and who collectively use devices like hemodialysis machines are in the risk group for HHV-6 infection [3]. HHV-6 isolates are divided into two groups as variant A and variant B in terms of their biological, immunological, and molecular characteristics. *In vitro* cell tropism of these variants, their effects on expression of T cell markers, their reactions with monoclonal antibodies, their restriction endonuclease profiles, their nucleotide sequences, sero-epidemiologies and their association with the diseases are different from each other. Although it is known that HHV-6 B is the causative agent of exanthema subitum and is commonly active in immunocompromised individuals, the precise relation of HHV-6 with any disease has not been defined thus far [4,5].

The present study aimed to determine the HHV-6 IgM and IgG sero-prevalence with the indirect fluorescence antibody (IFA) method and HHV-6 DNA prevalence and determination of variant species (variant A and variant B) with the PCR-RFLP molecular method in hemodialysis patients.

Materials and Methods

Ethics Statement: This study was approved by Fırat University, Faculty of Medicine, Ethics Committee to collect patient sample and reviewed by Refik Saydam National Public Health Agency reviewer board for laboratory study. Written informed consent was obtained from all patients.

Patients: As part of the study, 25 adult (25-60 years) patients undergoing hemodialysis in the Hemodialysis Unit of Fırat University Medical Faculty due to chronic renal failure and 25 healthy individuals that were matched in terms of age and gender as the control group were included to the study.

Preparation of blood samples: Five ml of blood without anticoagulant was taken from peripheral veins of the individuals in the patient and the control groups. After holding the blood at room temperature for 1-2 hours, they were separated into serums by centrifugation for 10 minutes at 3000 × g. The serum samples that were to be used for the PCR test were extracted and purified using DNA extraction and purification kits (Epicentre Technologies, Madison, Wisconsin). After the purification process, the purity control was performed on each sample by the spectrophotometric method. Each sample was kept at -20◦C until the study day. The serum samples that were to be used for serological examination by the IFA method were stored at -20◦C.

Reproduction of the target gene region with polymerase chain reaction (PCR): For amplification of HHV-6 DNA, a sequence of 830 base pair of LTP (large tegument protein) region was chosen as the target region on viral genome [4]. The region is protected among the HHV-6 species that demonstrate genetic variability, and does not react with other herpes viruses. For this purpose, the A: 5' - GAT CCG ACG CCT ACA AAC AC - 3' and C: 5' - CGG TGT CAC ACA GCA TGA ACT CTC - 3' primer sequences were used. The PCR mixture was prepared by combining apyrogenic water, PCR buffer (1X), PCR enhancer (1X), Mg Cl2 (2.5 mM), dNTP mixture (200 µM), each primer (20 p mol/µl), Taq DNA polymerase (5 U/µl), 5 µl of sample DNA that will be tested, to produce a final reaction mixture to be 50µl for each sample. For amplification, the tubes were stored at 94◦C for 3 minutes for initial denaturation, producing 40 cycles (denaturation at 94◦C for 45 sec., annealing at 62◦C for 45 sec., extension at 72◦C for 1.15 sec.), at final extension 72◦C for 4 minutes (Eppendorf Thermal Cycler, USA Scientific, Inc.) [6].

Examination of PCR reproduction products with electrophoresis: PCR products were placed on agarose gel of 1.5%. After this phase, the gel was kept in ethidium bromide solution (0.5 mg/ml), which was previously prepared, for 20 minutes and stained; the bands that were seen under an ultraviolent light source were evaluated. In all studies, negative controls were used. The samples demonstrating reproduction products under 830 bp magnification were taken to restriction enzyme analysis for typing (Figure I).

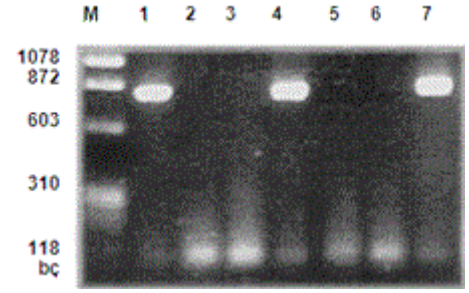

Figure 1: Amplification of HHV-6 DNA in clinical samples.

