Prevalence of Human Herpesvirus-8 (HHV-8) in untreated patients with early stage Mycosis Fungoides (A retrospective study)

Al Saif Fahad, MD, Dermatology [(1)]
Department of Dermatology
King Khalid University Hospital
King Saud University, Riyadh
Kingdom of Saudi Arabia

Abstract:

Background : Human herpesvirus 8 (HHV-8), also known as Kaposi's Sarcoma – associated herpesvirus (KSHV) was first identified and detected in 1994 in patients with Kaposi's Sarcoma. Recently, a strong association has been shown between HHV-8 and large-plaque parapsoriasis and mycosis fungoides(MF). This association has been attributed to either recent infection or reactivation of HHV-8 in patients who had extensive and/or who had an advanced stage of the diseases. This intriguing observation prompted us to perform a retrospective study in which tested previous histopathology specimens of untreated patients with early stage MF for presence of (HHV-8) by Polymerase Chain Reaction (PCR) technology

Objective: To investigate the presence of Human Herpesvirus-8 (HHV-8) in lesional skin of patients with early MF.

Method: Retrospective study of the presence of HHV-8 in patients with early stages (1a,1b) MF. Fifty Paraffin–embedded lesional skin specimens were selected, 27 specimens were from patients with MF stage la and lb, 21 specimens from patients with psoriasis as negative control and 2 specimens from patients with Kaposi's sarcoma as positive control. The presence of HHV-8 was analyzed from paraffin-embedded lesional tissue samples using a real time PCR technology.

Results: A low association of HHV-8 infection in early stages of MF was observed. Only two samples were tested positive, while none tested positive in psoriatic samples.

Conclusions: It may be concluded that HHV-8 is not significantly associated with early stages MF.

Key words: Human Herpesvirus-8, Mycosis Fungoides, Psoriasis, Kaposis Sarcoma, Saudi Arabia

Address for correspondence

Dr. Fahad Al Saif
Chairman and Consultant
Department of Dermatology
P.O. Box – 2925, Riyadh-11461
Email: fsaif1000@hotmail.com
Tel: 00966 1 4691427, 4691426
Fax: 00966 1 4691432

Introduction

Human herpesvirus 8 (HHV-8), also known as Kaposi's Sarcoma – associated herpesvirus (KSHV) was first identified and detected in 1994 in patients with Kaposi's Sarcoma[1]. There is marked heterogeneity of the affected population but it is common in parts of Africa and Europe especially Italy and Southern Mediterranean countries. [2-3] The mode of transmission seems to be through body fluids like saliva or anal secretion. Non sexual transmission is mainly through close interpersonal contact of non-intact skin or mucous membrane with blood containing secretions or saliva, Sexual transmission is through homosexual contact activity. HHV-8 is the causative agent for all forms of Kaposi's sarcoma(KS) [4], Primary effusion lymphoma (PEL) [5] and the plasmacytic form of Multicentric castleman disease,[6] Recently, a strong association has been shown between HHV-8 and large-plaque parapsoriasis and MF, though the etiologic and pathogenetic role of HHV-8 was inconclusive. This association has been attributed to either recent infection or reactivation of HHV-8 in patients who had extensive and/or who had an advanced stage of the diseases. This intriguing observation prompted us to perform a retrospective study in which tested previous histopathology specimens of untreated patients with early stage MF for presence of (HHV-8) by PCR technology.

Materials and Methods

Patients and specimens:

All specimens were obtained from the paraffin embedded lesional skin specimen blocks kept in department of pathology at King Khalid university hospital.

Between the year 2002 and 2007 a total of 50 paraffin-embedded lesional skin specimen were selected, 27 specimens were from patients with MF stage la and lb, 21

from patients with psoriasis as negative control, and 2 specimens from patients with Kaposi sarcoma as positive control. Diagnosis was based on clinical and histopathological features .The stage of MF was determined on the basis of the type and extent of skin involvement .Stage 1A refers to MF confined to the skin with patches and plagues covering less than 10% of the skin surface (T1N0M0 on the TNM classification) .Stage 1B refers to MF confined to the skin with equal or more than 10% of patches and plagues covering the skin surface (T2N0M0). Consents were taken from patients before skin biopsy. The protocol of the study was approved by the ethics review board of our department.

