

## Major Article

### Varicella Zoster Virus in Saliva of Patients With Herpes Zoster

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**Running title:** VZV in saliva of patients with zoster

Abstract word count: 202  
Text word count: 2311

## **FOOTNOTE PAGE**

### **Conflict of interest:**

The authors do not have a commercial or other association that might pose a conflict of interest.

### **Financial support:**

National Aeronautics and Space Administration (grants 111-30-10-03 and 111-30-10-06 to D.L.P.), and National Institutes of Health (grants AG06127 and NS32623 to D.H.G.).

### **Meeting presentations:**

Information included in this paper was presented at the International Herpesvirus Workshop, July 2007 in Asheville, North Carolina.

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## ABSTRACT

**Background.** VZV DNA is present in saliva of healthy astronauts and patients with Ramsay Hunt syndrome (geniculate zoster). We hypothesized that a prospective analysis of patients with zoster would detect VZV in saliva independent of zoster location.

**Methods.** We treated 54 patients with valacyclovir. On the first treatment day, 7- and 14-days later, pain was scored and saliva examined for VZV DNA. Saliva from six subjects with chronic pain and 14 healthy subjects was similarly studied.

**Results.** Follow-up data was available for 50/54 patients. Pain decreased in 43/50 (86%), disappeared in 37 (74%), recurred after disappearing in three (6%) and increased in four (8%). VZV DNA was found in every patient the day treatment was started, decreased in 47/50 (94%), transiently increased in three (6%) before decreasing, increased in two (4%) and disappeared in 41 (82%). There was a positive correlation between the presence of VZV DNA and pain, as well as between the VZV DNA copy number and pain ( $P<0.0005$ ). Saliva of two patients was cultured, and infectious VZV was isolated from one. VZV DNA was present in one patient before rash and in four patients after pain resolved, and not in any control subjects.

**Conclusion.** VZV DNA is present in saliva of zoster patients.

**Keywords:** varicella zoster virus, saliva, zoster

## INTRODUCTION

Varicella zoster virus (VZV) is a highly neurotropic, exclusively human alpha-herpesvirus that infects nearly all humans and causes chickenpox (varicella). After chickenpox, VZV becomes latent in cranial nerve, dorsal root, and autonomic nervous system ganglia along the entire neuraxis. Decades later, a declining VZV host immunity allows virus to reactivate, resulting in shingles (zoster), characterized by pain and rash usually restricted to one to three dermatomes. Zoster is often complicated by postherpetic neuralgia (PHN), pain that persists for months to years after rash resolves. Virus may also spread to the spinal cord and produce myelitis as well as to the blood vessels of the brain and cause a unifocal or multifocal vasculopathy, both of which are more common in immunocompromised individuals. The increased incidence and severity of zoster and its attendant neurological complications in elderly and immunocompromised individuals appear to be due to a VZV-specific host immunodeficiency. Thus, zoster can be viewed as a continuum, ranging from a natural decline in VZV-specific immunity with age, to more serious host immune deficits encountered in transplant recipients and patients with cancer and AIDS.

When zoster occurs within a few days of myelopathy or vasculopathy, the clinical diagnosis is straightforward. However, many cases of myelitis, vasculopathy and even chronic radicular pain caused by VZV develop without rash [1]. Because viremia is rare in zoster, proof that VZV causes neurological disease without rash requires virological analysis of cerebrospinal fluid (CSF) [2]. Detection of VZV by a non-invasive procedure might obviate the need for CSF examination to verify the diagnosis virologically. Because VZV DNA has been detected in saliva of astronauts without rash during and after space flight [3], we asked whether a prospective analysis of zoster patients might also reveal the presence of VZV in saliva.