M- Marker φ X174 Hae III MV

1-Positive control

2-Negative control

3, 5, 6- HHV-6 DNA (-) samples

4, 7- HHV-6 DNA (+) samples

Restriction fragment length polymorphism (RFLP): Restriction endonuclease enzyme analysis was performed to type the visible bands of the samples with positive PCR reaction results on agarose gel. The amplification product of each patient was cut with Hind III, Hinf I and Taq I enzymes. The prepared reaction mixture was incubated at 37◦C for one night for cutting with Hind III and HinfI enzymes and at 65◦C for cutting with the Taq I enzyme. Two percent agarose gel was prepared for the cutting products. Ten µl of cut samples and 5 µl of uncut samples of the same patient were loaded together with the loading tamponade to the wells on the gel. It was run at 110 volts for 40 minutes. After staining with ethidium bromide, the gel was examined with a UV-transilluminator [4-7] (Figure II).

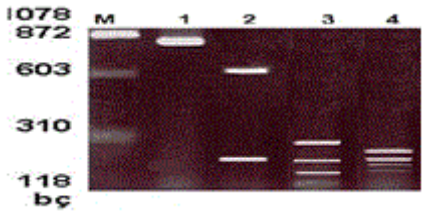

Figure 2: Cut of PCR positive samples with Hind III, Hinf I and Taq I enzyme.

M- Marker φX174 Hae III MV

1- HHV-6 DNA positive control

2-Amplified product that is cut with Hind III enzyme

3- Amplified product that is cut with Hinf I enzyme

4-Amplified product that is cut with Taq I enzyme

Indirect Immunofluorescence Antibody Test (IFAT): The HHV-6 specific Ig G and Ig M antibodies were investigated by using HHV-6 IgG (V3 HHV6) and HHV-6 IgM (V17 HHV6) IFA kits obtained from the Biotrin company. In all study phases of the test, the principles and the standards defined in the kit procedure were obeyed. Upon evaluation of the images with a fluorescence microscope under 200-500× magnification in a dark room, the degree of density of the fluorescence reaction was used as a base. The cells with green-yellow fluorescence on a black ground were accepted as (+), other cells were accepted as negative. According to the degree of the fluorescence reaction (+), cells were graded as very bright ++++, bright +++, intermediately bright ++, and weak + (Figure III, IV).

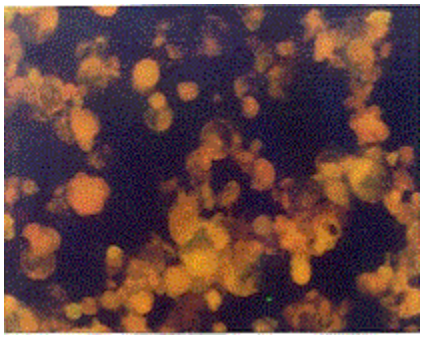

Figure 3: The negative appearance of HHV-6 antibodies with IFA.

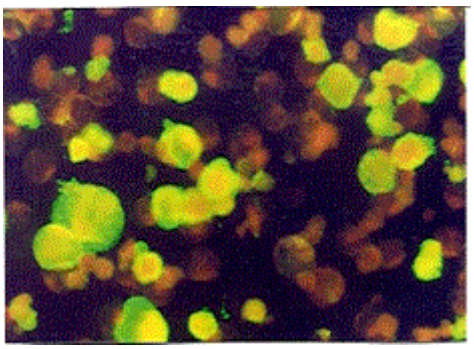

Figure 4: The positive appearance (++++) of HHV-6 antibodies with IFA.

Statistical analysis: All statistical analyses were performed with the SPSS, version 14.0. Upon analysis of the data, Fisher's exact chi-square tests were used. A P value of <0.05 was considered significant.

Results

IFAT results: HHV-6 IgG and HHV-6 IgM antibody positivity ratios in the patient and control groups were found to be 76% and 20% respectively. The results are presented in Table I.

Groups	HHV-6 IgG	HHV-6 IgM
	Positivity rate (%)	Positivity rate (%)
Hemodialysis patients (n: 25)	19/25 (76)	7/25 (28)
Healthy controls (n: 25)	19/25 (76)	3/25 (12)
Total (n: 50)	38/50 (76)	10/50(20)

Table 1: Ratios of HHV-6 IgG and IgM antibody sero-positivity in patient and healthy control groups

When the patient and the control groups were statistically compared, no significant difference was found between the groups in terms of HHV-6 IgG antibody positivity. Furthermore, no statistically significant difference was found between the groups in terms of HHV-6 IgM antibody positivity (p=0.29). HHV-6 IgM sero-positivity ratio in hemodialysis patients did not differ from the healthy control group.