PCR Studies: We used disposable microtome blades to prevent possible cross-contamination between samples, the human herpesvirus-8 genome was isolated from tissue using the High pure PCR Template preparation Kit. The A 142 bp fragment of the latent nuclear antigen ORF73 from the Human Herpesvirus-8 genome was amplified with specific primers and probes on Roche Diagnostic LightCycler® instrument using real time PCR technology.The light Cycler Carousel-Based system uses real-time PCR to quantify target materials .It is a rapid thermal cycler, combined with a micro-volume fluorimeter and supplied with a personal computer and user-friendly software, making analysis easy and accurate .PCR products can be detected fluorescently, using either an intercalating fluorescent dye, SYBR Green1, or fluorescently labeled, target probes. The resulting PCR fragment was analyzed with hybridization probes that designated in commercial kit called LightMix® kit by MOLBIOL company.

Results:

Fifty lesional skin specimens were enrolled in this retrospective study. 27 specimens were from patients with MF stage

la and lb, 21 from patients with psoriasis and 2 specimens from patients with Kaposi sarcoma. All patients were Saudi and most of them were from the central region where the vast majority of the Saudi population reside .Patients age, gender, duration of MF, clinical staging of MF and medical history are presented in (table 1).Out of 27 tissue samples of patients with MF only two tested positive for the presence of HHV-8 DNA. All specimens from patients with Psoriasis tested negative while both KS samples tested positive. The real time PCR results are shown in {Fig 1,2,3,}.

No statistically significant differences were found between the study and control group regarding HHV-8 prevalence.

Discussions:

Human herpesvirus-8 (HHV-8) was first found in tissues from AIDs-associated Kaposi's sarcoma (KS)[11]. The HHV-8 seroprevalence is variable throughout the world with high seropositvity in Mediterranean area especially in Italian individuals (23.8%)[9] and west Africa (23.7%)[2] while in United States it is about 20%. [10] Other countries have very low seropositivity ranging from 0.2% to 2% as reported in French and Japanese Studies.[11]. Infection with human herpesvirus-8 (HHV-8) is common among homosexual men in North America, South America and Europe[12].The mode of transmission of HHV-8 is not clear. DNA sequences have been detected in tissues, peripheral blood mononuclear cells and different types of body fluids (Saliva, Semen, rectal and prostatic secretions or tissues), therefore sexual and non sexual transmission may play a role in the viral transmission.

There are many ways for optimal detection of HHV-8 DNA. For plasma, peripheral blood, mononuclear cells and tissue specimens [13-14] The most sensitive method is Real time PCR, while

immunohistochemical analysis is more sensitive for detection of HHV-8 in endothelial cells, Keratinocytes or neoplastic T-cells

HHV-8 is an oncogenic virus with a long latency period which may get reactivated when the hosts are immunosuppressed. Recently, it has been shown to increase tumorigencity of infected cells in animal model. [15]. Human herpesvirus-8 is now considered to be the causative agent for all Kaposi's sarcoma types, primary effusion lymphoma and multicentric castleman disease. However the role of HHV-8 in the pathogenesis of other tumors remains controversial. On the other hand, older studies have shown a relationship between HHV-8 and autoimmune vesiculobullous disorders, though a recent study have shown absence of HHV-8 in pemphigus and Bullous pemphigoid.[16] During the last few years, several studies showed a strong association of HHV-8 with large-plaque parapsoriasis and mycosis fungoides.[7-8] Kreuter A and his colleagues explained that this high association could be due to geographic regions of high seroprevalence of HHV-8 or due to recent infection or reactivation of HHV-8 in patients who had extensive treatment and/or patients with advanced stages of the disease. The pathogenetic role of HHV-8 in CTCL is still unclear not, a recent study [17] showed that latent CMV infection may play a role in the susceptibility of MF in predisposed subjects by inducing T-cell proliferation and resistance to apoptosis. Other studies [18] have shown higher risk for developing lymphoma in patients with prior EB infection and other studies excluded the pathogenic role of staphylococcus aures and human herpesvirus-7 in CTCLs. Our study is unique in choosing patients, with early stages of MF with no history of treatment

