Oral Microbiology Immunology 2004: 19: 201–204
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Oral Microbiology and Immunology

Short communication

Presence of human herpes virus-8 in saliva and non-lesional oral mucosa in HIV-infected and oncologic immunocompromised patients

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Triantos D, Horefti E, Paximadi E, Kyriakopoulou Z, Karakassiliotis G, Papanastasiou K, Lelekis M, Panos G, Donta-Bakoyianni C, Rapidis A, Markoulatos P. Presence of human herpes virus-8 in saliva and non-lesional oral mucosa in HIV-infected and oncologic immunocompromised patients.
Oral Microbiol Immunol 2004: 19: 201–204. © Blackwell Munksgaard, 2004.

Background/Aim: Human Herpes Virus-8 (HHV-8) is a recently identified virus etiologically associated with Kaposi's sarcoma. Studies regarding its presence in the oral cavity have given variable results. This study attempted to determine the oral presence of HHV-8 in an area where classic Kaposi's sarcoma is primarily found such as Greece.
Methods: Three groups of patients were studied: 10 immunocompromised with hematologic malignancies, 10 immunocompromised with HIV infection and 20 immunocompetent as controls. Whole unstimulated saliva and scrapes from the lingual and the buccal mucosa were collected and polymerase chain reaction was applied to amplify HHV-8 DNA.
Results: None of the patients in any group had oral lesions. In the control group, all samples tested negative (0/60). HHV-8 DNA was detected in 5/30 (17%) of all samples from HIV-positive patients (the mean value of their $CD4^+$ T-lymphocytes being $385/mm^3$) and in 13/30 (43%) of all samples from oncologic patients (mean $CD4^+$ T-lymphocytes $51/mm^3$). HHV-8 DNA was found in 10% of saliva samples and 40% of lingual and buccal scrapes both of HIV-infected and of oncologic patients.
Conclusions: HHV-8 is present in the saliva and the non-lesional oral mucosa (not simultaneously) of patients with impaired immunity, with or without HIV co-infection. The oral epithelium seems to represent an independent location of viral residency and may be of viral replication; the clinical implications need further clarification.

Key words: hematologic malignancies; HHV-8; HIV; Kaposi's sarcoma; oral mucosa; saliva

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Accepted for publication November 10, 2003

Human Herpes Virus 8 (HHV-8) is a recently recognized γ-herpes virus etiologically related to Kaposi's sarcoma (10). Epidemiologic data are based mainly on the detection of anti-HHV-8 antibodies, which indicate a prevalence ranging from 2% to 10% in industrialized countries (15). HHV-8 infection is not widespread and its prevalence corresponds to the geographic distribution of Kaposi's sarcoma before the era of AIDS (20). While many studies into viral pathogenic mechanisms have been conducted, the mode of viral transmission is not yet clear. At the beginning of the AIDS pandemic, the prevalence of Kaposi's sarcoma was much higher in men who have sex with men comparing to other high risk groups, and was also related to specific sexual practices (orogenital contact, high number of sexual partners and history of previous sexually transmitted disease) (4).

Attempts to incriminate genital secretions (semen and prostatic fluid) as potential transmission vehicles have yielded conflicting results (15).

HHV-8 shows genetic similarity to Epstein-Barr virus, a herpesvirus shed into saliva and which is known to undergo lytic infection in the oropharyngeal epithelial cells (23). In Africa, the principal route of person-to-person transmission of HHV-8 seems to be through casual contact between children and adolescents (20). In addition, the seroprevalence of HHV-8 among women, children and brothers in families of patients with Kaposi's sarcoma was found to be higher than HHV-8 seropositive controls without familial relationship, suggesting that intrafamilial transmission through saliva and casual contact can also occur (15).

The possible non-sexual transmission of HHV-8 and variable presence of HHV-8 in sexually relevant secretions and sites has suggested that the mouth may be a site of HHV-8 residence and replication, but data concerning the presence of HHV-8 in saliva have been conflicting (1, 2, 5–7, 9, 12–14, 16, 22, 26). Since more research data are needed, especially from different geographic locations, the present study was conducted to detect the presence of HHV-8 in saliva and oral mucosae in healthy individuals and in HIV-infected and non-HIV infected immunocompromised (oncologic) patients in a region where classic Kaposi's sarcoma is primarily found, such as Greece.

