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Extensive oral shedding of human herpesvirus 8 in a renal allograft recipient

L. M. Al-Otaibi[1,2], M. H. Al-Sulaiman[3], C. G. Teo[2], S. R. Porter[1]

[1]UCL Eastman Dental Institute – Oral Medicine Department, [2]Health Protection Agency, Virus Reference Division, Centre for Infections, London, UK, [3]Armed Forces Hospital, Department of Nephrology, Riyadh, Saudi Arabia

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Introduction: Studies were conducted to investigate changes in the extent of human herpesvirus 8 (HHV-8) shedding and diversity of HHV-8 strains in the mouth of a renal allograft recipient who developed cutaneous post-transplantation Kaposi's sarcoma.
Methods: Matched oral and blood samples were obtained from a Saudi Arabian renal allograft recipient from 3 days before to 38 weeks after transplantation, and from his kidney donor. Polymerase chain reaction (PCR) protocols to amplify selected HHV-8 sub-genomic regions were applied to detect and quantify HHV-8 DNA. Sequence diversity was determined by cloning the PCR products and subjecting them to denaturing gradient gel electrophoresis and to nucleotide sequencing.
Results: Before transplantation, the recipient was seropositive for anti-HHV-8 immunoglobulin G, but the donor was seronegative; HHV-8 DNA could be detected in the recipient's blood, whole-mouth saliva (WMS) and buccal exfoliates, and the salivary viral load was estimated as 2.6 million genome-copies/ml. Post-transplantation, the recipient's salivary viral load initially increased to 4.1 million genome-copies/ml, and thereafter declined precipitously, coinciding with an increase in the dosage of valaciclovir given; HHV-8 DNA was detected most often in WMS compared with parotid saliva, and buccal and palatal exfoliates. Carriage of multiple HHV-8 strains was evident in blood and oral samples; whereas before transplantation strains belonging to genotypes A1 and A5 were observed, after transplantation genotype A5 strains became dominant and A2 strains emerged.
Conclusion: Immunosuppression and antiviral prophylaxis may interact to influence the spectrum of oral HHV-8 strains and the extent of post-transplantation HHV-8 shedding into the mouth.

Key words: human herpesvirus 8; Kaposi's sarcoma; renal transplantation; saliva

Lubna M. Al-Otaibi, UCL Eastman Dental Institute – Oral Medicine Department, 256 Gray's Inn Road London WC1X 8LD, UK
Tel.: +44 20 7915 1100;
fax: +44 20 7915 2341;
e-mail: lalotaibi@gmail.com
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Viral infections among renal transplant recipients continue to be a major cause of post-transplantation morbidity and mortality despite the availability of effective antiviral therapy (6, 20). Human herpesvirus 8 (HHV-8), the first human gamma-2 herpesvirus to be discovered, is associated with all epidemiological variants of Kaposi's sarcoma (KS), including post-transplantation (iatrogenic) KS. KS is a mesenchymal tumor involving blood and lymphatic vessels. Post-transplantation KS may arise from HHV-8 reactivation in patients who have been infected before reception of the graft, or from HHV-8 neo-infection from either contaminated blood products or the allograft (26, 29). Most cases of post-transplantation KS develop as a result of viral reactivation (2, 3, 7, 12).

Previous studies have shown that HHV-8 infection, identified by the presence of HHV-8 DNA sequences in peripheral blood mononuclear cells (PBMCs) or of antibodies to HHV-8 in the blood circulation, precedes and is predictive of the subsequent onset of KS (15, 36, 48). However, not all pre-transplantation HHV-8-seropositive patients develop KS even when they are undergoing

immunosuppression (4, 7, 37). In addition, it is not known if HHV-8 shedding from the oral cavity, a dominant site of shedding (45), can predict the development of KS in immunosuppressed people.

We investigated the extent of oral and blood HHV-8 shedding in a renal allograft recipient who developed KS after kidney transplantation. The study included an evaluation of the diversity of HHV-8 strains carried in the oral and blood compartments.

Subject and methods

Matched oral and blood samples were obtained from a 33-year-old Saudi Arabian renal allograft recipient and the kidney donor, his 21-year-old sister, undergoing kidney transplantation in 2004 at the Armed Forces Hospital in Riyadh, Kingdom of Saudi Arabia. Ethical approval for the investigation was obtained from the Ethics Committee of the Riyadh Armed Forces Hospital before the study. The renal allograft recipient was diagnosed with and treated for hepatitis C virus infection 2 years prior to the transplantation. Routine pre-transplantation screening for various infectious agents, including hepatitis B virus, hepatitis C virus, human immunodeficiency virus, and cytomegalovirus, by the hospital laboratory revealed that the donor and the recipient were seronegative for all viruses tested except for cytomegalovirus and hepatitis C virus, respectively.

