



No Evidence of Human Herpesvirus 8 among Iranian Patients Infected with HIV

*Amitis RAMEZANI¹, Elham SABOORI², Kayhan AZADMANESH³, Minoo MOHRAZ⁴, Monireh KAZEMIMANESH³, Afsaneh KARAMI⁵, Mohammad BANIFAZL⁶, Hanieh GOLCHEHREGAN⁴, *Arezo AGHAKHANI¹*

1. Dept. of Clinical Research, Pasteur Institute of Iran, Tehran, Iran
2. Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran
3. Dept. of Virology, Pasteur Institute of Iran, Tehran, Iran
4. Iranian Research Center for HIV/AIDS, Tehran, Iran
5. Zanzan University of Medical Sciences, Zanzan, Iran
6. Iranian Society for Support of Patients with Infectious Disease, Tehran, Iran

*Corresponding Author: Email: araghakhani@hotmail.com

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Abstract

Background: Kaposi's sarcoma is a vascular malignancy, which frequently occurs among immunocompromised individuals such as transplant recipients and patients with acquired immunodeficiency syndrome. Human herpesvirus 8 (HHV-8) is considered the etiological agent of all forms of Kaposi's sarcoma. Though some seroepidemiological studies conducted on the prevalence of HHV-8 in Iran, there are insufficient data on the prevalence of HHV-8 viremia in HIV infected patients. We therefore, aimed to determine the prevalence of HHV-8 viremia in general population and HIV infected patients without Kaposi's sarcoma in Tehran, Iran.

Methods: We conducted a cross sectional survey on 99 patients with HIV infection referred to Iranian Research Center for HIV/AIDS and 40 healthy controls in Tehran, Iran from January to April 2014. The presence of HHV-8 DNA was detected in buffy coat samples of enrolled subjects using nested PCR assay.

Results: A total of 99 HIV infected patients with mean age of 37.9 ± 10 yr and 40 healthy controls with mean age of 39 ± 11.5 yr were enrolled in the study. The mean CD4 count was 410.3 ± 211.4 cells/mm³. HHV-8 DNA was not detected in both healthy control and HIV patient groups.

Conclusion: This survey showed low rate of HHV-8 DNA in healthy controls and HIV patients. Considering our findings HHV-8 infection does not seem to be widespread in our population. Further studies focusing on different regions of Iran appear to be required to have a more accurate estimation.

Keywords: Human immunodeficiency virus (HIV), Human herpesvirus 8 (HHV-8), Prevalence, Viremia, Iran

Introduction

Human herpesvirus 8 (HHV-8) is a Rhadinovirus of the subfamily Gammaherpesvirinae, and belonged to Herpesviridae family (1). The association between HHV-8 and AIDS-related Kaposi's sarcoma has been clearly identified (1). Moreover HHV-8 consistently related to lymphoprolifera-

tive diseases, like primary effusion lymphoma and multicentric Castelman's disease (2- 4).

HHV-8 distribution is very variable and exhibits both geographical and behavioral risk factors as serological investigations reported high seroprevalence rate of 48% in blood donors of Tanzania

(5,6) and low prevalence of 2.8% to 7.3% in the United States blood donors (7,8).

The incidence rates of Kaposi's sarcoma (KS) consider to be increased in regions with a high prevalence rate of HHV-8. Immunosuppressed cases, such as patients with end-stage renal disease, solid organ transplant recipients, and HIV patients, are at risk of acquiring HHV-8 associated diseases (9, 10). Additionally HIV infection significantly increases the risk of HIV-KS development and worsens its natural course (11, 12). It is considered that 40% of HIV patients may develop cancer and KS is the most common neoplasm seen in patients infected with HIV (3, 13). Although with the introduction of highly active antiretroviral treatment (HAART) and its influence on reconstitution of immune system, KS incidence has decreased, the emergence of HIV drug resistant isolates grows the concern for a reemergence of cases with KS. In HIV patients, presence of HHV-8 viremia is predictive for future development of KS and used for monitoring of response to treatment (14).

While few Iranian studies showed high seroprevalence rate of HHV-8 among hemodialysis patients (16.9%), renal transplant recipients (25%) and HIV patients (45.7%) (15, 16), there are insufficient data on the prevalence of HHV-8 viremia in Iranian population. We therefore, aimed to determine the prevalence of HHV-8 viremia in general population and HIV infected patients without KS in Tehran.

Methods

Study population

In this cross-sectional study, 99 patients with HIV infection referred to Iranian Research Center for HIV/AIDS in Tehran, and 40 healthy controls were consecutively enrolled in the study from Jan to Apr 2014.

The study protocol was approved by Pasteur Institute of Iran Ethical Committee and informed consent was obtained from subjects prior to their enrollment.

