Detection of human herpesvirus 6 and 7 DNA in saliva from healthy adults from Rio de Janeiro, Brazil

Ivna M Magalhães, Rebeca VN Martins, João J Cossatis, Renata M Cavaliere, Larissa A Afonso, Natalia Moysés, Solange A Oliveira, Silvia MB Cavalcanti/+

Departamento de Microbiologia e Parasitologia, Instituto Biomédico, Universidade Federal Fluminense, Rua Ernani Melo 101 Laboratório 319, 24210030 Niterói, RJ, Brasil

In this study, we aimed to evaluate virus shedding in the saliva of healthy adults from the metropolitan region of the city of Rio de Janeiro, Brazil, in order to verify the prevalence of both human herpesviruses 6 and 7 (HHV-6, HHV-7). The studied group comprised 182 healthy individuals at Pedro Ernesto University Hospital, who were being seen for annual odontologic revisions. Saliva specimens were subjected to a multiplex polymerase chain reaction (PCR) to detect the presence of HHV-6A, HHV-6B and HHV-7. The total Roseolovirus *DNA prevalence was 22.4%. The PCR detected a HHV-6 prevalence of 9.8%, with HHV-6A detected in 7.1% of the samples and HHV-6B in 2.7%. HHV-7 DNA was revealed in 12.6% of the studied cases. Multiple infections caused by HHV-6A and 7 were found in 2.1% of the samples. No statistical differences were observed regarding age, but for HHV-7 infection, an upward trend was observed in female patients. Compared to studies from other countries, low prevalence rates of herpesvirus DNA were detected in saliva from the healthy individuals in our sample. PCR methodology thus proved to be a useful tool for* Roseolovirus *detection and it is important to consider possible geographic and populations differences that could explain the comparatively low prevalence rates described here.*

Key words: *Roseolovirus* - healthy adults - saliva

The β human herpesvirus-6 and 7 (HHV-6 and HHV-7) are closely related viruses, discovered in 1986 and 1990, respectively (Salahuddin et al. 1986, Frenkel et al. 1990). They are members of the *Roseolovirus* genus, characterized by tropism for T lymphocytes (Jarrett et al. 1990, Wyatt & Frenkel 1992). Although infection occurs frequently without symptoms, both viruses are accepted as etiological agents of exanthem subitum, a classical childhood illness characterized by spiking fever and rash (Yamanishi et al. 1988). Primary infection has also been associated with neurological symptoms such as seizures (Ward 2005). HHV-6 is further classified into two variants: A and B. While HHV-6B is recognized as the causative agent of exanthem subitum, no human disease has been clearly associated with HHV-6A (Tanaka-Taya et al. 1996). It is important to note that in young adults and adolescents, HHV-7 has also been associated with pityriasis rosea (Black & Pellett 1999).

To date, limited information is available concerning the excretion of these viruses from body sites during and after infection. It is well established that almost all children are HHV-6 seropositive by two years old (Ward et al. 1993) while HHV-7 infections usually occur later, being gradually acquired over the first five or six years of life (Huang et al. 1997). But infection may sometimes occur earlier, as reported in Brazil by Oliveira et al. (2003). Like other herpesviruses, latency and persistence can occur in the salivary glands, peripheral blood mononuclear cells (PBMC) and central nervous system (Braun et al. 1997). Some authors suggested that both viruses persist in the host and are shed in saliva throughout life (Hidaka et al. 1993). Viral reactivation occurs in immunocompromised patients and has been associated with fever, rash, encephalitis and bone marrow suppression (Caserta et al. 2001).

Clinical diagnosis is not accurate and differential laboratory tests are necessary to distinguish exanthem subitum from viruses such as rubella, measles, human parvovirus B19 and dengue fever (Oliveira et al. 2003). Primary infection is determined by the detection of low-avidity IgG antibodies, in immunofluorescent tests (Ward 2005). The results of studies using polymerase chain reaction (PCR) are controversial due to the possibility of reactivation and reinfection by such viruses. Finally, most prior studies described prevalence of the viruses in PBMCs but these results were compromised by the observation of chromosomal integration of HHV-6 within these cells (Daibata et al. 1999).

