Journal of Acute Disease

Original article

doi: 10.4103/2221-6189.272857 jadweb.org

Prevalence of human herpesvirus 8 infection in patients undergoing hemodialysis using nested-PCR

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ARTICLE INFO

Article history:
Received 18 July 2019
Revision 1 November 2019
Accepted 9 November 2019
Available online 16 December 2019

Keywords:
Human herpesvirus 8
Hemodialysis patients
Nested-PCR

ABSTRACT

Objective: To study the prevalence of HHV-8 infection in patients undergoing hemodialysis.
Methods: In this study, blood samples of 89 patients undergoing hemodialysis were collected. DNA was extracted from peripheral blood mononuclear cells and HHV-8 DNA was evaluated by nested-PCR.
Results: Of total 89 patients, 51 (57.3%) were males and 38 (42.7%) were females. The patients' age ranged from 24 to 90 years and the mean age was (57.5±1.4) years. HHV-8 DNA was found in 9 of 89 (10.1%) peripheral blood mononuclear cell samples, 8/51 (15.7%) in males and 1/38 (2.6%) in females (P=0.07). All patients who were positive for HHV8-DNA were more than 50 years old.
Conclusions: This study shows high prevalence of HHV-8. Since hemodialysis patients are candidates for kidney transplantation and due to the possibility of HHV8-reactivation and its serious complications in immunocompromised patients, routine screening for detection of the virus should be implemented for all hemodialysis patients.

1. Introduction

Human herpesvirus 8 (HHV-8), also known as Kaposi's sarcoma-associated herpesvirus, is one of the oncogenic viruses. HHV-8 is a member of the γ-herpesvirinae subfamily which belongs to the herpesviridae family. In recent years, HHV-8 has attracted more attention because it is linked to the development of several lymphoproliferative disorders including Kaposi's sarcoma (KS), multicentric Castleman's disease, plasmablastic lymphoma and primary effusion lymphoma[1,2]. It is also observed that the virus may be associated with squamous cell carcinoma of the conjunctiva. The mechanism by which HHV-8 may contribute to tumorigenesis is not completely understood. However, the virus may play an important role in cancer development through encoding several homologues of human proteins, such as viral interferon regulatory factors 1-4, viral G protein-coupled receptor, viral interleukin 6, and B-cell lymphoma 2 which are able to regulate programmed cell death pathways, resulting in suppress or promote apoptosis[3]. Like all herpesviruses, HHV-8 has two distinct phases of infection, known as lytic and latent. Both lytic and latent infections may play a direct role in cancer development[4]. During latent infection, some viral genes are expressed which helps the virus escape from the

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How to cite this article: Shokri S, Mahmoudvand S, Makvandi M, Shahani T, Samadi A, Shahbazian H. Prevalence of human herpesvirus 8 infection in patients undergoing hemodialysis using nested-PCR . J Acute Dis 2019; 8(6): 250-254.

host's immune system resulting in persistent viral infection. Thus, the immune system has a crucial role in determining the clinical consequences of diseases which is associated with Kaposi's sarcoma-associated herpesvirus. Since immunosuppression is considered to be a predisposing factor for KS development, HHV-8 has remarkable clinical significance for immunosuppressive subjects[5]. The definitive pathway for transmission of HHV-8 has not yet been completely determined, but so far various patterns of HHV-8 transmission have been reported. Sexual transmission is an important route of transmission, and other patterns of transmission include saliva, blood transfusion and organ transplantation[6,7]. Transmission by blood transfusion is a risk factor for HHV-8 infection. Since hemodialysis (HD) patients require frequent blood transfusions, they are prone to HHV-8 acquisition. On the other hand, HHV-8 DNA was reported to be present in peripheral blood mononuclear cells (PBMCs)[8,9]. Thus, screening of PBMCs can help to detect the virus among these patients.

Considering the association of HHV-8 with the development of several lymphoproliferative disorders and its increasing prevalence, the present study aimed to evaluate the prevalence of HHV-8 infection in PBMCs isolated from the blood of HD patients who are at higher risk for acquiring of HHV-8 infection.

2. Materials and methods

2.1. Participants

This was a cross-sectional study that assessed HHV-8 DNA in blood samples of 89 HD patients, including 38 females and 51 males, who referred to Golestan Hospital, Ahvaz, Iran. Verbal or written informed consent was obtained from all the subjects. This study was approved by the Ethics Committee of the Ahvaz Jundishapur University of Medical Sciences (No: IR.AJUMS.REC.1397.882), and this study was performed with registration number 97s41.

2.2. Eligibility criteria

Inclusion criteria include patients residing in Ahvaz city who undergoing hemodialysis and patients who have the mental capacity to provide written, informed consent. Exclusion criteria include patients who did not agree to follow the study.

