

Full Length Research Paper

Prevalence of human herpesvirus 8 infection in Iranian patients with hematological malignancies

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Human herpesvirus 8 (HHV-8) is a gamma-herpesvirus linked causally to Kaposi's sarcoma and many malignancies such as multicentric Castleman's disease, primary effusion lymphoma, some lymphoproliferative diseases and post transplantation bone marrow failure. The aim of this study was to determine the prevalence of HHV-8 infection in patients with malignant hematological diseases. From September, 2009 to April, 2010, 62 patients with hematological diseases were recruited for the study. Five milliliter of ethylenediaminetetraacetic acid (EDTA) anti-coagulated peripheral blood was collected from each subject. The presence of HHV-8 DNA was tested using a real time technology for the sequences from HHV-8 ORF65. The mean age of patients was 33.9 ± 18.0 years. Four of the patients were found to be HHV-8 polymerase chain reaction (PCR) positive using Real time-PCR and viral prevalence was 6.5%. HHV-8 was found in 1 (25%) patient with acute myelogenous leukemia (AML), 3 (75%) patients with chronic myelogenous leukemia (CML), and none was detected in patients with acute lymphoblastic leukemia (ALL) and lymphoma. The results of this study show that patients with malignant hematological diseases may have HHV-8 infection, therefore, it seems that considering HHV-8 infection in patients with hematological malignancies might be beneficial.

Key words: Human herpesvirus 8 (HHV-8), malignant hematological diseases, prevalence, Iran.

INTRODUCTION

Human herpesvirus 8 (HHV-8), also known as Kaposi's sarcoma-associated herpesvirus (KSHV), was initially identified in AIDS-associated Kaposi's sarcoma (KS) in 1994 (Chang et al., 1994). Human herpesvirus 8 has also been implicated in a number of hematological malignancies such as primary effusion lymphoma (Cesarman et al., 1995), multicentric Castleman's disease (Soulier et al., 1995), B cell lymphoma (DeLoose et al., 2005), germotrophic B cell plasmablast lymphoproliferative disorder (Du et al., 2002) and post transplantation bone marrow failure (Luppi et al., 2000) or lymphoproliferative disorders (Cesarman et al., 1999). Unlike most other human herpes viruses, KSHV infections presumably begin with a

primary infection of susceptible hosts; following this, latency is established, from which intermittent reactivation of replication is possible (Knipe et al., 2007). The global prevalence of HHV-8 infection can be categorized into three major patterns of prevalence: high (>50%) in sub-Saharan Africa and parts of Amazon basin, intermediate (between 5 and 20%) in Mediterranean, Middle East Countries, Caribbean, and low (up to 5%) in Northern and Western Europe and in the United States (Knipe et al., 2007).

Kaposi's sarcoma-associated herpesvirus may be transmitted by sexual intercourse, saliva, solid organ transplantation (Regamey et al., 1998) and blood transfusion

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(Parisi et al., 2007). Patients with hematological malignancies are usually associated with multiple transfusions and immunocompromised state. Whether they are at a higher risk of HHV8, infection has not been determined. In Iran HHV-8 have been studied in hemodialysis, renal transplant recipients and HIV patients diagnosed clinically with Kaposi's sarcoma (Somayeh et al., 2011), but have not been studied in patients with malignant hematological diseases. We conducted this study to determine the prevalence of HHV8 infection among 62 patients with hematological malignancies.

MATERIALS AND METHODS

Study population

In this cross-sectional study, 62 consecutive patients with established malignant hematological diseases who were referred from September, 2009 to April, 2010 to oncology Unit of Hazrate Rasol Hospital, Tehran, Iran, were used for this study. All of the patients received chemotherapy for their malignancy

Collection and preparation of samples

About 5 ml of peripheral blood of each subject were collected into ethylenediaminetetraacetic acid (EDTA)-containing vacutainer tubes. Blood buffy coat were separated from EDTA-treated blood by centrifugation and stored at -70°C until analysis. Informed consents were obtained from all of the subjects, which conform to the guidelines of the 1975 Declaration of Helsinki. EDTA anticoagulated peripheral blood specimens from 20 consecutive blood donors as well as genomic DNA from HHV8 harboring cells were used as negative and positive controls, respectively.

HHV-8 DNA detection in blood buffy coat samples by real time-polymerase chain reaction (PCR)

Detection of HHV-8 DNA in blood buffy coat specimens was performed by Real Time-polymerase chain reaction (Real Time - PCR) method. Briefly, DNA was extracted from 200 µl blood buffy coat sample using the High Pure Extraction Kit (Roche Diagnostics GmbH, Mannheim, Germany) in accordance with the instructions provided by the manufacturer and subjected to PCR with a Light Cycler system with the Light Cycler Fast Start DNA Master Hybridization probes kit (Roche Applied Science) and Taqman probes for HHV-8. The primer set for the HHV-8 ORF65 was HHV8-F: CCTCTGGTCCCCATTCATTG and HHV8-R: CGTTTCCGTCGTGGATGAG and the probe for HHV-8 ORF65 was HHV8-P: FAM-CCGGCGTCAGACATTCTCACAACC-TAMRA (Sugita et al., 2008). The thermal cycler profile is optimized and validated with Heat activation (15 min at 95°C) of hot-start; Taq polymerase was followed by 40 cycles of denaturation (30 s at 95°C), annealing (30 s at 50°C), and extension (30 s at 72°C).