Healthy adults can shed virus intermittently, behaving as the epidemiological source of infection among human populations. Nevertheless, previous articles have reported conflicting results: from 2.5-100% of patients showed HHV-6 and 7 in saliva (Cone et al. 1993). Hence, the aim of our study was to evaluate virus shedding in the saliva of healthy adults from the metropolitan region of the city of Rio de Janeiro, state of Rio de Janeiro (RJ), Brazil, in order to assess the prevalence of both HHV-6 and 7 among this population, because no epidemiological data are available.

Financial support: PROAP/UFF
+ Corresponding author: silviacavalcanti@vm.uff.br
Received 10 February 2010
Accepted 18 August 2010

To achieve this aim, we conducted a study in the Pedro Ernesto University Hospital of State University of Rio de Janeiro (HUPE/UERJ), a large tertiary public hospital in RJ. The studied group comprised 182 individuals residing in RJ, attending HUPE for annual odontologic revisions between May 2006-May 2008. The average age of participants was 24.6 years with an age range of 14-56 years old. This study was approved by the Ethical Committee from HUPE/UERJ (protocol 138-2005).

In order to detect HHV-6 and 7, DNA was extracted from 500 μL of whole saliva by using QIAmp kit (QIAgen, Germany) and tested for HHV-6A, B and 7 DNA with the use of a multiplex nested PCR assay as previously described (W Knowles, personal communication). A data bank was generated and data were analyzed using the EPInfo 2004 statistical software package (CDC, Atlanta, USA, 2004). Prevalence rates were compared by Chi-square tests with Yates correction. The significance level (p) of the statistical tests was set at 0.05.

Among the 182 studied individuals, 75 (41.2%) were men and 107 (58.8%) were women. After stratifying by age (10-year interval), no significant differences were found in prevalence rates ($p > 0.05$). PCR results showed a HHV-6 prevalence of 9.8% (18/182), with HHV-6A detected in 7.1% (13/182) of the samples and HHV-6B in 2.7% (5/182), HHV-7 DNA was revealed in 12.6% (23/182) of the studied cases. Multiple infections caused by HHV-6A and 7 were demonstrated in 2.1% (4/182) of the samples. The total herpesvirus DNA prevalence was 22.4% (41/182). Statistical analysis indicated no differences between men and women regarding the prevalence of these infections ($p < 0.05$), except for a slight trend of increased prevalence of HHV-7 in females, as previously described by Black and Pellett (1999). This greater prevalence among females could be attributable to hormonal imbalance throughout life.

As summarized by Caselli and Di Luca (2007), HHV-6 and 7 primary infection occurs in early childhood and causes short febrile diseases, sometimes associated with cutaneous rash (exanthem subitum). Both HHV-6 and 7 are highly prevalent in the healthy population, establish latency in macrophages and T-lymphocytes, are intermittently shed in the saliva of healthy donors and the pathogenic potential of reactivated virus ranges from asymptomatic infection to severe diseases in transplant recipients. These features have contributed to the notion that HHV-6 and 7 are more or less "harmless" viruses. Consequently, the medical and scientific interest originally prompted by their discovery has been gradually waning. As revised by Ward (2005), few papers concerning these viruses have been published and most of them reported significant differences in prevalence rates, outcome and in definitions of latency, reactivation and re-infection.

In our study, we found low prevalence rates of both HHV-6 and 7 when compared to other studies (Tanaka-Taya et al. 1996, Ward et al. 2002). Pereira et al. (2004) have already described similar results for HHV-6 prevalence using gingival crevicular fluid and parotid gland saliva (14%), suggesting that inhibitors present in saliva would interfere with DNA amplification, resulting in false-negatives. In fact, rates of DNA detection showed a wide variation among different studies and geographic regions (Zerr et al. 2000, Ward 2005). The reasons for this variation are unclear but point to differences in populations from different geographical areas, socioeconomic status, as well as to technical issues concerning PCR protocols, specimens of study and method of collection. Recently, de Pagter et al. (2010) conducted a multicenter external quality assessment to evaluate the available molecular methods for the detection of HHV-6. The qualitative results they described indicated 80% agreement between laboratories from 20 countries, presenting only 1.8% false-positive results. Hence, variations in prevalence rates described worldwide may reflect different geographic circulation of the *Roseolovirus*.

