Association between mycosis fungoides and human herpes virus 8: A case-control study

Yalda Nahidi, MD [1,2]
Naser Tayyebi Meibodi, MD [1,3]
Kiarash Ghazvini, MD, PhD [4,5]
Habiballah Esmaeeli, PhD [6]
Mahsa Nahidi, MD [7]

1. Cutaneous Leishmaniasis Research Center, Imam Reza Hospital, Mashhad University of Medical Sciences, Mashhad, Iran
2. Department of Dermatology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3. Department of Pathology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
4. Research Center for Microbiology and Virology, Mashhad University of Medical Sciences, Mashhad, Iran
5. Department of Microbiology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
6. Faculty of Public Health, Mashhad University of Medical Sciences, Mashhad, Iran
7. Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Corresponding Author:
Naser Tayyebi Meibodi, MD
Cutaneous Leishmaniasis Research Center, Imam Reza Hospital, Mashhad University of Medical Sciences, Mashhad, Iran
Email: tayebin@mums.ac.ir

Conflict of interest: none to declare

Received: 30 July 2015
Accepted: 20 October 2015

Background: Mycosis fungoides is the most common type of primary cutaneous T-cell lymphoma. Its etiopathogenesis, despite numerous studies, remains unclear. Environmental, viral, and genetic factors have been proposed as its potential etiologic factors. In recent years, viral agents of herpes virus family such as human herpes virus 8 (HHV-8) have been noticed.

Objective: The aim of this study was to evaluate the association between the presence of HHV-8 and mycosis fungoides.

Methods: This case-control study was done on skin biopsies of 42 mycosis fungoides cases referred to the Department of Pathology, Imam Reza Hospital, Mashhad, Iran from 2004 to 2012, and 42 age and sex matched samples of melanocytic nevi. PCR was performed to detect HHV-8 in biopsy samples of the patients and the control group. Statistical analysis was done using the IBM SPSS Statistics (IBM Corp., Armonk, NY, USA) 20.

Results: Of 42 patients with MF, 25 were male and 17 were female with a male to female ratio of 1.4. The mean age of the patients was 51.2 years. Most of the patients were in the age group over 60 years. Clinical lesions of the majority of the patients were papules and plaques. HHV-8 DNA was not found in any of the mycosis fungoides samples using the PCR method.

Conclusion: Based on our study results, HHV-8 does not play an important role in the pathogenesis of mycosis fungoides.

Keywords: mycosis fungoides, cutaneous T cell lymphoma, human herpes virus 8, PCR

Iran J Dermatol 2015; 18: 163-168

INTRODUCTION

Mycosis fungoides (MF) is the most common primary cutaneous lymphoma, and has been known as cutaneous T-cell lymphoma (CTCL). The etiopathogenesis of this type of lymphoma has remained unclear despite several investigations. Infectious, environmental, and genetic factors have

been introduced as potential etiologic agents of CTCL. Occasional CTCL cases in the elderly people and in a background of immune deficiency as well as few reports of occurrence of cases involving several individuals in a single household or spouses suggest a common infectious or environmental agent. Chronic antigenic stimulation by sustained viral or bacterial infections has been introduced in the pathogenesis of CTCL. In recent years, retroviruses and herpes virus family members have been considered to be associated with CTCL. In addition to the direct oncogenic capacity, these viruses appear to act as stable antigens in the skin, indirectly causing CTCL. In addition, the ability of these viruses to directly infect the T-cells and establish latency in host tissues has turned them into a new focus of investigation in CTCL [1]. Several studies have evaluated the role of HHV-8 in the etiopathogenesis of MF with contradictory results. Kreuter *et al.* demonstrated HHV-8 DNA in 70% of their studied samples using PCR [2]; however, many PCR and Southern blot studies have shown no HHV-8 DNA in CTCL tissues [3-14]. The aim of this study was to investigate the association of HHV-8 with this type of cutaneous lymphoma.