Sample collection

Samples from the donor were obtained pre-transplantation, whereas samples from the recipient were obtained 3 days before and up to 38 weeks after transplantation. Peripheral blood was obtained by venepuncture. Whole mouth saliva (WMS) was collected unstimulated by having the patient dribble into a sterile plastic container. Parotid saliva (PS) was collected using a Lashley's cup placed over the parotid duct after 5% citric acid solution was dropped onto the tongue. Buccal mucosa exfoliates (BE) and palatal exfoliates (PE) were obtained by rotating a cytology sampling brush (Cytotak®, Medical Wire and Equipment Co., Corsham, Wiltshire, UK) against the oral mucosa; the brush was then rinsed in 5 ml phosphate-buffered saline.

Sample processing

After separation of plasma from the blood, the $CD45^+$, $CD31^+$, $CD19^+$, $CD14^+$ and $CD2^+$ cell subsets were immunomagnetically fractionated using Dynabeads (Dynal A.D., Oslo, Norway) as described elsewhere (24). The cellular and supernate fractions of WMS (WMS^c and WMS^s, respectively) were separated by low-speed centrifugation. BE and PE were similarly pelleted. Following aspiration of the supernate, WMS^c, BE and PE were resuspended in 1 ml phosphate-buffered saline, and stored at −20°C until required. DNA was extracted from blood cell subsets and oral samples using the QIAamp DNA Blood Mini Kit (Qiagen Inc., Venlo, the Netherlands). The presence of DNA in each extract was verified by amplifying a fragment of the β-globin gene as previously described (41).

HHV-8 subgenomic DNA detection

A 211-base-pair segment from the KS330 region of open reading frame (ORF) 26, spanning positions 366 to 577, was amplified from sample extracts by nested polymerase chain reaction (PCR) as previously described (11). (KS330 position assignments are based on the prototype HHV-8 sequence, GenBank accession no. U75698.) A 247-base-pair segment of ORF K1 that encompasses the highly variable V1 region, spanning positions 573 to 820, was amplified by nested PCR as previously described (10). (K1/V1 position assignments are based on the BCBL-1 HHV-8 sequence, GenBank accession no. U86667.) Extracts were tested at least twice for KS330 and K1/V1, and considered positive only when PCR repeatedly yielded products from both KS330 and K1/V1 segments. To minimize contamination, pre- and post-PCR procedures were conducted in dedicated rooms. Appropriate negative and positive control specimens were included in each PCR. The PCR products were sequenced using a Beckman-Coulter CEQ2000 automated capillary array sequencer. Raw DNA sequence data were analysed using SEQMAN (DNAstar).

Denaturing gradient gel electrophoresis

To screen for inter- and intra-sample K1/V1 sequence differences, selected oral samples that concordantly amplified positively for KS330 and K1/V1 and showed a significant divergence in their K1/V1 sequences were subjected again to nested PCR by use of the EXPAND High Fidelity PCR System (Roche Diagnostics, Indianapolis, IN). Clones were then generated from the K1/V1 PCR products using a TOPO-TA® Cloning System (Invitrogen, Carlsbad, CA). From each amplificate, 14 colonies were randomly picked, and each colony was subjected to another round of PCR using a clamping primer in place of the inner sense primer, which contains a guanine/cytosine-rich domain with a high melting temperature to prevent complete denaturation of the PCR product. Polyacrylamide gels were prepared and the PCR products were subjected to denaturing gradient gel electrophoresis (DGGE) as described elsewhere (5), followed by nucleotide sequencing to confirm that differences in the rate of migration of DNA bands were the result of nucleotide sequence variations.

Quantitative PCR

A quantitative, fluorescence-based, real-time PCR assay targeting ORF 25, which is applicable to a variety of clinical specimens, including saliva, was applied to positive WMS^s extracts, as previously described (42). The samples were tested in duplicate.

Anti HHV-8 immunoglobulin G detection

An enzyme-linked immunosorbent assay (Advanced Biotechnologies Inc., Columbia, MD) was used for the detection of HHV-8 immunoglobulin G (IgG) antibodies in the plasma samples collected from both donor and recipient.

Results

HHV-8 DNA and anti HHV-8 detection

HHV-8 was detected in the recipient's PBMCs both pre- and post-transplantation. Of a total of 35 PBMC fractions tested, seven (20%) were concordantly positive for KS330 and K1/V1 (one $CD19^+$ fraction; one $CD31^+$ fraction; and five $CD45^+$ fractions). HHV-8 could also be detected in the recipient's oral samples pre- and post-transplantation. Of a total of 35 oral samples tested, 16 (46%) were concordantly positive for KS330 and K1/V1 (five for WMS^s; six for WMS^c; three for BE; and one each for PS and PE). Before transplantation, HHV-8 was present in WMS^s, WMS^c and BE. Post-transplantation, HHV-8 DNA was detected in every WMS sample, in BE at 2 weeks and 4 weeks, and in PS and PE at 2 weeks and 4 weeks, respectively. All pre- and post-transplantation plasma samples obtained from the recipient were reactive for anti-HHV-8 IgG. Figure 1 summarizes the laboratory findings in relation to the changes in dosages of immunosuppressive