## METHODS

***Patients and general procedures.*** All human study protocols were approved by the Committee for the Protection of Human Subjects of the Johnson Space Center, Houston, TX (control subjects), and the Institutional Review Board of the University of Texas Health Sciences Center, Houston, TX (zoster patients), and informed consent was obtained from all subjects. The 54 zoster patients consisted of 29 women (age 21 to 82 years) and 25 men (age 35 to 79 years) (table 1). All zoster patients were treated with oral valacyclovir, one gram three times daily, for seven consecutive days. Pain was described by all patients on a scale of 0 (no pain) to 10 (worst pain) [4]. Saliva samples from all 54 zoster patients were obtained using cotton rolls in Salivette tubes (Sarstedt, Inc., Newton, NC) as described [5]. To avoid contamination from skin lesions, patients did not touch the salivette cotton roll while collecting their saliva samples. Samples were collected on day 1 before antiviral therapy and again on days 8 and 15. Saliva of two patients with zoster was also cultured for virus isolation [6]. Table 2 describes six control subjects (five women, age 24 to 64 years and one 38-year-old man) with chronic pain due to malignancy or non-VZV inflammatory disease. Fourteen

additional healthy control subjects consisted of nine men and five women (age 34 to 70 years). Three saliva samples were collected at weekly intervals from all six control subjects with chronic pain and from the 14 healthy control subjects.

**DNA extraction and PCR.** Saliva specimens were concentrated with a Microsep 100K filtration unit (Filtron Technology Corporation, Northborough, MA) and DNA was extracted with non-organic extraction reagents (Qiagen Inc., Chatsworth, CA). Microcarrier gel (Molecular Research Center Inc., Cincinnati, OH) was added to facilitate DNA recovery. DNA was dissolved in 50  $\mu$ L of nuclease-free water (Amresco, Solon, OH). Quantitative real-time PCR was performed in a TaqMan 7700 sequence detector (Applied Biosystems, Foster City, CA) using fluorescence-based simultaneous amplification and product detection. Primers and probes specific for VZV, herpes simplex virus (HSV)-1 and glyceraldehyde 6-phosphate dehydrogenase DNA sequences have been described [3, 7].

**Isolation of VZV from saliva.** Saliva samples of patients 11 and 19 (table 1) were inoculated onto subconfluent monolayers of human fetal lung cell fibroblasts and observed for a cytopathic effect (CPE). Cells at the height of CPE were analyzed by PCR with HSV-1 and VZV-specific primers [3, 7] and by immunohistochemistry for viral antigens. Cells were fixed in 4% paraformaldehyde and incubated with either a 1:2000 dilution of rabbit antibody directed against VZV gene 63 protein or a 1:2000 dilution of rabbit antibody directed against HSV-1 VP22 for one hour at room temperature, washed with 150 mM NaCl, 3% bovine serum albumin, and 20 mM Tris-HCl, pH 7.5, followed by incubation for one hour at room temperature with a 1:10,000 dilution alkaline phosphatase-conjugated goat anti-rabbit IgG (Invitrogen, Carlsbad, CA). Specific antibody binding was detected by colorimetric development (NBT/BCIP, nitro-blue tetrazolium chloride/5-bromo-4-chloro-3'-indolyphosphate p-toluidine salt) as directed by the supplier (Roche, Boulder, CO).

**Statistical methods.** Random-effects logistic regression was used to correlate pain with the presence of VZV DNA in saliva of zoster patients [8]. Association between pain and the VZV DNA burden was determined using the Somers D test [9], a nonparametric analog to the regression coefficient in ordinary linear regression which is related to forms of Kendall's Tau [10].

## RESULTS

All control subjects and patients with zoster were seropositive for VZV (data not shown). Table 1 lists the level of pain reported by 54 zoster patients before and after treatment, as well as the number of VZV DNA copies detected by real-time PCR in saliva of the patients. For 50 of the 54 patients, follow-up data were available.