Whole blood sample from each subject was collected in an EDTA-containing sterile tube and centrifuged at room temperature at 1500 x g for 15 min to separate leukocyte band (buffy coat), plasma and concentrated red blood cells (RBCs). After centrifugation, buffy coat was aspirated with a sampler and collected in a sterile tube. For lysing of erythrocytes, one volume of blood was mixed with two volumes of 0.83% NH₄Cl solution and erythrocytes were lysed. The leukocytes were collected after centrifugation at 2500 x g for 10 min and two additional washes with PBS. The peripheral blood leukocytes (PBL) were resuspended in 500µl of PBS solution and stored at -20 °C until testing.

CD4 count was determined by flowcytometry and defined as cells/mm³.

DNA extraction and Nested PCR

Viral DNA was extracted from 200 µl of PBL using Invisorb®Spin Tissue Mini kit (Invitek, Berlin, Germany) and Invisorb® Spin Blood Mini Kit following the manufacturer's instructions. The extracted DNA was stored at -20 °C prior to PCR analysis.

The suitability of extracted DNA was confirmed by β-globin gene amplification using PCO3 (5'-ACACAACCTGTGTTCACTAGC -3') and PCO4 (5'-CAACTTCATCCACGTTCCACC -3') primers that amplify a 102-bp fragment. PCR was performed with a 25µl amplicon mixture containing 1µl extracted DNA, 15 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM MgCl₂, 200 µM dNTP, 200 µM each of primers, 1X PCR Buffer and 1 U Taq polymerase (YTA PCR Master Mix, Iran).

The detection of HHV-8 DNA was performed by nested PCR using primers specific for the ORF 26 primers of HHV-8, described previously (17) including primers KS1 (5'-AGCCGAAAGGATTCCACCATTCC -3') and KS2(5'- GTGTTGTCTACGTCCAG -3') for first PCR reaction and primers KS3 (5'-TATTCTGCAGCAGCTGTTGG -3') and KS4 (5'- TCTACGTCCAGACGATATGTGC -3') for second PCR reaction.

Briefly, a 5µl sample of DNA extract was added to 20µl of a same PCR mixture (above) with pri-

mer KS1 and KS2, which amplify a 233 bp fragment. The second reaction utilized the same mixture (above), but with KS3 and KS4 primers and 5 μ l of first-round PCR product. The expected size of the PCR products was 138 bp. For both first and second steps, dsDNA was initially denatured for 5 min at 95 °C, followed by 35 cycles of 95 °C for 40 sec, 59 °C for 40 sec and 72 °C for 40 sec and the final extension step at 72 °C for 5 min. Each batch included negative controls (distilled water) and positive samples from previous study (20) as positive control.

The PCR products were electrophoresed on a 1.5% agarose gel with the 100-bp DNA ladder (Sinaclon, Tehran, Iran) and stained with ethidium bromide and visualized by ultraviolet transillumination.

Statistical analysis

Statistical analyses were conducted using SPSS statistics software (version 16, Chicago, IL, USA). The Chi square test or Fisher's exact test was used to compare variables. Data are presented as mean \pm SD or, when indicated, as an absolute number and percentage. *P*-values <0.05 were considered statistically significant.

Results

A total of 99 HIV infected patients with mean age of 37.9 \pm 10 yr and 40 healthy controls with mean age of 39 \pm 11.5 yr were enrolled in the study.

In HIV positive group, 71.7% were male. The mean CD4 count was 410.3 \pm 211.4 cells/mm³. The reported routes of HIV transmission were intravenous drug use (54.6%), heterosexual contact (34.3%), infected blood and blood products transfusion (2%), vertical transmission (2%), homosexual (1%) and in 6.1% the route of HIV acquisition was not identified. 76.3% of cases were under HAART treatment.

HHV-8 DNA was detected in none of buffy-coat samples in both healthy control and HIV patient groups (Fig. 1).

Discussion

This study investigated the prevalence of HHV-8 viremia in HIV infected patients without KS and healthy controls in Tehran. We showed no evidence of HHV-8 viremia in general population and HIV patients. In the present study, lack of HHV-8 detection corroborates findings of prior studies conducted in low prevalence areas for HHV-8.