It is also interesting to note a dominance of HHV6-A over HHV6-B, in agreement with the findings presented by Kasolo et al. (1997) in Zambia. The latter report also described similar prevalence rates for HHV6-A, showing around 13% DNA detection, which differed from the rates described by Ward (2005).

In our study, the chosen clinical specimen was the whole saliva: a non-invasive sample that is easy to collect. Zerr et al. (2000) had already studied different samples and whole saliva was demonstrated as an appropriate fluid for genomic analysis, since throat swabs or blood can contain latent virus in local lymphocytes.

Previously, it was thought that in the immunocompetent human, HHV 6 DNA was present transiently in serum during early primary infection, but not thereafter. Recent data, however, showed that in contrast to acute primary infection, where only HHV-6B DNA is found transiently, both HHV-6A and 6B DNA are occasionally detected in the serum of individuals of all ages (Ward 2005). The significance of this newly described phenomenon in relation to diagnosis, clinical consequences and congenital infections must be further studied. Persisting DNA in saliva would represent reactivation of latent viruses, even though the individual is not immunocompromised. It could also be caused by integration of these viruses and hereditary transmission leading to chronic excretion of viruses, as seen in some individuals. In fact, HHV-6 is the only herpesvirus known to integrate and transmit in the chromosomes of one or both parents (Daibata et al. 1999). Nevertheless, no apparent morbidity has been associated with congenital infection (Hall et al. 2004). We believe that these results have important implications for diagnosis. Although diagnosis is best made by comparing antibody titers of paired sera and demonstrating seroconversion to low avidity antibodies, it has been proposed that the detection of HHV-6B DNA in saliva by PCR would be adequate to diagnose early primary infections as well as for epidemiological studies. Previous studies, based on viral isolation in tissue culture followed by antigen detection - a difficult and laborious procedure that detects only viable viral particles - showed a low prevalence of the virus. Therefore, despite its limitations, PCR is still the most suitable technique for HHV-6 detection in epidemiological surveys.

Regarding HHV-7, we detected 12.6% prevalence of viral DNA; a low rate, when compared to the results reported by Kidd et al. (1998). The latter study described viral DNA prevalence of 32% and showed that 80% of the cases presented co-infection with HHV-6. In fact, prevalence rates varied extensively in studies from different regions and standardization methods are still lacking (Black & Pellett 1999). Hence, further studies are necessary to determine HHV-7 prevalence in our population as well as its pathogenic potential in clinical syndromes observed in children and in immunosuppressed patients. In conclusion, although PCR methodology proved to be a useful tool for *Roseolovirus* detection, it is important to consider possible geographic and population variations that could account for the low prevalence rates described here.

ACKNOWLEDGEMENTS

To Wendy Knowles, from Health Protection Agency (London, UK), for providing protocols and HHV-6 and 7 DNA clones.

REFERENCES

Black JB, Pellett PE 1999. Human herpesvirus 7. *Rev Med Virol 9*: 245-262.

Braun DK, Dominguez G, Pellett PE 1997. Human herpesvirus 6. *Clin Microbiol Rev 10*: 521-567.

Caselli E, Di Luca D 2007. Molecular biology and clinical associations of roseoloviruses human herpesvirus 6 and human herpesvirus 7. *New Microbiol 30*: 173-187.

Caserta MT, Mock DJ, Dewhurst S 2001. Human herpesvirus 6. *Clin Infect Dis 33*: 829-833.

Cone RW, Huang ML, Ashley R, Corey L 1993. Human herpesvirus 6 DNA in peripheral blood cells and saliva from immunocompetent individuals. *J Clin Microbiol 31*: 1262-1267.

Daibata M, Taguchi T, Nemoto Y, Taguchi H, Miyoshi I 1999. Inheritance of chromosomally integrated human herpesvirus 6 DNA. *Blood 94*: 1545-1549.

de Pagter PJ, Schuurman R, de Vos NM, Mackay W, van Loon AM 2010. Multicenter external quality assessment of molecular methods for detection of human herpesvirus 6. *J Clin Microbiol 48*: 2536-2540.