**Pain in zoster patients.** Pain scores were available from all 54 patients on the day treatment was started (day 1), from 44 patients on day 8 and from 48 patients (not necessarily the same patients) on day 15. In 43 of 50 patients (86%), pain decreased

during the 14-day study period; in two patients (patients 33 and 45), pain transiently increased before decreasing. Ultimately, in 37 of the 50 patients (74%), pain disappeared entirely during the 14-day study period. Three of the 50 patients (6%) (patients 9, 41, and 50) developed an increase in pain after it had disappeared. In four of 50 patients (8%) (patients 6, 9, 16, and 34), pain increased throughout the 14-day study period.

**VZV DNA in saliva of zoster patients.** All 54 patients with herpes zoster had rash on day 1 when treatment was started (table 1). Saliva was obtained from all 54 patients before treatment was started on day 1, from 42 patients on day 8 and from 47 patients on day 15 (not necessarily the same patients). VZV DNA was detected in saliva of all 54 patients with zoster before treatment on day 1, independent of pain score, or the presence or absence of rash. Linear regression analysis to model the log VZV DNA copies on day 1 revealed no significant effect of age ( $P=0.225$ ) or gender ( $P=0.652$ ).

In 47 of 50 patients (94%), virus DNA decreased in saliva during the 14-day study period, although in three patients (6%) (patients 6, 31, and 45) virus DNA transiently increased before decreasing. Ultimately, virus DNA disappeared from saliva during the 14-day study period in 41 of the 50 patients (82%). No patients developed any increase in virus DNA after it had begun to decline or disappear from saliva. In two patients (patients 16 and 34), virus DNA increased throughout the 14-day study period. In four patients (patients 7, 9, 27, and 53), VZV DNA was detected in saliva after pain resolved. In one patient (patient 48), VZV DNA was present in saliva when the patient had pain, but before rash developed (table 3). There was a significant positive correlation between pain and the presence of VZV DNA in saliva ( $P<0.0005$ ) as well as between pain and the VZV DNA burden ( $P<0.0005$ ). Overall, reported pain levels were highest when the VZV copy numbers were high. Further, as VZV DNA disappeared, pain scores decreased and eventually became zero.

In the six patients with chronic non-dermatomal distribution pain, VZV DNA was not detected in any of 18 saliva samples (three from each patient) obtained over a two-week period (table 2). During a six-month follow-up period, none of these patients developed herpes zoster or exhibited an increase in anti-VZV specific IgG levels (Quest Laboratory, Houston, TX). In 14 other healthy control subjects, VZV DNA was not detected in any of 42 saliva samples (three from each individual) obtained over a two-week period (data not shown).

Saliva samples of patients 11 and 19 (table 1) were each inoculated onto subconfluent monolayers of human fetal lung cell fibroblasts and observed for CPE. After one subcultivation, a herpesvirus-specific CPE was observed in cells inoculated with saliva from patient 19, but not in patient 11 saliva cultures. Both PCR and immunohistochemistry revealed that the CPE was VZV-specific (not shown).

One 21-year-old patient with zoster whose pain preceded rash (patient 48) was studied extensively (table 3). She developed T12-distribution radicular pain (scored as 8) without rash at a time when VZV DNA was detected in both her saliva and plasma;

three days later, her pain increased to a score of 9, a T12-distribution zoster rash developed, and VZV DNA was again detected in both saliva and plasma. She was treated immediately with oral valacyclovir (one gram three times daily for seven days). Two days after antiviral treatment, pain decreased to 7, and VZV was detected in saliva and peripheral blood mononuclear cells (PBMC). Seven days after antiviral treatment, her pain level was still 7, but VZV DNA was no longer detected in saliva, but was found in her PBMC. Three weeks after onset of pain, the patient became pain-free, and no VZV DNA was detectable in her saliva.