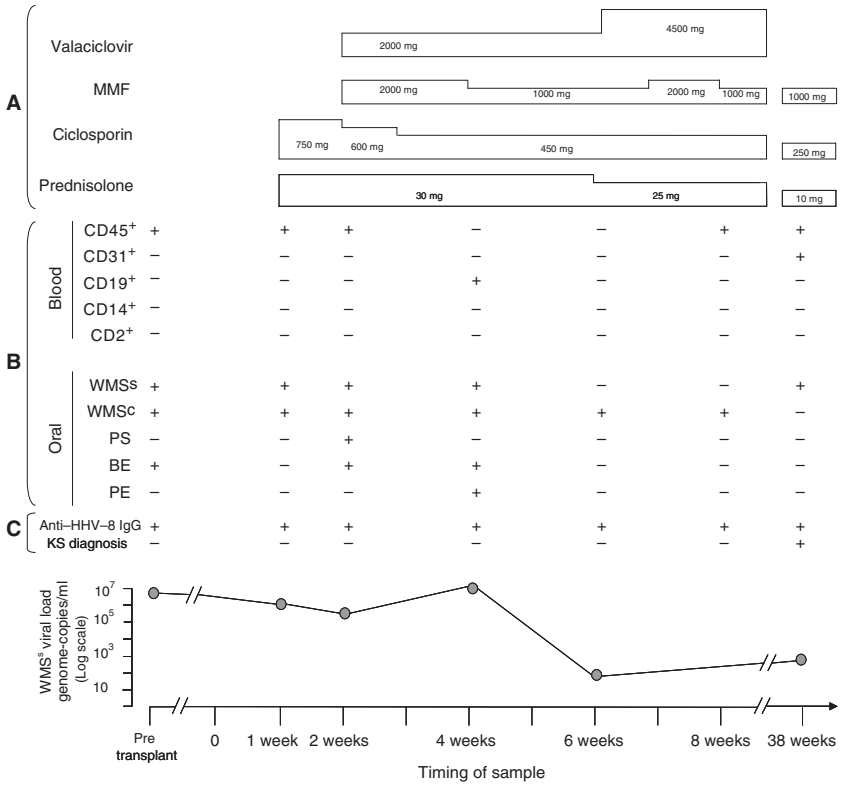

Fig. 1. Time chart illustrating change in supernate whole mouth saliva (WMS^s) viral loads in relation to (A) daily dosage of administered drugs; (B) human herpesvirus 8 (HHV-8) DNA polymerase chain reaction findings; and (C) anti-HHV-8 immunoglobulin G and Kaposi's sarcoma diagnosis. Viral load measurement values (in genome-copies/ml) were as follows; pre-transplant sample: 2,606,250; post-transplant samples: 2,270,000 (1 week), 788,750 (2 weeks), 4,106,500 (4 weeks), 170 (6 weeks), and 1495 (38 weeks). Abbreviations: MMF, Mycophenolate Mofetil; $CD45^+$, $CD31^+$, $CD19^+$, $CD14^+$, and $CD2^+$, immunomagnetically fractionated cell subsets; WMS^s, supernate fraction of whole-mouth saliva; WMS^c, cellular fraction of whole-mouth saliva; PS, parotid saliva; BE, buccal exfoliate; PE, palatal exfoliate.

and antiviral prophylactic drugs administered to the recipient. The donor of the allograft was negative for anti-HHV-8 IgG and for HHV-8 DNA.

HHV-8 subgenomic nucleotide sequence diversity

A narrow inter-sample KS330 sequence diversity (≤1.2%) was observed. The KS330 genotype of HHV-8 in all blood and oral samples taken at different intervals was identical and could be assigned to genotype B/C.

A dendrogram displaying the diversity of consensus K1/V1 sequences and their genotypic assignments is shown in Fig. 2. Inter-sample sequence diversities ranged between 0 and 9.5%. Various blood and oral samples, taken before transplantation, and at 2 weeks and 8 weeks post-transplantation, segregated into two genotypes (A1 and A5, or A2 and A5), while those taken at 1, 4, 6 and 38 weeks post-transplantation all belonged to the A5 genotype.

Inter- and intra-sample K1/V1 nucleotide sequence differences

The recipient's pre-transplantation WMS^s sample showed a 9.5% K1/V1 consensus sequence divergence from the pre-transplantation BE sample. DGGE of PCR clones derived from the K1/V1 amplificates of these two samples (Fig. 3A) and confirmation by sequencing showed a maximum divergence of 9.5% among the BE clonal sequences and 0.5% among the WMS^s samples. The majority sequence (V) was identical to the consensus ORF K1/V1 sequence from the pre-transplantation WMS^s. The BE sequences could be assigned to two genotypes (A1 and A5), while the WMS^s all belonged to A5. In addition, sequence diversity of up to 9.5% between the pre-transplant WMS^s clones and the majority of buccal clones was also observed. A dendrogram displaying the diversity of sequences generated after sequencing a selected number of clones, indicated by an asterisk in Fig. 3A, is displayed in Fig. 3B. An alignment of the nucleotide sequences is shown in Fig. 3C.

