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Search for Varicella Zoster Virus DNA in Saliva of Healthy Individuals Aged 20 to 59 Years

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Abstract

All neurological and ocular complications of varicella zoster virus (VZV) reactivation can occur without rash. Virological verification requires detection of VZV DNA or anti-VZV IgG antibody in cerebrospinal fluid (CSF), or anti-VZV IgM antibody in serum or CSF. If VZV were readily detected in other tissue in patients with neurological disease without rash and found to correlate with tests listed above, more invasive tests such as lumbar puncture might be obviated. Saliva is a potential source of VZV DNA. To study the potential diagnostic value of detecting VZV DNA in saliva from patients with neurological disease, saliva of healthy adults searched for VZV DNA. A single saliva sample obtained by passive drool was centrifuged at 16,000 × g for 20 mins. DNA was extracted from the supernatant and cell pellet and examined in triplicate for VZV DNA by real time PCR. A single random saliva sample from 80 healthy men and women aged 20–59 years revealed no VZV DNA (Table 1), but was uniformly positive for cell (GAPdH) DNA. Because VZV DNA was not found in a random saliva sample from 80 individuals 20–59 years-old, a VZV-positive sample during neurologic disease may have potential significance. Further studies will determine whether VZV DNA in saliva correlates with VZV DNA or anti-VZV antibody in CSF in patients with neurological disease.

Keywords

VZV; saliva; diagnosis; PCR

INTRODUCTION

Varicella zoster virus (VZV) is a ubiquitous neurotropic alphaherpesvirus. Primary infection usually produces varicella (chickenpox), after which virus becomes latent in ganglionic neurons along the entire neuraxis [Gilden et al., 1983; Kennedy et al., 1998; 1999]. Decades later, reactivation produces zoster (shingles) which may be further complicated by meningoencephalitis, myelopathy, vasculopathy and multiple ocular disorders. Importantly, these neurological and ocular complications of VZV reactivation can occur without rash [Gilden et al., 2010]. In such instances as well as in zoster sine herpete (radicular pain in the absence of rash), virological verification relies on detection of VZV DNA or anti-VZV IgG

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antibody in cerebrospinal fluid (CSF), or less often, the presence of VZV DNA in blood mononuclear cells or anti-VZV IgM antibody in serum or CSF. If VZV were readily detected in other tissue samples (e.g., saliva or tears) in patients with neurological disease in the absence of rash and shown to correlate with the standard tests listed above, more invasive tests such as lumbar puncture might be obviated.

Saliva is a potential source of VZV DNA in such patients [Gershon 2011]. First, VZV DNA was present not only in saliva in all of 54 patients with acute herpes zoster involving the face, trunk, upper and lower extremities [Mehta et al., 2008], but also in saliva of patients with acute peripheral facial palsy without rash [Furuta et al., 2000; 2001; 2005], indicative of geniculate zoster (Ramsay Hunt syndrome) sine herpete. VZV DNA was also present in saliva up to 28 days after zoster immunization of people aged >60 years with no history of rash [Pierson et al., 2011]. Finally, the frequency of VZV DNA detection in saliva samples from individuals aged >60 years was significantly higher in all patients with a history of zoster than in age-matched subjects with no zoster history [Nagel et al., 2011].

MATERIALS AND METHODS

To further study the potential diagnostic value of detecting VZV DNA in saliva of adults, this study searched for the presence of VZV DNA in saliva of healthy adults, aged 20–59 years, without a history of zoster, immunosuppression or current pregnancy. After informed consent and with approval of the University of Colorado Denver Institutional Review Board, saliva was collected saliva from 80 healthy individuals, aged 20–59 years, during November and December 2012. Ten women and 10 men in each decade of life (20–29, 30–39, 40–49 and 50–59 years) were randomly selected (Table 1). Among the subjects, 75% were Caucasian, 10% were Asian, 6% were Hispanic, 4% were Indian and 3% were Afro-Americans. Exclusion criteria included a history of zoster, current immunosuppressive conditions (cancer, HIV, diabetes and immunosuppressive medications, including steroids), and current pregnancy or under antiviral treatment.

A single saliva sample was collected from each person at any time of day, at least one hour after a meal and after rinsing the mouth with water. Samples were obtained by passive drool, wherein individuals sat comfortably and allowed saliva to accumulate for approximately one min, after which saliva was allowed to dribble into a wide-mouth polypropylene tube; the process was repeated until at least 2 ml of saliva was collected. On average, 4.7 ml of saliva was collected from each individual (range 2.8 –7.5 ml), with the total collection time varying from 3 to 15 mins (average 5 mins). Saliva was stored at −20°C until analyzed.