The study involved three groups of individuals: 10 inpatients of the Clinic of Hematologic Diseases, Hellenic Anticancer Institute "St. Savvas" Athens, Greece, 10 HIV-1-seropositive patients undergoing clinical and laboratory assessments in the Infectious Diseases Unit, Athens General Hospital "G. Gennimatas" Athens, Greece and 20 immunocompetent individuals (as inferred by medical records and/or patients' self-defined health status), attending for regular dental check-ups at the Department of Oral Diagnosis, Dental School, University of Athens, Greece. In all patients, data were obtained regarding sex, age, disease under therapy, risk factors for HIV-infection, hematologic and immune status (including absolute $CD4^+$ T-lymphocyte count and HIV viral load), type and duration of therapeutic regimen, other diseases and the findings from the oral examination. Individuals were randomly selected and those who refused to give clinical samples were not included in the study population. Patients receiving antiviral therapy were also excluded from the study. The clinical examination was carried out by the same individual (D.T), a clinician with special training in oral mucosal diseases, using diagnostic criteria described earlier (11). Each patient was informed about the objectives of the study and gave written consent for his/her participation. The sampling was also carried out by the same clinician (D.T) from three different oral components. Each patient was first asked to rinse with 10 ml distilled water for 1 min and spit out saliva into a Universal tube. The samples were stored at –30°C until further processing and scrapings of the lateral aspects of the tongue and the buccal mucosa were then collected with a tongue spatula, the samples transferred into 1 ml distilled water in Eppendorf tubes and stored at –30°C.

Processing of the samples was carried out in the Department of Virology of Hellenic Institute Pasteur. DNA extraction from saliva was carried out according to the method described by Markoulatos et al. (17), while the extraction from the buccal and the lingual mucosal specimens was carried out as described by Casas et al. (8). Polymerase chain reaction was carried out using as primers the sequences 5'-TTCCACCATTGTGCTCGAAT-3' (left) and 5'-TACGTCCAGACGATATGTGC-3' (right) of the open reading frame 26 of the HHV-8 genome as described by Chang et al (10). Briefly, 10 μl DNA was mixed with 40 μl reaction mixture consisting of, 10 pmol for each primer, 5 μl 10× reaction buffer, 1.5 mM $MgCl_2$, 1.5 units μl Taq DNA polymerase (Promega, Madison, WI) and 1 mM dNTPs. The reaction was performed for 35 cycles (denaturation in 95°C for 10 s, annealing at 60°C for 0 s and extension at 72°C for 10 s). Before the onset of each reaction, the samples were denatured for 3 min at 95°C; after the last cycle, one more was performed for 15 min at 78°C. The polymerase chain reaction products (210 base pairs in length) were subjected to agarose gel electrophoresis, stained by ethidium bromide and the gel was photographed under ultraviolet light transillumination. HHV-8 DNA, isolated from infected lymphoma cells (BC-3) and the human B cell line (BJAB), was used as positive and negative controls, respectively, both kindly provided by L. Arvanitakis (Hellenic Pasteur Institute). In each sample, polymerase chain reaction was performed twice. In the case of ambiguous results (in two lingual specimens), the samples were run in triplicate, and the majority finding constituted the final polymerase chain reaction result.

Table 1. HHV-8 detection in immunocompromised patients

Patients	Specimens: Mucosal: Buccal	Specimens: Mucosal: Lingual	Specimens: Salivary	Total	$CD4^+$ T-lymphocytes, mean (range)
10 HIV+	4*	1*	0*	5/30* (17%)	385/mm^3 (161–880)
10 oncologic	5	6	2	13/30 (43%)	51/mm^3 (10–89)
Total	16/40 (40%)		2/20 (10%)		

*No. of patients showing HHV-8.

The results of the detection of HHV-8 DNA are summarized in Table 1. None of the patients had mucocutaneous Kaposi's sarcoma at the time of examination. The group of healthy controls comprised 13 males and 7 females, mean age 49 years (range 21–75). In this group, all samples were negative for HHV-8. The group of HIV-infected patients comprised nine males and one female, mean age 39 years (range 25–58); eight were men who had sex with men, while two were heterosexual partners of HIV-positive individuals. In this group, HHV-8 was not found in saliva, but was detected in buccal scrapes of four patients and the lingual scrape of one patient. The group of oncologic patients comprised nine males and one female, mean age 55 years (range 21–73). Five patients were under therapy for non-Hodgkin's lymphoma, two for acute myelogenous leukaemia and one patient each for acute lymphoid leukaemia, Hodgkin's disease and multiple myeloma. HHV-8 was detected in the saliva of two patients, in the buccal mucosa of five patients and in the lingual mucosa of six patients.