WMS^s viral load

The recipient's pre-transplantation WMS^s sample carried 2,606,250 genome-copies/ml. At 1 month post-transplantation, the WMS^s viral load increased to 4,106,500 copies/ml, thereafter dropping significantly (Fig. 1).

Clinical progress

Twenty-six weeks following allograft receipt, the recipient developed cutaneous KS on the upper trunk, arms and legs (which was confirmed histologically). KS affecting the stomach and colon were diagnosed at 61 weeks and 68 weeks post-transplant, respectively. No oral KS was evident.

Discussion

Salivary HHV-8 shedding in immunocompromised individuals may be extensive, even before immunosuppression is administered, and may increase further when immunosuppression is started. The salivary HHV-8 viral load of the renal allograft recipient studied here was found to increase by 60% 1 month after immunosuppression, thereafter dropping significantly, coinciding with an increase in the dosage of valaciclovir given. Valaciclovir, an ester of aciclovir, is licensed for herpes zoster virus and herpes simplex virus infections of the skin and mucous membranes. Aciclovir has been found to have some effect, albeit weak, on the inhibition of HHV-8 replication (21, 28). This relatively insubstantial effect may explain why, in this case, higher doses of valaciclovir were required to achieve a significant reduction in the WMS^s HHV-8 viral load. The observation that the HHV-8 load in WMS^s later rebounded following the cessation of valaciclovir is noteworthy. Nevertheless, valaciclovir administered between week 2 and week 8 post-transplantation, as prophylaxis against herpesvirus infection, could not prevent the onset of KS. This outcome is in agreement with previous studies showing that aciclovir has no effect on the development of KS in AIDS patients (16, 33).

Previous studies have detected HHV-8 DNA in the buccal mucosa and palate of KS patients (5, 9, 10), and in the normal oral mucosa of immunocompetent individuals (13). The present study supports these findings, but in the context of an

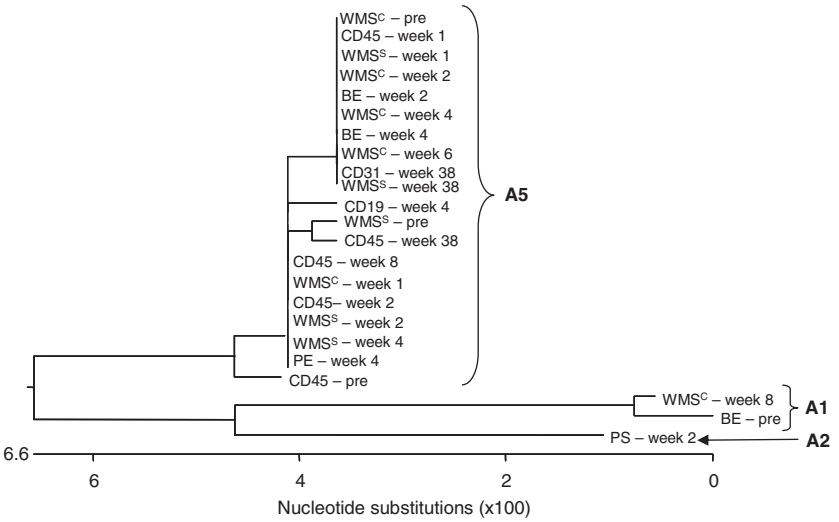

Fig. 2. Dendrogram showing diversity of consensus K1/V1 sequences. Abbreviations: CD45^{+}, CD31^{+}, CD19^{+}, CD14^{+} and CD2^{+}, immunomagnetically fractionated cell subsets; WMS^{s}, supernate fraction of whole-mouth saliva; WMS^{c}, cellular fraction of whole-mouth saliva; PS, parotid saliva; BE, buccal exfoliate; PE, palatal exfoliate.

individual who has been given immunosuppressants. HHV-8 DNA was found in both pre- and post-transplantation BE coinciding with time points when WMS^{s} viral loads were ≥788,750 copies/ml, and in post-transplantation PE when the WMS^{s} viral load was the heaviest, indicating that HHV-8 in the buccal and palatal cells contributed to the increased WMS^{s} HHV-8 viral load. The inability to detect HHV-8 DNA in the BE or PE at other times does not rule out the possibility that the virus was replicating at locations distant from the sampling sites. The limited HHV-8 DNA detection rate in saliva collected from the parotid duct supports previous findings indicating that the major salivary glands are not important contributors to HHV-8 shedding (38). The observation that WMS yielded significantly higher PCR detection rates than other oral samples may reflect HHV-8 shedding from other sites, such as tonsils or tongue (8, 23, 47, 49).