2.3. Sample collection and processing

2.3.1. Preparation of PBMCs

Totally 6 mL of blood was collected from each patient in sterile tubes containing ethylenediaminetetraacetic acid and then PBMCs were separated from the whole blood by a standard procedure using Ficoll density gradient centrifugation (Bahar Afshan CO., Tehran, Iran). The process of PBMC isolation was as follows: First, blood samples diluted with phosphate-buffered saline (PBS) (1:1) and then the diluted blood was layered on top of the Ficoll. After centrifugation at 400 *g* for 30 min at 18 ℃, PBMCs were isolated. Then, the cells were washed three times in PBS by centrifugation at 400 *g* for 10 min at 18 ℃, and the pellets were re-suspended in 200 μL PBS. Until DNA extraction, the cells were stored at -20 ℃.

2.3.2. DNA extraction

DNA was extracted from PBMC samples using High Pure Nucleic Acid Kit (Roche Applied Science, Germany) according to the manufacturer's instructions. The extracted DNA was stored at -20 ℃ till used. Then, Thermo Scientific™ NanoDrop™ One Microvolume UV-Vis spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA) was used for assessing the concentration and the purity of all extracted DNA samples.

2.4. Nested-PCR for detection of HHV-8 DNA

All samples were subjected to nested-PCR for detection of HHV-8 DNA using following primers derived from the Open Reading Frame 26. The sequences of the outer primers were 5'-AGCCGAAAGGATTCCACCAT-3' and 5'-TCCGTGTTGTCTACGTCCAG-3'. The sequences of the inner primers were 5'-TTCCACCATTGTGCTCGAAT-3' and 5'-TACGT CCAGACGATATGTGC-3'[10]. The outer primers and inner primers are expected to produce a 233 bp and a 211 bp fragments, respectively. The reaction mixture contained 1.5 mM MgCl2, PCR buffer 10 (Roche, Germany), 200 mM each dNTP (Roche, Germany), 10 pmol of each primer and 1 U of Taq DNA polymerase (Roche, Germany). Thermocycler was programmed with the following conditions: The first round was started with an initial denaturation at 94 ℃ for 5 min, followed by 30 cycles of 94 ℃ for 45 s, 58 ℃ for 30 s and 72 ℃ for 30 s and final extension at 72 ℃ for 5 min. All conditions including reaction mixture and thermal cycling conditions were the same as the first-round amplification. The PCR products were analyzed by electrophoresis in 2% agarose gels stained with safe stain (CinnaGen, Iran).

2.5. Statistical analysis

In order to assess the detection rate of HHV-8 DNA among the gender and age, Fisher's exact test was used to calculate *P*-value. The collected data was analyzed by SPSS 16 Package software for statistical analysis (SPSS Inc., Chicago, IL, USA). *P*-value less than 5% was considered to be statistically significant.

3. Results

The current study was conducted on 89 HD patients, and HHV-8 DNA was found in 9 of 89 (10.1%) PBMCs samples. The demographic characteristics of the study subjects are shown in Table 1. Although, in the present study, HHV-8 DNA positivity was more prevalent in males than females, statistical analyses showed

Table 1. Distribution of HHV-8 DNA between males and females.

Genders	Mean age (years)	Number [*n* (%)]	HHV-8 DNA positive [*n* (%)]
Male	57.6±1.53	51 (57.3%)	8 (15.7%)
Female	57.4±1.14	38 (42.7%)	1 (2.6%)

that there is no significant difference in the frequency of HHV-8 positive subjects regarding genders (P=0.07) (Table 1). All HD patients had multiple blood transfusion history. Out of 89 patients, 67 (75.3%) cases were older than 50 years old. All patients who were positive for HHV8-DNA were older than 50 years old. No significant differences were observed in the detection rate of HHV-8 DNA between subjects >50 and <50 years old (P=0.1).

4. Discussion

HHV-8 is an oncogenic virus infecting mostly B-cells and it contributes to the development of several malignancies. There is an association between HHV-8 and the development of KS and post-transplant lymphoproliferative disorders in immunocompromised subjects such as transplanted and HD patients[11]. In HD patients, HHV-8 may be reactivated during states of relative immune dysregulation leading to worse consequences. During the hemodialysis process, the blood of subjects who had kidneys dysfunction, especially patients in end-stage renal failure, is purified and renal transplantation is considered an alternative treatment for these patients[12]. Further, HD patients require frequent blood transfusions and some studies showed that blood transfusion is a risk factor for HHV-8 acquisition/transmission[13]. As a result, the patients are at higher risk for reactivation and acquiring HHV-8 infection.