PCR - RFLP results: In the study, HHV-6 DNA was examined with PCR in 50 serum samples of hemodialysis patients and healthy individuals. HHV-6 DNA was positive in 15 samples and negative in 35 out of a total of 50 serum samples. When the HHV-6 DNA positivity ratio was evaluated according to the study groups, it was positive in seven of 25 samples of hemodialysis patients (28%) and it was positive in eight of 25 samples of the control group (32%).The results are presented in Table II.

Groups	HHV-6 DNA	HHV-6 DNA
	Positivity rate (%)	Negativity rate (%)
Hemodialysis patients (n:25)	7(14)	18(36)
Healthy controls (n: 25)	8 (16)	17(34)
Total (n:50)	15(30)	35(70)

Table 2: HHV-6 DNA positivity ratios in hemodialysis patients and healthy individuals with PCR method.

The positive (+) or negative (-) evaluation of the samples that were amplified with the PCR method in terms of HHV-6 DNA was performed with agarose gel electrophoresis. In agarose gel electrophoresis, the presence of a band in a region of 830 bp was evaluated as PCR (+) and absence of a band in 830 bp was evaluated as PCR (-) (Figure III). For the Hind III enzyme, the isolates that did not show any cut region (830 bp) were accepted as variant A, the isolates that were separated into fragments of 610 and 220 bp were accepted as variant B. For the Hinf I enzyme, the isolates that were separated into fragments of 530, 110, 100, and 90 bp were accepted as variant A and the isolates that were separated into fragments of 300, 200, 150, 100, and 90 bp were accepted as variant B. For the Taq I enzyme, the isolates that were separated into fragments of 630 and 200bp were accepted as variant A, the isolates that were separated into fragments of 270, 200,180 and 160 bp were accepted as variant B (28) (Figure IV). The variant distribution in healthy individuals and hemodialysis patients after cut of positive PCR samples with the Hind III enzyme is demonstrated in Table III.

Groups	**Variant A**	**Variant B**
	Positive (%)	Positive (%)
Hemodialysis patients (n:25)	0/7 (0)	7/7 (100)
Healthy controls (n:25)	0/8 (0)	8/8 (100)
Total (n:50)	0/15 (0)	15/15(100)

Table 3: HHV-6 variant distribution in hemodialysis patients and healthy individuals

While variant B is detected at a rate of 100% in all study groups, no variant a type was observed as a result of RFLP analysis.

Discussion

Although generally asymptomatic, the HHV-6 infection can vary depending on the socio-economic nature of the countries and the age of the patient. The first serological marker in HHV-6 infected

individuals is anti-HHV-6 IgM positivity and it can be determined in blood within 5-6 days after the appearance of clinical signs. After reaching the maximum level within the first 2-3 weeks, it generally disappears within 2 months and reappears as a result of reactivation. Two weeks after the beginning of the infection, HHV-6 IgG becomes positive and remains life-long positive. After the development of the antibody in individuals with HHV-6, the virus remains in the latent phase in tissues and polymorphonuclear leucocytes in blood. HHV-6 IgM antibodies are not specific markers of primary infection; rather they might become positive in the reactivation of the latent virus and in reinfection states [8]. Therefore, the individuals whose immune system is compromised due to various reasons are at risk of HHV-6 infection. In recent years, this topic has been examined in detail, especially in the recipients of kidney, bone marrow, and liver transplantation, and HIV infections. In the studies about recipients of kidney transplantation conducted in different centers, Okuno et al. detected a high rate of increase in HHV-6 antibody titers at a rate of 38% in individuals who had allograft rejection following transplantation [9]. On the other hand, Yoshikawa et al. demonstrated that there was an increase in HHV-6 antibody titers at a rate of 55% within the first three months following transplantation in the recipients of kidney transplantation [10]. In the current study, similar results with the control group were obtained in terms of HHV-6 IgG antibody positivity rates in hemodialysis patients who are candidates for renal transplantation. Additionally, while the HHV-6 IgM antibody was 12% in the control group, it was 28% in the hemodialysis patients. As the results in the present study were obtained qualitatively by using the IFA technique, the comparison of the serum HHV-6 IgG antibody titers of the patient and the control groups could not be made. There was no statistically significant difference was found between groups in terms of HHV-6 IgM antibody positivity. However, the slightly higher rates of HHV-6 IgM sero-positivity in the patient group when compared to the control group suggested that there might be a development of primary infection during multiple blood transfusions or reactivation of the primary infection due to immune suppression in these patients. In a study conducted by Altay et al. on 35 hemodialysis patients, 36 peritoneal dialysis patients, and 20 healthy volunteers, the rates of HHV-6 IgM positivity were 25.7% (9/35), 22.2% (8/35), and 10% (2/10), respectively. HHV-6 IgG sero-positivity was significantly higher in hemodialysis patients at a rate of 20% compared to the peritoneal dialysis patients (5.6%) and the control group (0%) [11].