PARTICIPANTS AND METHODS

In this case-control study, using simple non-probability sampling, paraffin embedded blocks and microscopic slides of 105 samples taken from MF patients were selected from the pathology archive of the Department of Pathology, Imam Reza Hospital, Mashhad, Iran from 2004 to 2012 and reviewed. Definite cases of MF confirmed by immunohistochemistry were isolated. As HTLV-1 is endemic in Mashhad, HTLV-1 serologic evaluation was done for all the patients to rule out ATLL. All patients were negative for HTLV-1. Exclusion criteria included systemic therapy or phototherapy of the patients, insufficient tissue for PCR in paraffin embedded blocks, and negative beta-globin test results. According to these criteria, skin samples of 42 patients with MF were finally enrolled in the study. For the control group, 42 normal age- and sex-matched skin samples around melanocytic nevi removed for cosmetic reasons were selected. Skin samples from three patients confirmed with Kaposi's sarcoma were used as the positive control, and distilled water was used as the negative control. Using a disinfected microtome blade, six 5-micron sections were prepared from the paraffin embedded blocks and placed in sterile 1.5 ml Eppendorf microtubes. The following steps were then performed for DNA extraction and PCR.

Deparaffinization

The xylol/ethanol method was used. In this method, 1 ml xylol was added to 1.5 ml microtubes containing tissue samples that were then constantly shaken at room temperature for 30 minutes. The microtubes were centrifuged at 13000 rpm for 10 minutes, and the supernatant was discarded. These two steps were repeated once. Then, 500µl of 100% ethanol was added to the obtained precipitate. After several inversions, the microtubes were centrifuged at 13000 rpm for 10 minutes, and the supernatant was discarded. This step was repeated once. The precipitate was kept at room temperature for the extra ethanol to completely dry out.

DNA extraction

The Genet Bio kit (Korea) was used for DNA extraction from the tissue. In short, 200 µl TL buffer and 20 µl proteinase K were added to the precipitate obtained from deparaffinization and mixed well. The mixture was incubated at 56 °C until the tissue was completely lysed. Then, 200 µl GB buffer was added and incubated at 56 °C for 10 minutes. Afterwards, 200 µl absolute grade ethanol was added and vortexed, and the solution was set in extraction columns. The columns were centrifuged at 8000 rpm for 1 minute. Later, GW1 and GW2 buffers and subsequently GE buffer were added. The microtubes were centrifuged at 5000 rpm for 10 minutes, and the supernatant was used for PCR.

PCR procedure

After DNA extraction from paraffin embedded blocks, the quality of the extracted DNA samples was studied using GH20 and PC04 primers of the beta-globin gene. These primers amplified a 260 bp segment, and their sequence was as follows:

GH20: 5́ GAA GAG CCA AGG ACA GGT AC 3́

PC04: 5́ CAA CTT CAT CCA CGT TCA CC 3́

The samples which produced the 260 bp segment

using the desired primers were considered as optimum samples.

To evaluate the presence or absence of the HHV-8 sequence in the extracted DNA samples, KS1 and KS2 primers were used, which yielded a 233 bp product from the ORF26 gene of HHV-8. The primer sequence was as follows:

KS1: 5́ AGC CGA AAG GAT TCC ACC AT 3́

KS2: 5́ TCC GTG TTG TCT ACG TCC AG 3́

After amplification, 5 µl of each sample was electrophoresed on a 2% agarose gel, stained with Greenview dye, and evaluated to track the 233 bp segment of HHV-8. The samples producing the 233 bp segment using the desired primers were considered positive for HHV-8.

After collection and classification, the data were entered into IBM SPSS Statistics (IBM Corp., Armonk, NY, USA)[20]. Description of data was done using frequency tables and graphs as well as mean ± standard deviation (SD), and the data were analyzed using chi square, *t*, Fisher's exact, Mann-Whitney *U*, and Kruskal-Wallis tests. The level of significance was set at 0.05 in all the tests.