## DISCUSSION

The diagnosis of herpes zoster was established in 54 patients by the presence of dermatomal distribution rash. When rash developed, every patient was treated immediately with valacyclovir and then studied for two weeks. VZV DNA was found in the saliva of all 54 patients. During the two-week study period, the VZV DNA copy number declined in nearly all patients and disappeared in 82% of the patients. In two zoster patients, one of whom was being treated with immunosuppressive drugs for cancer (patient 16), both salivary virus DNA and pain increased throughout the 14-day study period. In two other patients (patients 31 and 45), salivary VZV DNA also increased from day one to day 8, but both virus and pain disappeared by day 15. In addition to the detection of VZV DNA in saliva of all patients with zoster, infectious VZV was isolated from one of two zoster patients whose saliva was cultivated in tissue culture. In contrast, PCR revealed no VZV DNA in saliva sampled identically three times over a two-week period from six control subjects with chronic pain or in any of 14 healthy adults. The observed decline in salivary VZV DNA in zoster patients, matched by reduction of pain in nearly all patients and ultimate disappearance of pain in 74% of patients by the end of the two-week study period, most likely reflects a boost in cell-mediated immune responsiveness to VZV that occurs in adults with zoster [11] combined with oral antiviral treatment.

VZV DNA has been detected in saliva of patients with Ramsay Hunt syndrome (zoster oticus and peripheral facial palsy) [12, 13]. Because this syndrome results from virus reactivation in the geniculate ganglion, the detection of VZV DNA in saliva of such patients is readily explained anatomically since visceral efferent parasympathetic fibers of the seventh cranial nerve pass through the geniculate ganglion before innervating the salivary glands. None of our patients had geniculate zoster and no known anatomic pathways explain the detection of VZV DNA in saliva of our patients with zoster in trigeminal, cervical, thoracic and lumbar dermatomes remote from geniculate ganglia. One possibility might rest in VZ viremia. VZV DNA can be found in blood mononuclear cells one to 23 days after zoster [14, 15], and isolation of infectious VZV from the blood of an immunocompetent patient with zoster has been reported [16]. Infectious VZV can also be recovered from blood mononuclear cells of immunosuppressed cancer patients two to six days after zoster [17]. Another possibility is that VZV reactivated from geniculate ganglia [18] simultaneously with VZV reactivation from ganglia in the dermatome where zoster occurred. VZV is latent in ganglia at all levels along the

human neuraxis [19]. The notion of simultaneous VZV reactivation from multiple ganglia is provided by the classic work of Lewis [20] who described dermatomal distribution radicular pain in areas distinct from pain with rash, as well as by virological verification of VZV vasculopathy in a dermatome distant from the original site of zoster [21].

Our findings demonstrate the usefulness of saliva to detect virus in zoster patients. VZV DNA was present in saliva of every zoster patient early in disease. Interestingly, there were a few instances when VZV DNA was found in saliva of zoster patients after pain had disappeared, and once at a time when radicular pain preceded rash. Since there have been multiple reports of virologically confirmed VZV-induced neurological disease without any history of zoster rash, including myelitis [22], cerebellar ataxia [23, 24], meningoencephalitis [25], VZV vasculopathy [26] and zoster sine herpete [27], it will be important to see if VZV DNA can be detected in saliva of such patients. To date, definitive virological confirmation required blood and CSF examination for VZV DNA and anti-VZV IgG, including reduced serum/CSF ratios of anti-VZV IgG compared to reduced serum/CSF ratios of albumin or total IgG [2, 21]. Finally, correlative clinical-virological analyses have suggested that postherpetic neuralgia (PHN), the most common complication of zoster, might be due to a persistent low-grade ganglionitis [28]. The detection of VZV DNA in saliva of PHN patients, but not in control zoster patients who did not develop PHN, would provide further evidence for the presence of low-grade ganglionitis and point to a need for more aggressive antiviral treatment.

## **ACKNOWLEDGMENTS**

We thank Dr. John Blaho for providing HSV-1-specific antiserum, Marina Hoffman for editorial review, and Cathy Allen for assistance in the preparation of the manuscript.



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