

RESEARCH ARTICLE

Molecular Detection of Human Herpes Virus Type 8 among HIV Patients in Khartoum State Sudan

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Abstract

Introduction: Human Herpes Virus- 8 (HHV-8) also known as Kaposi sarcoma –associated herpes virus, is a gamma herpes virus associated with the development of Kaposi's sarcoma, and Body-Cavity-Based-Lymphoma.

Objectives: The aim of the present study was to detect the presence of HHV-8 genome among HIV patient in Khartoum State, Sudan during the period September to November 2016.

Methods: The study was carried out in Omdurman Teaching Hospital, Khartoum Teaching hospital and Bashaer Hospital, Khartoum State, Sudan. A total of (70) HIV positive (30 males and 40 females) cases with no symptoms of cancer development were included. HHV-8 was detected using real time PCR.

Results: Among 70 HIV positive samples, 3 samples (4.2%) were found positive for HHV-8 using real time PCR.

Conclusion: Our findings indicated low prevalence of HHV-8 DNA among the study group. In addition, our study reveals the need for further investigations in different parts of the country to determine the extent of the problem.

Keywords: Real-Time PCR; Sudan; HHV8; HIV

Introduction

Human herpes virus- 8 (HHV-8) also known as Kaposi sarcoma-associated herpes virus (KSHV) is a linear double stranded DNA virus [1,2]. HHV-8 is a gamma herpes virus associated with the development of Kaposi's sarcoma, Body-Cavity-Based-Lymphoma (BCL), and some forms of Multicentric Castleman's Disease (MCD) [3-5].

KS is an angio-proliferative disease that is particularly frequent and aggressive in patients with AIDS. It commonly presents as multifocal disease, frequently in the upper body, head and neck, with a rapid course regarding both local progression of lesions to tumors and visceral dissemination, leading to organ dysfunction and high mortality [6]. The most common sites for visceral involvement by KS are the lungs (37%), gastrointestinal tract (50%) and lymph nodes (50%) [7].

Four major clinical forms of KS have been described: 1- AIDS-associated KS, an aggressive form of KS that occurs mainly in homosexual and bisexual men, 2- classic KS affects typically elderly men in Mediterranean area, 3- endemic KS, affecting children and young men in central Africa and 4- iatrogenic KS, observed in some patients under immunosuppressive therapy [8-10].

Since HHV-8 has not been isolated in cell cultures, HHV-8 infection is identified by means of either serological methods or molecular biology assays. Several qualitative and quantitative amplification techniques for HHV-8 detection in different biological samples have been developed [11,12].

Application of novel molecular techniques revealed the strong association of HHV-8 with AIDS- related KS [1]. Some investigators extended these findings to a search for HHV-8 DNA sequences in other tissues from KS patients, as well as in KS lesions from patients without HIV infection. They found that HHV-8 was not solely an opportunistic infection in patients with AIDS but that the other forms of KS seen in patients without HIV infection were caused by the same infectious agent [13].

Sero-epidemiological studies demonstrated a low prevalence of anti-HHV-8 antibodies in the general population in the United States and Asia (1 to 2%), an intermediate seroprevalence in Italy and other Mediterranean countries (5 to 35%), and the highest seroprevalence was found in Southern and Central Africa (30 to 60%) and among Brazilian Amerindians (41 to 65%) [14].

Although, the prevalence of HHV-8 in the general population may be low in some areas, however many studies have suggested that there is a close association between HHV-8 and KS [15-17].

Materials and Methods

Study Area

The study was conducted in Khartoum State of Central Sudan..., Patients involving HIV positive, at HIV treatment center during period September to November 2016.

Data Collection

Demographic data of the patients were collected using a structured questionnaire, which included the following criteria: Age, gender, and place of sample collection. From each patient, 5 ml of blood in EDTA was collected from the cubital vein then centrifuged at 4000 r.p.m to obtain the plasma. The clear plasma was taken immediately for analysis or stored at -20 °C until tested.

DNA Extraction

Commercial DNA extraction kits (analytikjena, Germany) were used to extract DNA of HHV-8 from the plasma samples according to procedure described by the manufacturer.