RESULTS

According to the inclusion and exclusion criteria, 84 samples including 42 samples of patients with MF and 42 healthy controls with melanocytic nevi were enrolled in this study.

The mean age of the participants in the case and control group was 51.2 ± 15.1 and 51.4 ± 15.1 years, respectively. No significant difference in the mean age between the two groups was observed (P=0.954). The highest frequency of disease was observed in patients older than 60 years. Moreover, 40.5% of the patients were female and 59.5 % were male. Papules and plaques were the most frequent forms were.

The DNA of HHV-8 virus was not found in any of the cases and controls using PCR.

DISCUSSION

Herpes virus family members can play an important role in the activation and proliferation of T lymphocytes because of their latent form, which induces the production of proteins or transcripts of latency in infected cells. Similar to other herpes viruses, HHV-8 has a number of specific genes to control the host cell, and has a weird viral cycle. The virus can remain in host cells as a "latent" infection, which is characterized by limited gene expression. During replication (lytic process), many genes homologous with host genes involved in immune response in the individual are expressed, which cause virus transmission to new cells and protect the virus against the host immune system [15,16]. HHV-8 genome contains 86 reading frames, 25% of which encode proteins with immune regulation capacity. These proteins can disrupt many aspects of the immune response such as T cell and B cell function, complement activation, innate antiviral interferon response, and natural killer cell activity [17]. Furthermore, studies have shown that HHV-8 is capable of immune system manipulation using viral micro-RNA molecules [18]. At least 11 ORFs of HHV-8 encode proteins homologous with cellular proteins are involved in signal transduction, cell cycle regulation, apoptosis inhibition, and immune regulation [19]. Thus, HHV-8 possesses the genetic features of an oncogenic virus, and is an oncogene virus with a long incubation period which may be reactivated when the host immune system is suppressed. Recently, it has been shown that this virus increases the incidence of tumor in infected cells in animal models [20]. B lymphocytes, monocytes, and CD34+ cells in the blood of patients infected with HHV-8 contain viral sequences [21-23]. The presence of HHV-8 in lymphoma like germinotropic disease, CD3 positive cells of pleural T-cell lymphoma, as well as primary effusion lymphoma with T-cell phenotype [24-26] led to the hypothesis that HHV-8 may play a role in the pathogenesis of T cells in these lymphomas, and might be involved in development of T cell lymphomas of the skin via a similar mechanism. However, few cases of the simultaneous occurrence of Kaposi sarcoma (KS) and MF have been reported [27,28], and an increase in the frequency of human leukocyte antigen (HLA)-DRB1* 11 has been documented in patients with MF and KS [29]. Therefore, a common etiologic factor (probably KSHV) may be involved in both diseases.

In this study, none of the samples from patients with MF showed HHV-8 virus genome in PCR. Accordingly, HHV-8 is not likely to play an etiologic or pathogenic role in the development of MF. Like our study, most previous studies have not indicated the role of HHV-8 virus in the pathogenesis of MF.

In a study by Quereux *et al.*, none of the 49 skin biopsies of small and large plaques of parapsoriasis showed HHV-8 and HHV-6 viruses [5]. In a study conducted by Cesarman, 8 cases of CTCL were all negative for HHV-8 by PCR and Southern blot [7]. In one study performed by Sander on 20 cases of MF, 6 cases of parapsoriasis, 20 cases of peripheral T cell lymphoma, 4 cases of follicular lymphoma, 1 case of anaplastic large cell lymphoma, 1 case of diffuse large B-cell lymphoma, and 9 cases of atypical lymphoid infiltrates using PCR and Southern blot, HHV-8 was only detected in 10 cases [8].