To assure broad representation of the blood subpopulations, some of which have previously been reported to support HHV-8 persistence (17, 19, 22, 24, 31, 34, 39), immunomagnetic fractioning of CD45^{+}, CD31^{+}, CD19^{+}, CD14^{+} and CD2^{+} cell subsets was conducted. HHV-8 DNA was detected in the PBMCs before the development of clinically evident KS and even before renal transplantation, contrary to previous reports of the HHV-8 genome being present mainly in PBMCs of people with active KS (32, 40). The detection rate of HHV-8 in PBMCs varied according to the cell subsets studied, with the CD45^{+} cells generating the most positive results (5/7), followed by CD19^{+} cells (1/7) and CD31^{+} cells (1/7). The CD2^{+} and CD14^{+} cells, previously reported to harbor HHV-8 in patients with KS (19, 39), were negative for HHV-8 DNA throughout the sampling period. This result may reflect a specific HHV-8 cellular tropism or low-copy-number-carriage of viral DNA that was below the PCR detection limit, or intermittent HHV-8 shedding (25).

The extreme polymorphism of ORF K1 was exploited to investigate the genomic diversity of HHV-8 through amplification of K1/V1. Sequencing led to the assignment of sequences originating from the recipient's pre-transplant BE and 8-week post-transplantation WMS^{c} samples to the A1 genotype, from the recipient's 2-week post-transplantation PS to the A2 genotype and the remaining samples to the A5 genotype. These findings point to intra-compartmental (oral) carriage of multiple HHV-8 strains, the spectrum of which changed over the period of observation. The dominant A5 variant, reported here, has previously been identified in African (18, 46, 50) and Brazilian (35) samples, but not in Saudi Arabian samples.

Direct sequencing studies, however, have an important limitation in that they are unable to produce consensus sequence data that will allow sequences of minority variants to be identified. Samples with high HHV-8 genome loads favor the generation of sequences reflecting the dominant variants (44). Accordingly, the combined DGGE screening-nucleotide sequencing protocol was applied to K1/V1 amplicons generated from clonal inserts. To reduce the degree to which PCR generates nucleotide misincorporation, high-fidelity PCR was applied to sample extracts. Applying this combined DGGE-sequencing protocol showed that the recipient's pre-transplant BE clones exhibited a significant nucleotide sequence diversity of up to 9.5%, segregating BE clones into two genotypes (A1 and A5), with the minority clade (A5) comprising sequences that were genotypically distinct from the majority clade (A1). The findings from this study are supported by other reports that have revealed intra-person variation in the sub-genomic sequences of HHV-8 (1, 5, 14), but contrast with others that have not (27, 30, 43, 50). The KS biopsy samples for the recipient could not be retrieved, so the origin of the viral strain that led to the KS could not be defined.

This study has identified the oral cavity as a major site of HHV-8 shedding in a prospectively studied patient undergoing renal transplantation. The extensive amount of HHV-8 shed into the mouth points to oral fluid as posing a risk of nosocomial transmission in pre-transplantation settings (e.g. in renal dialysis clinics) and post-transplantation settings (e.g. shared hospital wards). Monitoring allograft recipients closely for oral HHV-8 shedding may guide decisions to customize the dosages of immunosuppressive and antiviral prophylactic drugs administered. Whether antiviral prophylaxis needs to be administered more aggressively to prevent the development of KS requires larger scale, case–control studies. The possible role antiviral prophylaxis has, in the context of drug-induced immunosuppression, to select for HHV-8 strains that are disposed to lead to the development of KS, may also merit further investigation.

Nucleotide sequence accession numbers

The sequences determined in this study have been deposited in the EMBL Nucleotide Sequence Database (accession numbers AM745354 to AM745428).