prior to skin biopsies. In this study HHV-8 DNA was detected in specimens of two mycosis fungoides patients out of 27, while in the control group none of the patients was HHV-8 DNA positive. There was no statistical difference in HHV-8 prevalence in patients with early stages of MF and negative control group. It may be concluded that HHV-8 is not associated with early stages of M.F.

ACKNOWLEDGEMENT:

The author would like to thank our laboratory technologist, Mr. Ahmad alhasimi for his excellent technical assistance during the PCR processing of this study. The secretarial skills of Mr. Abu Baker are also greatly appreciated.

References:

1.Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, Moore PS: Identification of herpesvirus - like DNA sequences in AIDs-associated Kaposi's Sarcoma. Science 1994: 266(5192): 1865-1869

2. Andrew A, Henry B, Foster G, Isaac B, Clement A, : Seroprevalence of HHV-8, CMV, and EBV among the general population in Ghana, West Africa. BMC infect dis 2008;8:111.

3. Plancoulaine S, Abel L, Gessain A. Epidermiology of human herpes Virus 8 (HHV-8) or the herpes virus associated with Kaposi's sarcoma (KSHv) (in French). Pathol Biol 2002;50:496-502.

4.Antman K, Chang Y. Kaposi's Sarcoma. N Engl, Med 2000:342(14):1027-1038.

5. Cesarman E, Chang y, Mooreps, Said DW, Knowles Dm. (1995a) Kaposi's sarcoma-associated herpes virus-like DNA sequences in AIDs-related body –Cavity-based lymphomas. N Engl J Med 332:1186-1191

6. Dupin N, Diss TL, Kallam P, et al. HHV-8 is associated with a plasmoblastic variant of castleman disease that is linked to HHV-8-positive plasmablastic lymphoma. Blood.2000;95:1406-1412.

7. Trento E, castilletti C, Ferr OC, et al. Human herpesvirus infection in patients with cutaneous lymphoproliferative disease. Arch Dermotol. 2005; 141 (10): 1235-1242

8. Alexander K, Sebastian B, Marina S, Ulrike W, et al. High association of human Herpesvirus 8 in large plaque parapsoriasis and mycosis fungoides, Arch Dermatol. 2008; 144(8):1011-1016.

9. Cattani P, Capauno M, Lesnoni LA, et al. Human herpesvirus 8 in Italian HIV-Seronegative patients with Kaposi's Sarcoma. Arch Dermatol 1998;134:695-699.

10. Lennett E, Blackbourm D, Levy JA. Antibodies to human herpesvirus 8 in general population and in Kaposi's sarcoma patients. Lancet. 1996;348:858-861.

11. Nishimoto S, Inogi R, Yamanish K, Hosokawa K, Kakibuchi K, Yoshikawa K. Prevalence of human herpesvirus 8 in skin lesions, Br J Dermatol.1997;137:179-184.

12. Guariur JV, casper c, Lama JR, et al. Prevalence and correlates of human herpesvirus 8 infection among Peruvian men who have sex with men. J acquir Immune Defic Syndr, 2008 Dec 15; 49 (5):557-562.

13. Rosamaria T, Alessia M, Eltore B. et al. Human herpesvirus 8 DNA quantification in matched plasma of patients with HHV-8 related lymphoproliferative disease. Journal of Clinical virology. 2008;43(11):255-259.