Frenkel N, Schirmer EC, Wyatt LS, Katsafanas G, Roffman E, Danovich RM, June CH 1990. Isolation of a new herpesvirus from human $CD4^+$ T cells. *Proc Natl Acad Sci USA 87*: 748-752.

Hall CB, Caserta MT, Schnabel KC, Boettrich C, McDermott MP, Lofthus GK, Carnahan JA, Dewhurst S 2004. Congenital infections with human herpesvirus 6 (HHV6) and human herpesvirus 7 (HHV7). *J Pediatr 145*: 472-477.

Hidaka Y, Liu Y, Yamamoto M, Mori R, Miyazaki C, Kusuhara K, Okada K, Ueda K 1993. Frequent isolation of human herpesvirus 7 from saliva samples. *J Med Virol 40*: 343-346.

Huang LM, Lee CY, Liu MY, Lee PI 1997. Primary infections of human herpesvirus-7 and herpesvirus-6: a comparative, longitudinal study up to 6 years of age. *Acta Paediatr 86*: 604-608.

Jarrett RF, Clark DA, Josephs SF, Onions DE 1990. Detection of human herpesvirus-6 DNA in peripheral blood and saliva. *J Med Virol 32*: 73-76.

Kasolo FC, Mpabalwani E, Gompels UA 1997. Infection with AIDS-related herpesviruses in human immunodeficiency virus-negative infants and endemic childhood Kaposi's sarcoma in Africa. *J Gen Virol 78*: 847-855.

Kidd IM, Clark DA, Bremner JA, Pillay D, Griffiths PD, Emery VC 1998. A multiplex PCR assay for the simultaneous detection of human herpesvirus 6 and human herpesvirus 7, with typing of HHV-6 by enzyme cleavage of PCR products. *J Virol Methods* 70: 29-36.

Oliveira SA, Turner DJ, Knowles W, Nascimento JP, Brown DW, Ward KN 2003. Primary human herpesvirus-6 and -7 infections, often coinciding, misdiagnosed as measles in children from a tropical region of Brazil. *Epidemiol Infect 131*: 873-879.

Pereira CM, Gasparetto PF, Corrêa ME, Costa FF, de Almeida OP, Barjas-Castro ML 2004. Human herpesvirus 6 in oral fluids from healthy individuals. *Arch Oral Biol 49*: 1043-1046.

Salahuddin SZ, Ablashi DV, Markham PD, Josephs SF, Sturzenegger S, Kaplan M, Halligan G, Biberfeld P, Wong-Staal F, Kramarsky B, Gallo RC 1986. Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. *Science 234*: 596-601.

Tanaka-Taya K, Kondo T, Mukai T, Miyoshi H, Yamamoto Y, Okada S, Yamanish K 1996. Seroepidemiological study of human herpesvirus-6 and -7 in children of different ages and detection of these two viruses in throat swabs by polymerase chain reaction. *J Med Virol 48*: 88-94.

Ward KN 2005. The natural history and laboratory diagnosis of human herpesviruses-6 and -7 infections in the immunocompetent. *J Clin Virol 32*: 183-193.

Ward KN, Couto Parada X, Passas J, Thiruchelvam AD 2002. Evaluation of the specificity and sensitivity of indirect immunofluorescence tests for IgG to human herpesviruses-6 and -7. *J Virol Methods 106*: 107-113.

Ward KN, Gray JJ, Fotheringham MW, Sheldon MJ 1993. IgG antibodies to human herpesvirus-6 in young children: changes in avidity of antibody correlate with time after infection. *J Med Virol 39*: 131-138.

Wyatt LS, Frenkel N 1992. Human herpesvirus 7 is a constitutive inhabitant of adult human saliva. *J Virol 66*: 3206-3209.

Yamanishi K, Okuno T, Shiraki K, Takahashi M, Kondo T, Asano Y, Kurata T 1988. Identification of human herpesvirus-6 as a causal agent of exanthema subitum. *Lancet 1*: 1065-1067.

Zerr DM, Huang ML, Corey L, Erickson M, Parker HL, Frenkel LM 2000. Sensitive method for detection of human herpesviruses 6 and 7 in saliva collected in field studies. *J Clin Microbiol 38*: 1981-1983.