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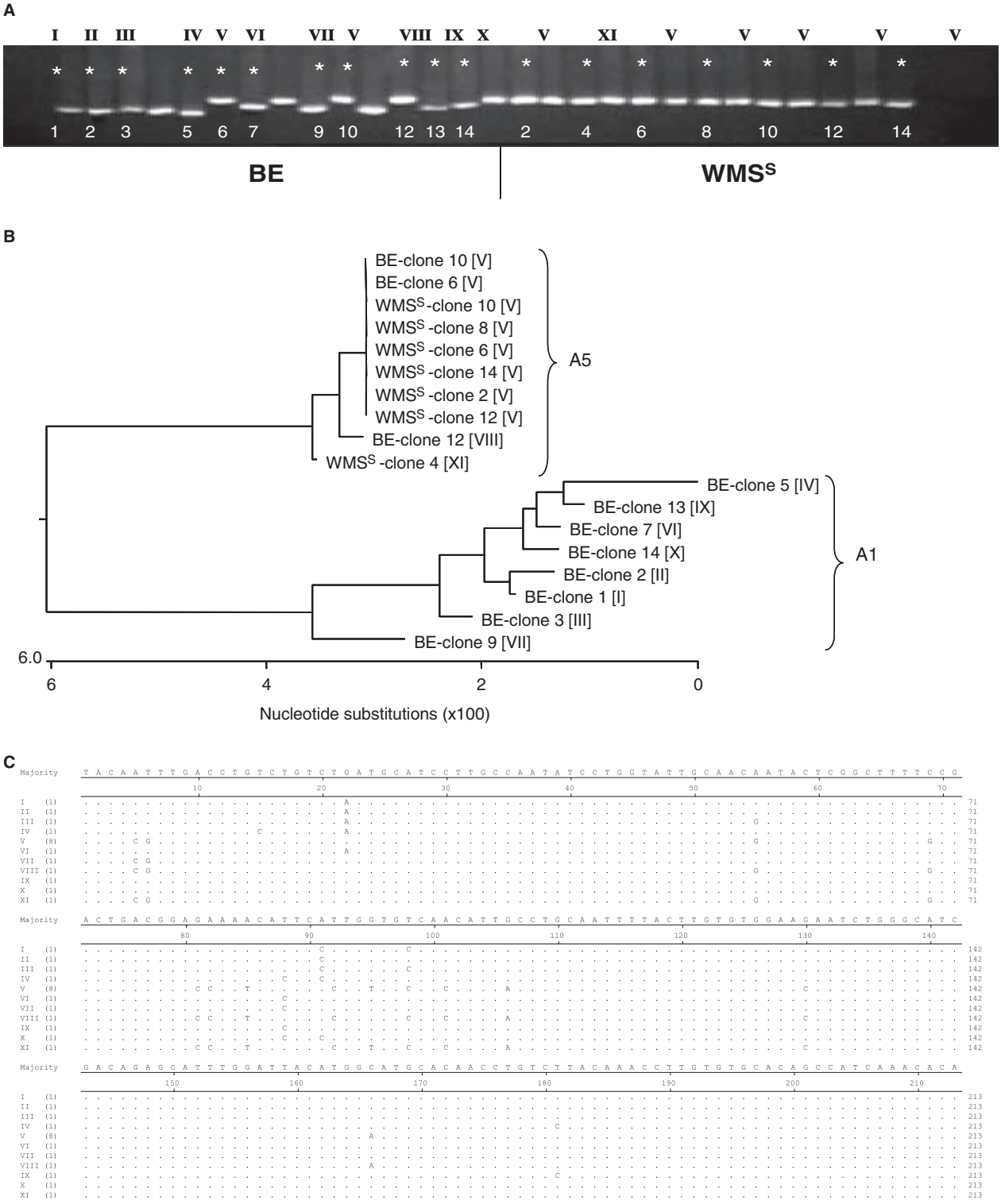

Fig. 3. (A) Denaturing gradient gel electrophoresis photograph accommodating K1/V1 DNA amplified from 14 clones generated from pre-transplant supernate fraction of whole-mouth saliva (WMS^s) and buccal exfoliate (BE) samples**.** (B) Dendrogram showing phylogenetic distribution of clonal K1/V1 nucleotide sequences of pre-transplant WMS^s and BE samples indicated in (A) as asterisks. (C) Comparison of clonal K1/V1 nucleotide sequences. Arabic numerals denote lane positions, Roman numerals denote unique sequences and numbers in the parenthesis denote the number of clones with the unique sequences.

References

1. Al Otaibi LM, Ngui SL, Scully CM, Porter SR, Teo CG. Salivary human herpesvirus 8 shedding in renal allograft recipients with Kaposi's sarcoma. J Med Virol 2007: **79**: 1357–1365.
2. Andreoni M, Goletti D, Pezzotti P et al. Prevalence, incidence and correlates of HHV-8/KSHV infection and Kaposi's sarcoma in renal and liver transplant recipients. J Infect 2001: **43**: 195–199.
3. Becuwe C, Euvrard S, Bosshard S et al. [Kaposi's sarcoma and organ