Pawson *et al.* evaluated peripheral blood mononuclear cells and skin biopsy specimens of 21 cases of MF, 4 cases of lymphomatoid papulosis, 5 cases of Sézary disease, and 4 cases of other cutaneous T-cell lymphomas and found that they were all negative for HHV-8 using nested PCR [9]. In a study by Hengold *et al.* on 16 cases of MF and 7 cases of lymphomatoid papulosis, HHV-8 was not found in any of the samples by PCR [10]. Nagore *et al.* investigated 32 cases of MF that were negative for HHV-8 in PCR amplification [11]. Kempf *et al.* studied 37 cases of lymphomatoid papulosis that were negative for HHV-8 by nested-PCR [12]. In a study by Dupin *et al.* on 47 HIV- negative patients with cutaneous lymphoma or large plaque parapsoriasis (LPP), PCR followed by hybridization with digoxigenin-labeled probe and nested PCR detected HHV-8 DNA sequence in only one patient with LPP [13]. In a study by Guttman-Yassky *et al.* on two patients that were 56 and 72 years old with both MF and KS diseases, nested PCR detected no DNA sequences of HHV-8 virus in MF lesions, while viral DNA was found in the KS tissue of these patients and direct involvement of KSHV in the pathogenesis of MF was rejected [6]. In another study by Fahad, only two samples obtained from 27 untreated cases of MF were positive for HHV-8 genome in RT PCR, and it was concluded that HHV-8 was not involved in the early stages of MF [4]. Erkek *et al.* conducted a study on 50 MF samples and found that they were all negative for HHV-8 genome by PCR [14]. In a study by Amitay-Laish *et al.* on 11 patients with LPP and 35 early-stage MF patients in Israel by TaqMan-based real time PCR specific for K6 gene region, only 2 adult samples of sporadic MF were positive [3].

In contrast, two studies showed a strong relationship between HHV-8 with LPP and MF. In one of these studies performed by Kreuter *et al.* on 53 patients treated for lymphoproliferative skin diseases, including 14 patients with LPP, 31 patients in various stages of MF and 8 patients with lymphomatoid papulosis (LyP), the presence of HHV-8 in paraffin embedded tissue samples was shown in 87% of LPP tissue samples and 70% of MF tissue samples using nested PCR. However, HHV-8 was not detectable by immunohistochemical markers of LANA or ORF73 [2]. In this study in Germany where the prevalence of HHV-8 in sera is low, the presence of HHV-8 in samples obtained from MF and LPP patients was justified by immune system disorders due to long term treatment of the disease and/or advanced disease, and HHV-8 was introduced as a cofactor in the progression of CTCL. However, due to discrepancies between PCR and IHC results, the presence of HHV-8 in endothelial cells, keratinocytes, or neoplastic cells was not indicated, and thus demonstration of the etiology and pathogenic role of this virus is subject to further studies [2]. In another study by Trento *et al.*, a highly significant association was found between the seroprevalence of HHV-8 infection and LPL (100%, *vs.* 25% for MF). In nested PCR, peripheral blood mononuclear cells were positive for HHV-8 in 8 out of 10 patients with LPP and 2 out of 12 patients with MF. All the tissue lesions of LPP and 2 out of 12 MF cases were positive for HHV-8. The results of these studies indicate that HHV-8 may play a role in LPP, a disease with an unknown etiology and development which is considered an early stage of MF. LPP has been assumed to be an entity apart from MF [30]. The high association rate of HHV-8 with LPP in this study may be explained because in Italy, HHV-8 seropositivity is prevalent [21].

Our results are consistent with the findings of the majority of previous studies, which failed to show the presence of HHV-8 DNA in MF lesions. In the study by Kreuter *et al.*, all patients were subject to various types of immunosuppressive therapies during sampling. Immunodeficiency or an altered immune system due to treatment or advanced disease can result in excessive production of inflammatory cytokines and consequent amplification of HHV-8 transcription. Therefore, finding HHV-8 may indicate an opportunistic infection secondary to the disease or its treatment and not necessarily the underlying cause of the disease. The strong point

of our study was excluding the patients receiving a variety of systemic, immunosuppressive, and phototherapy treatments.