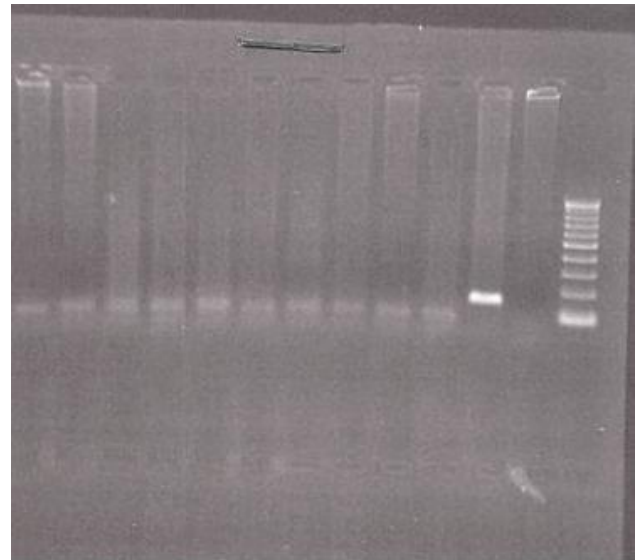


Fig. 1: Agarose gel electrophoresis of products obtained by nested PCR. Lanes from right to left: DNA size marker, negative control, positive control and samples

HHV-8 could be reactivated in immunocompromised situations such as patients with end-stage renal disease, transplant recipients, and HIV patients and it is associated with Kaposi's sarcoma and some hematological diseases (2, 9, 10).

The seroprevalence of HHV-8 is very variable and there is an association between HHV-8 antibodies and the incidence of KS. High prevalence of HHV-8 infection found in areas where Kaposi's sarcoma is endemic, for instance more than 50% in the general population of Central and Eastern Africa (5). While low prevalence of infection detected in the general population of the United States (<5%) and Northern Europe (<10%; except among homosexual men with AIDS) with low incidence of KS (18). In South-

ern Italy where classic KS is relatively frequent, intermediate prevalence rates of HHV-8 infection (10%-25%) have been reported (19, 20).

Several investigations were conducted on HHV-8 frequency in blood donors and normal population with variable results. For instance, in Italy (21) showed HHV-8 DNA in 9% of PBMC samples from blood donors. This rate was reported as 16.5% (22) in PBMCs of HHV-8 seropositive healthy adults. In Brazil, HHV-8 antibody was detected in 4% of blood donors while only one case showed HHV-8 DNA in PBMC and plasma samples (23). In contrast, several investigations from North America could not find HHV-8 viremia in PBMCs of healthy donors by PCR assays (8, 24, 25). The negative PCR results in the general population detected in present study are in concordance with the studies from low prevalence areas such as the USA (8), where all tested healthy blood donors were HHV-8 DNA negative.

Prior surveys mainly focused on the seroprevalence of HHV-8 in healthy blood donors and data regarding the HHV-8 viremia in HIV infected patients without KS are scarce. Situation such as HIV infection can raise the prevalence of HHV-8 especially in developing countries (26). This issue is important because virus detection in PBMCs of HIV infected individuals can predict future development of KS lesions (14).

In a study (27), 8.9% of HIV infected patients in Taiwan showed HHV-8 DNA in plasma. Moreover, they observed, higher prevalence of plasma HHV-8 DNA among men and patients younger than 40 yr old. In Japan (28), from, HHV-8 viremia evaluated in HIV infected patients with or without KS using real-time PCR. HHV-8 DNA was detected in 29.6% of individuals and prevalence and load of HHV-8 DNA were higher in patients with KS than patients without it (not significant). HHV-8 viremia was found in PBMCs of 50%-60% of patients with both types of classic and HIV-associated KS (29). In India, none of 309 treatment-naïve HIV cases and 70 normal healthy subjects tested was positive for HHV-8 DNA (30).

Although, published reports have shown different rate of HHV-8 virus replication among patients from different countries, the low HHV-8 replication detected in this paper could be in agreement with Sachithanandham et al. (30) findings from India on HIV patients and compatible with low seroprevalence of this infection in Iranian healthy blood donors (0-2%) (15, 31). However, we should consider that HAART reduce HHV-8 DNA loads like HIV-RNA and 76.3% of our cases were under HAART treatment. The reduction of HIV replication with HAART results to restoring of immune system and producing effective immune response against HHV-8. Moreover, Kaposi's sarcoma is rare in Iran and annual age-standardized incidence of KS varying from 0.06 to 0.08 per 100,000 in females to 0.10 to 0.17 per 100,000 in males (32). Therefore, regarding to the low prevalence of HHV-8 in blood donors and low incidence of KS in Iran, the lack of HHV-8 detection in our cohort of HIV patients is not unexpected and may indicated that virus is not widespread in this population.

Conclusion

This study investigated the prevalence of HHV-8 viremia in HIV infected patients without KS and normal population in Iran. We reported no evidence of HHV-8 viremia in general population and HIV patients. Therefore, Iran may be considered as a region with low endemicity for HHV-8, due to low seroprevalence of HHV-8 (1.6%) in blood donors and no evidence of HHV-8 DNA in normal population and HIV patients. However, further epidemiological studies focusing on different regions of Iran appear to be required to reach a more accurate estimation.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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