14. F. Lallmand, N.Desire, W.Rozenboum, et al. Quantitative analysis of human herpesvirus-8 viral load using a real time PCR assay. J clin Microbial 2000; 38(4):1404-1408

15. N cloutier, O. Van Eyll, M- E Janelle, S. lefort, S.-j Gao, L.flamand. Increased tumorigenicity of cells carrying recombinant human herpes virus-8 Arch virol 2008;153:93-103.

16. Golan A, Hui P, McNiff JM. Absence of human herpesvirus-8 in pemphigus and Bullous pemphigoid. Int J Clin Exp Pathol, 2009; 2(5): 456-62.

17. Ballanger F. Bressollette C, Volteau C, planche L, Breno B. Cytomegalovirus: its potential role in the development of cutaneous T-cell lymphoma. Ext Dermatol 2009 (3).

18. Kunishinge JH, McDonald H, Alvarez G. Johnson M, prieto V, Duvic M. Lymphomatoid papulosis and associated lymphomas; a retrospective case series of 84 patients. Clinc Exp Dermatol. 2009 Jan 16.

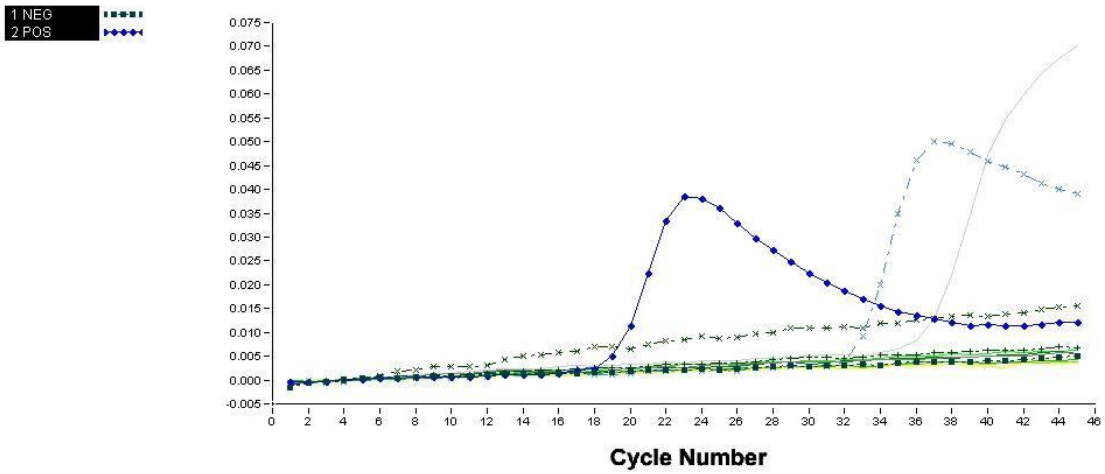

Fig- I Real Time PCR of lesional skin specimens of patients with Mycosis Fungoides (MF)