Real time Polymerase Chain Reaction

The primers were as designed by to amplify a 68-bp region of the ORF 65 gene of HHV-8 [14]. The primer sequences were HHV8-F: CCTCTGGTCCCCATTCATTG and HHV8-R: CGTTCCGTCGTGGATGAG, the sequence of the probe was FAM-CCGGCGTCAGACATCTCACAACC-TAMRA. The Master Mix was Innu MIX qMasterMix Probe (analytikajena_German). The reaction mixture content was 10µ of master mix 0.5µl of probe, 2µl of primer, 2.5µl of H₂O and 5µl of DNA (Total volume of Mix is 20µl). The PCR reaction was carried out as described by with slight modification [14]. Briefly, after 2 min of incubation at 50 °C, and 5sec of denaturation at 95 °C the PCR mixture was subjected to 45 cycles of 95 °C for 20 sec and 60 °C for 1 min. The intensities of the fluorescent dyes in each reaction were read automatically during PCR cycling in Topical thermo cycler (analytikajena, Germany).

Ethical Approval

The study has been approved by the local ethics committee of Alneelain University. All participants or their guardians in the study were given a written informed consent considering the aims of the study, Sample and clinical information's were used anonymously.

Result

HHV-8 DNA was detected in 3/70 (4.2%) of the samples tested. In terms of gender; 1 (33.3%) was a male while 2 (66.7 %) were females (Table 1). Based on age group, the 3 patients positive for HHV-8 were only found in the age group 37–62 years old, none was found in the younger 9-36 age group (Table 2).

	Real time PCR		
	Positive	Negative	Total of Samples
Male	1 (3.3%)* (33.3%)**	29 (43.3%)	30 (42.9%)
Female	2 (5%)* (66.7%)	38 (56.7%)	40 (57.1%)
Total	3 (100%)	67 (100%)	70 (100%)

*Out of the total no of respective gender (30 males & 40 females)

**Out of total no of positive HHV8 samples (n=3)

Table 1: Frequency of the HHV-8 according to gender

Age group	Number of patients with HHV-8 (%)		
	Positive	Negative	Total of Samples
9-36 year	0 (0%)	24 (100%)	24
37-62year	3 (6.5%)	43 (93.5)	46
Total	3	47	70

Table 2: Frequency of the HHV-8 according to age

Discussion

Immuno-suppression and co-infection with oncogenic viruses substantially increase the risk of cancers in HIV-infected patients such as cervical cancer, non-Hodgkin's lymphoma and (KS) [18].

Kaposi sarcoma is the most common tumor in HIV-infected individuals in Africa and is preceded by infection with (KSHV) [19].

In the present study seventy samples were collected from HIV positive patients and a low rate (4.2%) of HHV-8 infection was detected by using RT- PCR. Similar results were recorded in Southeast Asia in which, the virus was detected in 4.4% of the cases [20]. However, higher prevalence rates were reported from Johannesburg, South Africa where 139 out of 404 (48%) proved to be positive [21].

In our study HHV-8 infection was found in both male and female but females showed higher (5%) prevalence than males (3.3 %). These results are not in line with previous studies from sub-Saharan Africa which have shown higher incidence rates in HIV-infected and uninfected men compared to women [22].

This gender difference of KS incidence rate in sub-Saharan Africa is not well understood. In contrast to populations in the US; HHV-8 prevalence in sub-Saharan Africa appears to be similar in both HIV-infected men and women [23]. For example, in HIV-patients in Johannesburg, about 46% of men and 48% of women tested positive for HHV-8 [3]. It therefore seems unlikely that HIV infection explains the gender difference observed in our study although in the absence of adequate data on HHV-8 infection in Sudan we cannot exclude it.

The results obtained in this study show the need for wider surveillance at national level, in order to fully elucidate the true status of HHV-8 infection in Sudan. This study could serve as a baseline for future plans aiming to study HHV-8 in the country.

In conclusion, the prevalence and existence of HHV-8 in Sudan was documented through the detection of HHV-8 indicating a low prevalence among HIV patients in the country, Also in this study, the use of RT- PCR as a reliable method for the detection of HHV-8 among HIV patients was established.

Finally, this study represents the first report on HHV8 infection in HIV patients in Sudan.

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