Sexual transmission for HHV-8 has been confirmed although there is a non-sexual way of vertical transmission in endemic areas [31]. The seroprevalence of HHV-8 varies from almost 100% in some African countries to 15-20% in the Mediterranean region and 2% to 5% in northern Europe and United States [32]. Cultural and religious differences of our country with other countries as well as the relative infrequency of promiscuous sexual relationships in Mashhad may be the reasons for very low prevalence of HHV-8 in patients and controls.

In a study conducted in Italy [30] which demonstrated the presence of HHV-8 in all LPP patients, no significant association with MF was observed. The presence of the virus in the skin (keratinocytes, endothelial cells, and lymphocytes) was confirmed by immunohistochemical analysis only in 4 patients. Given the discrepancies between the results of PCR and IHC, a definitive conclusion about the role of HHV-8 in CTCL was not obtained in this study.

A limitation of this study was lack of access to patients for simultaneous assessment of HHV-8 in the peripheral blood and serum antibodies against HHV-8.

The results of this study support the results of previous studies, implying no direct role for HHV-8 in the pathogenesis of MF.

REFERENCES

1. Mirvish JJ, Pomerantz RG, Falo LD Jr, et al. Role of infectious agents in cutaneous T-cell lymphoma: facts and controversies. Clin Dermatol 2013;31:423-31.
2. Kreuter A, Bischoff S, Skrygan M, et al. High association of human herpesvirus 8 in large-plaque parapsoriasis and mycosis fungoides. Arch Dermatol 2008;144:1011-6.
3. Amitay-Laish I, Sarid R, Ben-Amitai D, et al. Human herpesvirus 8 is not detectable in lesions of large plaque parapsoriasis, and in early-stage sporadic, familial, and juvenile cases of mycosis fungoides. J Am Acad Dermatol 2012;66:46-50.
4. Fahad AS. Prevalence of Human Herpesvirus-8 (HHV-8) in untreated patients with early stage Mycosis Fungoides (A retrospective study). Int J Health Sci (Qassim) 2010;4:128-38.
5. Quéreux G, André-Garnier E, Knol AC, et al. Evaluation of the role of human herpes virus 6 and 8 in parapsoriasis. Exp Dermatol 2009;18:357-61.
6. Guttman-Yassky E, Bergman R, Cohen A, et al. Absence of Kaposi's sarcoma-associated herpesvirus in lesions of mycosis fungoides in patients with concomitant Kaposi's sarcoma. J Am Acad Dermatol 2004;51(2 Suppl):S133-4.
7. Cesarman E, Chang Y, Moore PS, et al. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. N Engl J Med 1995;332:1186-91.
8. Sander CA, Simon M, Puchta U, et al. HHV-8 in lymphoproliferative lesions in skin. Lancet 1996;348:475-6.
9. Pawson R, Catovsky D, Schulz TF. Lack of evidence of HHV-8 in mature T-cell lymphoproliferative disorders. Lancet 1996;348:1450-1.
10. Henghold WB, Purvis SF, Schaffer J, et al. No evidence of KSHV/HHV-8 in mycosis fungoides or associated disorders. J Invest Dermatol 1997;108:920-2.
11. Nagore E, Ledesma E, Collado C, et al. Detection of Epstein-Barr virus and human herpesvirus 7 and 8 genomes in primary cutaneous T- and B-cell lymphomas. Br J Dermatol 2000;143:320-3.
12. Kempf W, Kadin ME, Kutzner H, et al. Lymphomatoid papulosis and human herpesviruses--A PCR-based evaluation for the presence of human herpesvirus 6, 7 and 8 related herpesviruses. J Cutan Pathol 2001;28:29-33.
13. Dupin N, Franck N, Calvez V, et al. Lack of evidence of human herpesvirus 8 DNA sequences in HIV-negative patients with various lymphoproliferative disorders of the skin. Br J Dermatol 1997;136:827-30.
14. Erkek E, Sentürk N, Dinçer I, et al. Identification of herpes simplex virus DNA and lack of human herpesvirus-8 DNA in mycosis fungoides. Acta Derm Venereol 2002;82:214-6.
15. Schulz TF. Kaposi's sarcoma-associated herpesvirus (human herpesvirus-8). J Gen Virol 1998;79:1573-1591.
16. Alcami A, Koszinowski UH. Viral mechanisms of immune evasion. Trends Microbiol 2000;8:410-8.
17. Aresté C, Blackbourn DJ. Modulation of the immune system by Kaposi's sarcoma-associated herpesvirus. Trends Microbiol 2009;17:119-29.
18. Nachmani D, Stern-Ginossar N, Sarid R, et al. Diverse herpesvirus microRNAs target the stress-induced immune ligand MICB escape recognition by natural killer cells. Cell Host Microbe 2009;5:376-85.
19. Lazzi S, Bellan C, Amato T, et al. Kaposi's sarcoma-associated herpesvirus/ human herpesvirus 8 infection in reactive lymphoid tissues: a model for KSHV/HHV-8-related lymphomas? Hum Pathol 2006;37:23-31.
20. Cloutier N, van Eyll O, Janelle ME, et al. Increased tumorigenicity of cells carrying recombinant human herpesvirus 8. Arch Virol 2008;153:93-103.
21. Dupin N, Fisher C, Kellam P, et al. Distribution of human herpesvirus-8 latently infected cells in Kaposi's sarcoma, multicentric Castleman's disease, and primary effusion lymphoma. Proc Natl Acad Sci USA 1999;96:4546-51.
22. Henry M, Uthman A, Geusau A, et al. Infection of circulating CD34+ cells by HHV-8 in patients with Kaposi's sarcoma. J Invest Dermatol 1999;113:613-6.
23. Monini P, Colombini S, Stürzl M, et al. Reactivation and persistence of human herpesvirus-8 infection in B cells