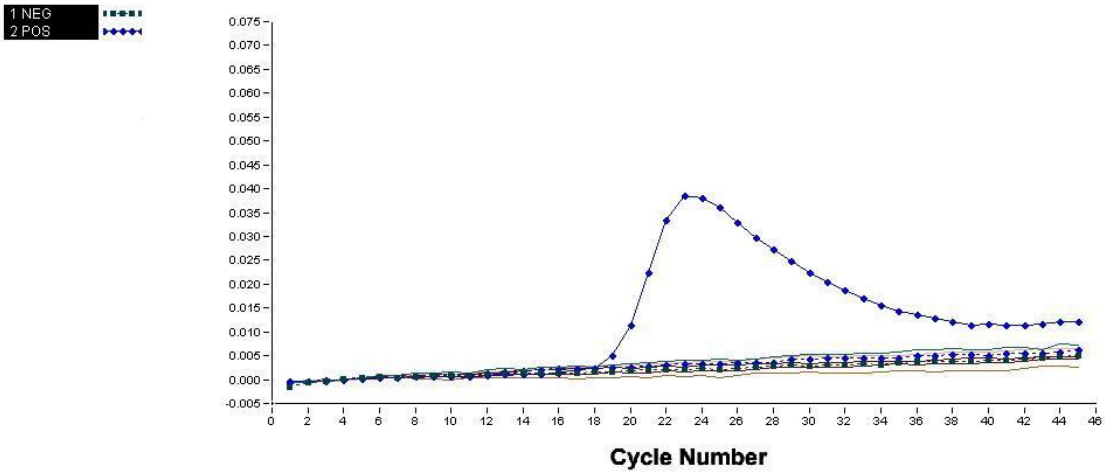

Fig-2 Real Time PCR of lesional skin specimens of patients with Psoriasis.

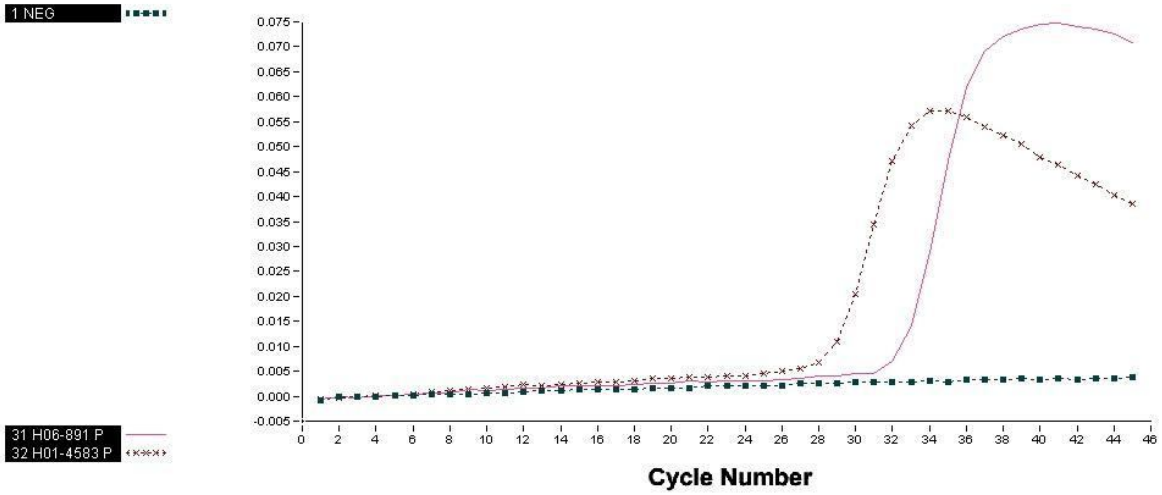

Fig-3 Real Time PCR of lesional skin specimens of patients with Kaposi's Sarcoma

Table – I : Clinical Characteristics of patients with Mycosis Fungoides

Patient Age	Gender	Duration of MF/year	MF Stage	Medical History
54	M	2y	la	-ve
60	M	1y	la	D.M,HTN
27	M	2y	la	-ve
30	F	3y	la	-ve
40	F	5y	lb	-ve
11	F	6/12y	la	-ve
45	M	4y	lb	-ve
18	M	3y	lb	-ve
40	M	1y	la	HTN
23	F	7/12y	lb	-ve
54	M	3y	la	-ve
26	F	6/12y	la	-ve
55	M	30y	lb	Dm
49	M	2y	la	-ve
45	M	3y	lb	D.M.HTN
21	F	2y	la	-ve
6	M	3y	la	-ve
10	M	2y	lb	-ve
35	F	1y	la	-ve
40	M	6/12	la	Seizers
30	M	10y	la	-ve
50	M	2y	lb	-ve
12	M	10/12y	la	-ve
53	M	2y	lb	htn
37	M	6/12y	lb	-ve
6	M	7y	la	-ve
40	F	6/12y	lb	Depression
Abbreviation: D.M : Diabetes Mellitus				
HTN: Hypertension				