transplantation: 22 cases]. Ann Dermatol Venereol 2005: **132**: 839–843.
4. Bergallo M, Costa C, Margio S et al. Human herpes virus 8 infection in kidney transplant patients from an area of northwestern Italy (Piemonte region). Nephrol Dial Transplant 2007: **22**: 1757–1761.
5. Beyari MM, Hodgson TA, Cook RD et al. Multiple human herpesvirus-8 infection. J Infect Dis 2003: **188**: 678–689.
6. Briggs JD. Causes of death after renal transplantation. Nephrol Dial Transplant 2001: **16**: 1545–1549.
7. Cattani P, Capuano M, Graffeo R et al. Kaposi's sarcoma associated with previous human herpesvirus 8 infection in kidney transplant recipients. J Clin Microbiol 2001: **39**: 506–508.
8. Chagas CA, Endo LH, Sakano E, Pinto GA, Brousset P, Vassallo J. Detection of herpesvirus type 8 (HHV8) in children's tonsils and adenoids by immunohistochemistry and in situ hybridization. Int J Pediatr Otorhinolaryngol 2006: **70**: 65–72.
9. Cook RD, Hodgson TA, Molyneux EM, Borgstein E, Porter SR, Teo CG. Tracking familial transmission of Kaposi's sarcoma-associated herpesvirus using restriction fragment length polymorphism analysis of latent nuclear antigen. J Virol Methods 2002: **105**: 297–303.
10. Cook RD, Hodgson TA, Waugh AC et al. Mixed patterns of transmission of human herpesvirus-8 (Kaposi's sarcoma-associated herpesvirus) in Malawian families. J Gen Virol 2002: **83**: 1613–1619.
11. Di Alberti L, Ngui SL, Porter SR et al. Presence of human herpesvirus 8 variants in the oral tissues of human immunodeficiency virus-infected persons. J Infect Dis 1997: **175**: 703–707.
12. Diociaiuti A, Nanni G, Cattani P et al. HHV8 in renal transplant recipients. Transpl Int 2000: **13**(suppl 1): S410–S412.
13. Duus KM, Lentchitsky V, Wagenaar T, Grose C, Webster-Cyriaque J. Wild-type Kaposi's sarcoma-associated herpesvirus isolated from the oropharynx of immunecompetent individuals has tropism for cultured oral epithelial cells. J Virol 2004: **78**: 4074–4084.
14. Gao SJ, Zhang YJ, Deng JH, Rabkin CS, Flore O, Jenson HB. Molecular polymorphism of Kaposi's sarcoma-associated herpesvirus (Human herpesvirus 8) latent nuclear antigen: evidence for a large repertoire of viral genotypes and dual infection with different viral genotypes. J Infect Dis 1999: **180**: 1466–1476.
15. Garcia-Astudillo LA, Leyva-Cobian F. Human herpesvirus-8 infection and Kaposi's sarcoma after liver and kidney transplantation in different geographical areas of Spain. Transpl Immunol 2006: **17**: 65–69.
16. Glesby MJ, Hoover DR, Weng S et al. Use of antiherpes drugs and the risk of Kaposi's sarcoma: data from the Multicenter AIDS Cohort Study. J Infect Dis 1996: **173**: 1477–1480.
17. Harrington WJ, Bagasra O, Sosa CE et al. Human herpesvirus type 8 DNA sequences in cell-free plasma and mononuclear cells of Kaposi's sarcoma patients. J Infect Dis 1996: **174**: 1101–1105.
18. Hayward GS, Zong JC. Modern evolutionary history of the human KSHV genome. Curr Top Microbiol Immunol 2007: **312**: 1–42.
19. 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**: 613–616.
20. Hwang EA, Kang MJ, Han SY, Park SB, Kim HC. Viral infection following kidney transplantation: long-term follow-up in a single center. Transplant Proc 2004: **36**: 2118–2119.
21. Kedes DH, Ganem D. Sensitivity of Kaposi's sarcoma-associated herpesvirus replication to antiviral drugs. Implications for potential therapy. J Clin Invest 1997: **99**: 2082–2086.
22. Kikuta H, Itakura O, Taneichi K, Kohno M. Tropism of human herpesvirus 8 for peripheral blood lymphocytes in patients with Castleman's disease. Br J Haematol 1997: **99**: 790–793.
23. Koelle DM, Huang ML, Chandran B, Vieira J, Piepkorn M, Corey L. Frequent detection of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) DNA in saliva of human immunodeficiency virus-infected men: clinical and immunologic correlates. J Infect Dis 1997: **176**: 94–102.
24. Kumar N, McLean K, Inoue N et al. Human herpesvirus 8 genoprevalence in populations at disparate risks of Kaposi's sarcoma. J Med Virol 2007: **79**: 52–59.
25. Laney AS, Dollard SC, Jaffe HW, et al. Repeated measures study of human herpesvirus 8 (HHV-8) DNA and antibodies in men seropositive for both HHV-8 and HIV. AIDS 2004: **18**: 1819–1826.
26. Marcelin AG, Calvez V, Dussaix E. KSHV after an organ transplant: should we screen? Curr Top Microbiol Immunol 2007: **312**: 245–262.