and monocytes by Th-1 cytokines increased in Kaposi's sarcoma. Blood 1999;93:4044-58.

24. Du MQ, Bacon CM, Isaacson PG. Kaposi sarcoma-associated herpesvirus/human herpesvirus 8 and lymphoproliferative disorders. J Clin Pathol 2007;60:1350-7.
25. Carbone A, Gloghini A. KSHV/HHV8-associated lymphomas. Br J Haematol 2008;140:13-24.
26. Said JW, Shintaku IP, Asou H, et al. Herpesvirus 8 inclusions in primary effusion lymphoma: report of a unique case with T-cell phenotype. Arch Pathol Lab Med 1999;123:257-60.
27. Sharpe RJ, Kadin ME, Harmon DC, et al. Complete resolution of Kaposi's sarcoma with systemic etretinate therapy in a patient with mycosis fungoides. J Am Acad Dermatol 1989;20:1123-4.
28. Ulbright TM, Santa Cruz DJ. Kaposi's sarcoma: relationship with hematologic, lymphoid, and thymic neoplasia. Cancer 1981;47:963-73.
29. Jackow CM, McHam JB, Friss A, et al. HLA-DR5 and DQB1*03 class II alleles are associated with cutaneous T-cell lymphoma. J Invest Dermatol 1996;107:373-6.
30. Trento E, Castilletti C, Ferraro C, et al. Human herpesvirus 8 infection in patients with cutaneous lymphoproliferative diseases. Arch Dermatol 2005;141:1235-42.
31. Martin JN, Ganem DE, Osmond DH, et al. Sexual transmission and the natural history of human herpesvirus 8 infection. N Engl J Med 1998;338:948-54.
32. Chatlynne LG, Ablashi DV. Seroepidemiology of Kaposi's sarcoma-associated herpesvirus (KSHV). Semin Cancer Biol 1999;9:175-85.