In the two groups of immunocompromised patients, HHV-8 was detected in 5/30 samples (17%) of HIV-positive patients (mean value of $CD4^+$ T-lymphocytes was 385/mm^3 (range 161–880)) and in 13/30 samples (43%) of the oncologic patients (mean value of $CD4^+$ T-lymphocytes was 51/mm^3 (range 10–89)). In total, HHV-8 was detected in 10% of the salivary samples (2/20 samples) and 40% of the oral scrapes (16/40 samples) of the immunocompromised groups. HHV-8 was present in all oral samples of only one patient, a 70-year-old male with non-Hodgkin's lymphoma. The estimates of HIV-viral

load were not available for all patients, and these were excluded from the final analysis.

Since the previous studies have centered upon populations with HIV-related or the endemic form of Kaposi's sarcoma, the present study is the first to examine the oral carriage of HHV-8 in groups of individuals from Greece, a region commonly associated with classic Kaposi's sarcoma. In Greece, the incidence rates of Kaposi's sarcoma during the pre and post-AIDS period (1974–1989) were estimated to be 0.62 and 0.32 cases per 100,000 for males and females, respectively, twice and four times as high as the incidences for males and females, respectively (24), compared with those in USA for the same time period.

The study findings are significant as HHV-8 was detected in the non-lesional oral mucosal epithelium (buccal and lingual) of 40% of immunocompromised patients – without simultaneous presence in saliva – suggesting that at least in immunodeficient groups (even non-HIV infected), HHV-8 oral carriage can arise. Nevertheless, the low (10%) percentage of HHV-8 detection in saliva in the majority of immunocompromised patients was unexpected. This low detection cannot be attributed to the inhibitory effect of salivary constituents against polymerase chain reaction (21), as all samples were performed using alpha-tubulin primers as quality controls of suitability of DNA extraction (18); in this way, false-negative results attributed to failure of DNA extraction and/or failure to remove any DNA inhibitors were avoided (19). Furthermore, the possibility of drug-induced inhibition of HHV-8 replication is unlikely (15) since patients under anti-viral therapy were excluded from the study. More likely, the low detection rate may reflect the transient presence of HHV-8 in saliva as it is shed from oral epithelial cells following their turnover, which varies among subjects (12).

The presence of HHV-8 in saliva of patients with Kaposi's sarcoma may be due to the shedding of HHV-8-infected neoplastic cells or their remnants. The detection rate of the virus in the saliva of patients without Kaposi's sarcoma in the present study is similar to others (12), suggesting that Kaposi's sarcoma lesions are not the only source. It has been shown that HHV-8 is located in B-lymphocytes of peripheral blood cells and also in the lingual and palatal tonsillar tissue, which bear crypts in contact with the oropharyngeal epithelium (3). In addition to the lymphoid pool, HHV-8 has been shown to replicate in the epithelial cells of oropharynx, a phenomenon well described for Epstein-Barr virus (23). HHV-8-specific RNA has been detected in oral epithelial cells of persons without Kaposi's sarcoma, who shed the virus in saliva (25). This finding, combined with the demonstration of HHV-8 in saliva of patients without simultaneous detection in the mononuclear cells of peripheral blood (13) or in other body compartments (14, 22), suggests that the epithelium of oropharynx represents an independent site of viral replication.

The absence of HHV-8 DNA in saliva and oral epithelium of healthy individuals is in agreement with other studies and reflects the rarity of HHV-8 infection in the general (immunocompetent) population (7, 12, 14, 16). However, healthy people can carry the virus in a latent state and the lack of oral shedding may suggest that the process of viral replication is under tight immunologic control. The significance of possessing an intact immunologic system is also suggested from our findings. Viral DNA was detected in 17% of HIV-seropositive persons and in 43% of the more severely immunocompromised oncologic patients, demonstrating that the reactivation of HHV-8 is not related to the HIV infection *per se* but to the degree of immunologic impairment. Similar findings have been reported for other herpes viruses (7, 16). To our knowledge, the present study is the first to detect HHV-8 DNA in the oral cavity of patients with hematologic malignancies, a significant finding, as the virus has been linked to other hematologic malignancies such as primary effusion lymphoma, multiple myeloma and other lymphoproliferative diseases (15).

This preliminary study confirms that the oral mucosa is a site of at least HHV-8 residence and its higher detection rate in patients with greater immunosuppression suggests that the virus may be replicating in the oral mucosa. Additional studies are needed in groups of HIV and non-HIV infected individuals to further elucidate the mechanisms and possible co-factors involved in the spread of HHV-8.

Acknowledgments

We are grateful to Profs. Crispian Scully and Stephen Porter (Eastman Dental Institute, University of London, UK) and to Dr. Chong Gee Teo (Public Health Laboratory Service, London, UK) for the critical reading of the manuscript and to the State Scholarships Foundation, Republic of Greece, for the partial financial support of the study.

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