Before DNA extraction, 1.7 ml of each saliva specimen was centrifuged at 16,000 × g for 20 mins, and DNA was extracted from the cell pellet using the QIA-Amp DNA Kit (Qiagen, Inc, Chatsworth, CA) as described [Mehta et al., 2004]. DNA concentration was determined (NanoDrop ND-1000 Spectrophotometer; NanoDrop Technologies, Inc. Wilmington, DE), followed by real-time PCR performed in triplicate to determine VZV DNA copy number [Mehta et al., 2008]. Based on VZV DNA standards, the sensitivity of PCR was 10 copies of VZV DNA/ng salivary DNA. PCR primer and probe sequences for VZV ORF 63 at a sensitivity of 10 copies of VZV DNA per 500 nanograms of total DNA and GAPdH were as described [Cohrs et al., 2008]. PCR was carried out in blinded fashion with respect to identification of clinical specimens and positive controls.

RESULTS

The average DNA yield was 56 ng/μl saliva (range 2.3 – 214 ng/μl; median 39 ng/μl); Table 1 lists the DNA yield per saliva sample for each decade of age. A positive control, stored at

−20°C, for each PCR analysis consisted of 5 ml of donor saliva mixed with 10 μl of tissue culture fluid from VZV-infected human lung fibroblasts harvested at the height of a cytopathic effect, was treated identically to each saliva sample as described above. A single random saliva sample from 80 healthy men and women aged 20–59 years revealed no VZV DNA but was uniformly positive for cell (GAPdH) DNA (Table 1).

DISCUSSION

The potential diagnostic value of detecting VZV DNA in saliva of patients with neurologic disease known to be caused by VZV in the absence of rash depends on its absence in saliva of healthy adults in the same age group. In this study, the search for VZV DNA in a single randomly collected saliva sample from each of 80 healthy adults aged 20–59 years was uniformly negative, data extending our earlier report of the absence of VZV DNA in 88 saliva samples collected from 10 healthy subjects 35 to 50 years old [Mehta et al., 2004]. Even in the 20–29 year age group in which the amount of total DNA obtained from saliva was ~1.5 times greater than in the other age groups, no VZV DNA was found. Since all subjects in this study were at least 20 years old, any VZV DNA that would have been detected would have to have been wild-type, since VZV vaccination of children was not FDA-approved until 1995. Importantly, VZV DNA has been detected in saliva of young people without rash only in unusual circumstances, i.e., in astronauts during and shortly after spaceflight [Cohrs et al., 2008; Mehta et al., 2004], in children admitted to intensive care units [Papaevangelou et al., 2013] and in medical residents on night call [Uchakin et al., 2011]; all of these individuals were considerably stressed. Finally, VZV DNA was found in saliva of one of 53 normal subjects ranging in age from 18 to 75 years-old; the age of the single subject whose saliva was positive for VZV DNA was not provided [Wang et al., 2010].

In an earlier analysis of saliva for VZV DNA in individuals over age 60, 7 saliva samples collected over a 2-week period revealed VZV DNA in saliva of 2/17 (12%) subjects and in 21/32 (67%) subjects in the same over-60 age group who had a history of zoster [Nagel et al., 2011]. Analysis of 7 saliva samples from each person enhanced the likelihood of obtaining at least one VZV DNA-positive sample; however, when neurologic disease develops without rash that could be due to VZV, it is unlikely that 7 saliva samples would be obtained. Thus, in light of the absence of VZV DNA in a random saliva sample from 80 individual’s age 20–59 years, a VZV-positive saliva sample at the time of neurologic disease may have potential significance.

Currently, the diagnostic gold standard for a VZV cause of neurological disease in the absence of rash is detection of VZV DNA or antibody to VZV in CSF. The next important studies must determine whether detection of VZV DNA in saliva in patients with neurologic disease correlates with the presence of VZV DNA or anti-VZV antibody in CSF, thereby potentially obviating invasive and expensive diagnostic procedures.

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TABLE 1

Absence of VZV DNA in saliva of healthy individuals aged 20–59 years

Age (years)	# subjects (men/woman)	Mean age (range)	Total DNA (ng) in saliva (mean±SD)	VZV+
20–29	20 (10/10)	24.9 (21–28)	4080 +/− 2940	0/20
30–39	20 (10/10)	34.9 (30–39)	2740 +/− 2345	0/20
40–49	20 (10/10)	43.9 (40–49)	2395 +/− 1830	0/20
50–59	20 (10/10)	54.2 (50–59)	2030 +/− 1735	0/20