27. Mbulaiteye S, Marshall V, Bagni RK et al. Molecular evidence for mother-to-child transmission of Kaposi sarcoma-associated herpesvirus in Uganda and K1 gene evolution within the host. J Infect Dis 2006: **193**: 1250–1257.
28. Medveczky MM, Horvath E, Lund T, Medveczky PG. In vitro antiviral drug sensitivity of the Kaposi's sarcoma-associated herpesvirus. AIDS 1997: **11**: 1327–1332.
29. Mendez JC, Paya CV. Kaposi's sarcoma and transplantation. Herpes 2000: **7**: 18–23.
30. Meng YX, Sata T, Stamey FR et al. Molecular characterization of strains of human herpesvirus 8 from Japan, Argentina and Kuwait. J Gen Virol 2001: **82**: 499–506.
31. Mesri EA, Cesarman E, Arvanitakis L et al. Human herpesvirus-8/Kaposi's sarcoma-associated herpesvirus is a new transmissible virus that infects B cells. J Exp Med 1996: **183**: 2385–2390.
32. Metaxa-Mariatou V, Chiras T, Loli A, Gazouli M, Vallis D, Nasioulas G. Molecular analysis of Kaposi's sarcoma occurring during haemodialysis. Clin Exp Dermatol 2004: **29**: 188–191.
33. Mocroft A, Youle M, Gazzard B, Morcinek J, Halai R, Phillips AN. Anti-herpesvirus treatment and risk of Kaposi's sarcoma in HIV infection. Royal Free/Chelsea and Westminster Hospitals Collaborative Group. AIDS 1996: **10**: 1101–1105.
34. Monini P, Colombini S, Sturzl 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**: 4044–4058.
35. Nascimento MC, Wilder N, Pannuti CS, Weiss HA, Mayaud P. Molecular characterization of Kaposi's sarcoma associated herpesvirus (KSHV) from patients with AIDS-associated Kaposi's sarcoma in Sao Paulo, Brazil. J Clin Virol 2005: **33**: 52–59.
36. Nsubuga MM, Biggar RJ, Combs S et al. Human herpesvirus 8 load and progression of AIDS-related Kaposi sarcoma lesions. Cancer Lett 2008: **263**: 182–188.
37. Parravicini C, Olsen SJ, Capra M et al. Risk of Kaposi's sarcoma-associated herpes virus transmission from donor allografts among Italian posttransplant Kaposi's sarcoma patients. Blood 1997: **90**: 2826–2829.
38. Pauk J, Huang ML, Brodie SJ et al. Mucosal shedding of human herpesvirus 8 in men. N Engl J Med 2000: **343**: 1369–1377.
39. Pellet C, Kerob D, Dupuy A et al. Kaposi's sarcoma-associated herpesvirus viremia is associated with the progression of classic and endemic Kaposi's sarcoma. J Invest Dermatol 2006: **126**: 621–627.
40. Purvis SF, Katongole-Mbidde E, Johnson JL et al. High incidence of Kaposi's sarcoma-associated herpesvirus and Epstein–Barr virus in tumor lesions and peripheral blood mononuclear cells from patients with Kaposi's sarcoma in Uganda. J Infect Dis 1997: **175**: 947–950.
41. Saiki RK, Scharf S, Faloona F et al. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 1985: **230**: 1350–1354.
42. Stamey FR, Patel MM, Holloway BP, Pellett PE. Quantitative, fluorogenic probe PCR assay for detection of human herpesvirus 8 DNA in clinical specimens. J Clin Microbiol 2001: **39**: 3537–3540.
43. Stebbing J, Wilder N, Ariad S, Abu-Shakra M. Lack of intra-patient strain variability during infection with Kaposi's sarcoma-associated herpesvirus. Am J Hematol 2001: **68**: 133–134.
44. Tedeschi R, Enbom M, Bidoli E, Linde A, De Paoli P, Dillner J. Viral load of human herpesvirus 8 in peripheral blood of human immunodeficiency virus-infected patients with Kaposi's sarcoma. J Clin Microbiol 2001: **39**: 4269–4273.
45. Teo CG. Conceptual emergence of human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus) as an oral herpesvirus. Adv Dent Res 2006: **19**: 85–90.
46. Treurnicht FK, Engelbrecht S, Taylor MB, Schneider JW, van Rensburg EJ. HHV-8 subtypes in South Africa: identification of a case suggesting a novel B variant. J Med Virol 2002: **66**: 235–240.
47. Triantos D, Horefti E, Paximadi E et al. 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.
48. Whitby D, Luppi M, Barozzi P, Boshoff C, Weiss RA, Torelli G. Human herpesvirus 8 seroprevalence in blood donors and lymphoma patients from different regions of Italy. J Natl Cancer Inst 1998: **90**: 395–397.
49. Widmer IC, Erb P, Grob H et al. Human herpesvirus 8 oral shedding in HIV-infected men with and without Kaposi sarcoma. J Acquir Immune Defic Syndr 2006: **42**: 420–425.
50. Zong J, Ciufo DM, Viscidi R et al. Genotypic analysis at multiple loci across Kaposi's sarcoma herpesvirus (KSHV) DNA molecules: clustering patterns, novel variants and chimerism. J Clin Virol 2002: **23**: 119–148.