In the present study, a correlation was found between HHV-IgG sero-positivity and duration of hemodialysis in hemodialysis patients. Upon the examination of presented study groups by the PCR-RFLP method, HHV-6 DNA positivity in both hemodialysis patients and the control group were at a lower rate than the other studies. This might result from the fact that the nested PCR method is more sensitive than the method that is used in the current study [12].In the present study, PCR and the IgM positivity as a marker of acute infection at a rate (28%) confirming each other in the hemodialysis patients could be interpreted as acute infection and higher rates of PCR positivity (32%) and lower rates of IgM positivity (12%) in the control group when compared with the hemodialysis patients could be interpreted as a marker of latent infection. There are two different variants of HHV-6. These variants are HHV variant A and variant B [13]. The primary infection is seen mostly due to variant B, however it may develop due to variant A. HHV-6 can be seen in transplantation patients as contamination of the donor, reactivation of the latent infection or reinfection. The most common HHV-6 variant isolated in blood samples of renal transplant patients is variant B, however variant A is more virulent [13-15]. Upon molecular typing of HHV-6 DNA positive samples by RFLP test, the variant B type is found at a rate of 100% in all samples and variant B type was not observed; whereas Yalçın et al. reported HHV-6 DNA positivity at a rate of 63% in renal transplant recipients [12]. Upon type differentiation conducted by the RFLP method they detected that 70% were variant B and 30% were variant A. Similarly to other studies, the current findings demonstrate that variant B is more frequent than variant A. Although HHV-6 variant B reactivation is frequently reported in renal transplant patients, in a study conducted by Csoma et al. in 200 patients who had renal transplantation, they found that HHV-6 variant A viremia is dominant, contrary to previously reported results [13].In the current study, the HHV-6 variant A in patients with active infection was significantly higher than the patients with latent infection. HHV-6 reactivation is common in the immunocompromised patient group, especially in renal transplant patients. Lempinen et al. investigated the presence of HHV-6 and CMV with immunohistochemical methods in gastroduodenal and colon biopsy samples of 81 renal transplantation patients and 46 chronic dialysis patients [16].In this study, the HHV-6 variant B positive cell ratio in gastroduodenal biopsy samples was reported as 34% and 28% in renal transplant patients and dialysis patients, respectively. CMV positivity ratio in the same tissue samples were 53% and 28% in renal transplant patients and hemodialysis patients, respectively. In the same study, while HHV-6B positive cell ratio in colon mucosa samples was 36% in renal transplant patients, it was 22% in hemodialysis patients. CMV positivity ratios were 36% and 17% in renal transplant patients and hemodialysis patients, respectively.In this study, HHV-6 variant A positivity was not reported. Both HHV-6 variant B and CMV positive cells were found at the same time in 50 patients who had renal transplantation. In the current study, detected HHV-6B positivity in serum samples at a rate of 28% (7/25) in hemodialysis patients is similar to the study of Lempinen et al. [16]. The HHV-6 variant A was not detected in either the patient group or the control group. As a result, the researchers believe that there is a need for controlled studies including more samples to determine the clinical importance of HHV-6 infection and HHV-6 variants in hemodialysis patients who are immunocompromised hosts, require frequent blood transfusions, and are candidates for renal transplantation.

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