Statistical analysis

All data were analyzed using SPSS software version 11.0 (SPSS Incorporated, Chicago, IL, USA). The analyses were carried out using descriptive statistical indexes including standard deviation, mean, confidence interval at 95%, and *t* test. *P* < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Sixty-two (62) patients with established malignant hematological diseases were recruited in this study. The mean age of patients was 33.9 ± 18.0 years. Of the 62 patients, 45 (72.6%) were male. According to the type of hematological malignancy, 27 (43.5%) with acute myelogenous leukemia (AML), 22 (35.5%) with chronic myelogenous leukemia (CML), 3 (4.8%) with lymphoma, and 10 (16.1%) with acute lymphoblastic leukemia (ALL) consist our study population. The Molecular method of Real time-PCR that amplifies sequences from the ORF65 provided a viral prevalence of 6.5%.

Human herpesvirus type 8 is an unusual herpesvirus because it encodes a huge number oncoproteins or cell signaling proteins. There is a large body of evidence linking HHV-8 to at least three malignancies, Kaposi's sarcoma, multicentric Castleman's disease, and primary effusion lymphomas (Mikala et al., 1999). HHV-8 infections presumably begin with a primary infection of susceptible hosts; following this, latency is established (mostly in B cells), from which intermittent reactivation of replication is possible. The information and understanding of HHV-8 prevalence in different population and patients groups is crucial because it may be useful in establishing prophylactic measures to decrease rates of viral transmission from infected individuals.

Most HHV-8 epidemiological studies have been based on antiviral antibodies detection but detection of HHV-8 genome in the specimen of patients is more reliable than that used in the present study and demonstrate that the prevalence of HHV-8 infection in patients with malignant hematological diseases in Iran is 6.5% (Table 1).

In Iran, HHV-8 has not been studied in patients with malignant hematological diseases and most studies found HHV-8 seroprevalence. In Iranian population a noticeable higher seroprevalence of HHV8 has been reported in hemodialysis (16.9%), renal transplant recipients (25%) and HIV (45.7%) patients compared to blood donors (2%) (Jalilvand et al., 2011a). Einollahi et al found Kaposi's sarcoma is the most common malignancy after renal transplantation in Iran (Eyn et al., 2001). Jalilvand et al. (2011b) found that the HHV-8 variants among classic Iranian Kaposi's sarcoma are largely related to Eurasian genotypes previously identified in Kaposi's sarcoma from Mediterranean, Middle East, and East Asian regions.

Lin et al. (2008) study showed that the prevalence of plasma HHV-8 DNA was 10.6% for HIV infection through sexual contact and 7.1% for HIV infection through intravenous injection. There are several reports in world which most of all determined viral prevalence in patients with lymphocytic (B cell) disorders and a small number of studies demonstrated information about lymphocytic and myelogenous malignancies. Chen et al. (2005) reported the prevalence of HHV-8 DNA in peripheral blood

Table1. Demographic characteristic and prevalence of HHV-8 infection among Iranian patients with Hematological Malignancies.

Hematological malignancy	Mean age	Male/female	Patient No	Age (years)	HHV-8 PCR positive (%)
Acute Myelogenous Leukemia (AML)	29.2 ± 12.0	19/8	15	<30	0
			11	30 - 60	0
			1	61 - 90	1
Chronic Myelogenous Leukemia (CML)	50.5 ± 14.4	17/5	1	<30	0
			16	30 - 60	0
			5	61 - 90	3
Lymphoma	27.3 ± 2.5	2/1	3	<30	0
			-	30 - 60	0
			-	61 - 90	0
Acute Lymphoblastic Leukemia (ALL)	12.3 ± 6.2	7/3	10	<30	0
			-	30 - 60	0
			-	61 - 90	0
Total	33.9 ± 18.0	45/17	62	-	4 (6.5)

Table 2. Demographic characteristics of patients positive to HHV-8.

Case	PCR result	Syndrome	Duration of blood transfusion in month	Age/Gender
1	+	AML	2	68/M
2	+	CML	2	83/F
3	+	CML	2	76/M
4	+	CML	1	65/M

mononuclear cells (PBMCs) in Taiwanese leukemia populations 10.29%. Tattevin et al. (2002) reported a HHV-8 infected patient with concomitant chronic lymphocytic lymphoma and multiple myeloma. Vey et al. (2001) in France report a HHV-8-infected patient with chronic myelogenous leukemia. Wang et al. (2001) in a study for the determination of prevalence of HHV-8 DNA in acute myelogenous leukemia (AML) found HHV-8 DNA sequences in one case.

In the present study, difference was seen between patients with and without HHV-8 infection but this difference was not statistically significant ($p < 0.04$). Therefore, HHV-8 infection may be as a result of transfusion with contaminated blood or blood products. The present study suggests that serious consideration must be given to prevent this infection via transfusion in hematological malignant patients, and assessing HHV-8 serology assay of the patients, before any transfusion, is a useful tool to get information about patient HHV-8 status.

Hudnall et al. (2003) study showed that there is a significant association between seropositivity of HHV-8 and older age. Interestingly in the present study, a significant difference was seen between HHV-8 DNA

positive results and older age ($p < 0.01$), although increasing HHV-8 positive results (seroprevalence or/and DNA) with age is consistent lifelong persistence of antibody against this virus as seen with other herpesvirus infections and with life-long susceptibility to this infection (Table 2).

In conclusion, the results of this study suggest that patients with malignant hematological diseases may have HHV-8 infection. Therefore, the possibility of HHV-8 infection should be considered in patients who suffer from hematological malignancies.

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Abbreviation: HHV-8, Human herpesvirus 8; EDTA, ethylenediaminetetraacetic acid; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; ALL, acute lymphoblastic leukemia.

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