The seroprevalence of HHV-8 varies between different countries and geographic. Low seroprevalence has been reported in North America and Northern Europe. Intermediate seroprevalence was found in Asia (up to 24%) and higher rates of HHV-8 seropositivity was found in the Middle East, Mediterranean (up to 35%) and sub-Saharan Africa (up to 50%)[14]. In the general population, KS is responsible for only 0.02% to 0.07% of all cancers[15]. The risk of post-transplantation KS is 23 to 28 percent in HHV-8-seropositive patients before kidney transplantation[16]. The incidence of KS in renal transplant recipients was 0.7%, 1.7% and 3.9% in Taiwan, Greece, and South Africa, respectively[17-19]. In Iran, KS is one of the most common cancers followed by renal transplantation, and its incidence varied from 0.45% to 2.4%[15].

There is limited information on the prevalence of HHV-8 among HD patients in Iran. Taken together, the present study showed that the prevalence of HHV-8 DNA is high in HD patients (10.11%). Several investigations were conducted on HHV-8 prevalence in HD patients with variable results. For instance, in a study performed by Gharehbaghian *et al.* in Iran, HHV8 antibody has been reported to be 16.9% (20 of 118) in HD patients[20]. In a study performed by El-Glil *et al.* in Egypt, HHV-8 DNA was found in 21.7% (13 of 60) and 3.3% (1 of 30) of the HD patients and the control group, respectively[21]. In a study conducted by Caterino-de-Araujo *et al.* in Brazil, the prevalence of HHV-8 antibody was 22.9% (16 of 70) in HD patients[22]. In study performed by Al-Otaibi *et al.* in Saudi Arabia, the prevalence rate of anti-HHV-8-IgG was 16.7% (12 of 72) among HD patients[23]. In the study conducted in Taiwan, HHV-8 antibody was detected in 19.5% of HD patients and 3% of healthy blood donors[24]. A study conducted in Italy showed HHV-8 antibody in 9.3% (9 of 97) of serum samples from HD patients[25]. In Greece, HHV-8 antibody was detected in 7.2% (35 of 485) of HD patients[26].

Differences in the prevalence of HHV-8 in various studies may be due to the different types of populations, geographical region, age, gender, genetic risk factors, sensitivity and specificity of the diagnosis tests. Further, situations such as HIV infection can raise the prevalence of HHV-8 infection.

The serological tests are widely used for the diagnosis of HHV-8 infection. Besides, detection of HHV-8 by PCR is a more reasonable method to detect the virus[27]. Serology tests shows lower sensitivity and poorer accuracy than PCR test, which explains why serology is not used in clinical practice. The determination of the quantity of HHV-8 viral load by real-time PCR in clinical samples is useful for understanding disease progression and risk of subsequent KS[28].

The peak incidence of this virus has been reported at the ages of 50-79 years[29]. HHV-8 diseases may appear in older patients or occasionally during immunosuppressive therapy. The present survey revealed that HHV-8 is highly prevalent in patients over 50 years (P=0.1). Our results are consistent with those of other studies which reported that the detection rate of HHV-8 infection was higher in older subjects compared to the younger subjects[21,30-32]. This can be due to poor immune responses in elderly subjects. In fact, changes in adaptive and innate immune responses in older individuals may contribute to the development of infection[33].

In various studies, the association between HHV-8 seropositivity and gender remains controversial[34-37]. In the present study, the rate of HHV-8 infection was higher in men (15.7%) than women (2.6%), but this difference was statistically insignificant (P=0.07). Our findings indicated that men may be more susceptible to HHV-8 infection than women patients. It is consistent with the results of two studies conducted by Begré *et al.* and Biryahwaho *et al.* which showed that HHV-8 infection is more prevalent in men than women[34,35]. No significant difference was found by Cattani *et al.* in the rates of HHV-8 in men and women[36]. However, the results of our study are in contrast with those of Sheldon *et al.* who reported a higher prevalence in females (12.9%) than males (4%)[37].

Studies have shown that ganciclovir, foscarnet, and cidofovir have activity against HHV-8 infection[38]. Currently, there is no licensed vaccine to prevent HHV-8 infection. Several clinical trials for a vaccine were conducted in the world. Thus, preventive measures for the spread of HHV-8 and screening tests to reduce the further complications of infection are needed.

In conclusion, in this study HHV8 DNA was detected in 10.11% of HD patients. Considering HHV8 reactivation-associated serious complications in these patients, further studies with sensitive methods are needed to detect the virus. Since HD patients are candidates for kidney transplantation, routinely screening for detection of HHV-8 should be done for all HD patients.

Conflict of interest statement

The authors report no conflict of interest.

Acknowledgments

This study was financially supported by Student Research Committee, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Authors' contribution

S.S. drafted the manuscript; S.M. designed the study, performed experimental, and edited the final version of the manuscript; M.M. suggested the research idea and corrected the manuscript; T.S. acquisition of data; A.S. analysis and interpretation of data; H.S. preparation of samples.

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