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Varicella-Zoster Virus in the Saliva of Patients with Herpes Zoster

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Abstract

Fifty-four patients with herpes zoster were treated with valacyclovir. On treatment days 1, 8, and 15, pain was scored and saliva examined for varicella-zoster virus (VZV) DNA. VZV DNA was found in every patient the day treatment was started and later disappeared in 82%. There was a positive correlation between the presence of VZV DNA and pain and between VZV DNA copy number and pain ($P<.0005$). VZV DNA was present in 1 patient before rash and in 4 after pain resolved and was not present in any of 6 subjects with chronic pain or in 14 healthy subjects. Analysis of human saliva has potential usefulness in the diagnosis of neurological disease produced by VZV without rash.

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

Methods

All human-study protocols were approved by the Committee for the Protection of Human Subjects of the Lyndon B. Johnson Space Center (control subjects) and by the Institutional Review Board of the University of Texas Health Sciences Center (patients with herpes zoster). Informed consent was obtained from all subjects. The 54 patients with herpes zoster consisted of 29 women (21–82 years old) and 25 men (35–79 years old) (table 1). All patients with herpes zoster were treated with oral valacyclovir (1 g 3 times daily) for 7 consecutive days. Pain was described by all patients on a scale of 0 (no pain) to 10 (worst pain) [1]. Six control subjects consisted of 5 women (24–64 years old) and 1 man (38 years old) with chronic pain due to malignancy or non-VZV inflammatory disease. Fourteen additional healthy control subjects consisted of 9 men and 5 women (34–70 years old). Three saliva samples were collected at weekly intervals from all 6 control subjects with chronic pain and from the 14 healthy control subjects.

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Saliva samples from all subjects were obtained by having them suck on a cotton plug for 1–2 min, after which the saturated cotton was placed in a salivette tube (Sarstedt). The salivette tube was centrifuged at 1303 *g* for 10 min to collect saliva in the outer part of the salivette tube. Saliva was then concentrated with a Microsep 100K filtration unit (Filtron Technology) by centrifugation at 4552 *g* for 2 h. DNA was extracted with nonorganic extraction reagents (Qiagen). Microcarrier gel (Molecular Research Center) was added to facilitate DNA recovery. DNA was dissolved in 50 μL of nuclease-free water (Amresco). Quantitative real-time polymerase chain reaction (PCR) was performed in a TaqMan 7700 sequence detector (Applied Biosystems) using fluorescence-based simultaneous amplification and product detection. Primers and probes specific for VZV, herpes simplex virus (HSV)–1 and GAPDH DNA sequences have been described elsewhere [2,3].

To avoid contamination from skin lesions, patients did not touch the salivette cotton roll while collecting saliva. Samples were collected on day 1 before antiviral therapy and again on days 8 and 15. Months after the study was under way and we knew that VZV was present in saliva of patients with herpes zoster, saliva from 2 patients (patients 11 and 19 in table 1) were inoculated onto human fetal lung-cell fibroblasts and observed for cytopathic effect (CPE) [3]. At the height of CPE, cells were analyzed by PCR with HSV-1–specific and VZV-specific primers [2,3] and by immunohistochemistry for viral antigens.

Random-effects logistic regression was used to correlate pain with the presence of VZV DNA in the saliva of patients with herpes zoster [4]. Association between pain and the VZV DNA burden was determined using the Somers *D* test [5], a nonparametric analog to the regression coefficient in ordinary linear regression, which is related to forms of Kendall's τ [6].

Results

All control subjects and patients with herpes zoster were seropositive for VZV (data not shown). Table 1 lists the level of pain reported by 54 patients with herpes zoster before and after treatment, as well as the VZV DNA copy number per milliliter of saliva, as detected by real-time PCR. For 50 of the 54 patients, follow-up data were available. 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 (86%) of 50 patients, pain decreased during the 14-day study period; in 2 patients (patients 33 and 45), pain transiently increased before decreasing. Ultimately, in 37 (74%) of the 50 patients, pain disappeared entirely during the 14-day study period. Three (6%) of the 50 patients (patients 9, 41, and 50) developed an increase in pain after it had disappeared. In 4 (8%) of 50 patients (patients 6, 9, 16, and 34), pain increased throughout the 14-day study period.

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). Despite repeated phone calls, some patients did not return for saliva collection but did provide pain scores. Independent of pain score, VZV DNA was detected in saliva from all 54 patients with herpes zoster on day 1 before treatment was started. Linear regression analysis to model log VZV DNA copies on day 1 revealed no significant effect of age ($P = .225$) or sex ($P = .652$). No HSV DNA was found in saliva from any of the 54 patients with herpes zoster or control subjects, whereas GAPDH DNA was present in all saliva samples.

In 47 (94%) of 50 patients, virus DNA copy numbers decreased in saliva during the 14-day study period, although in 3 patients (6%; patients 6, 31, and 45) virus DNA copy numbers transiently increased before decreasing. Ultimately, virus DNA disappeared from saliva during the 14-day study period in 41 (82%) of the 50 patients. No patients developed any increase in

virus DNA copy number after it had begun to decline or disappear from saliva. In 2 patients (patients 16 and 34), virus DNA copy number increased throughout the 14-day study period. In 4 patients (patients 7, 9, 27, and 53), VZV DNA was detected in saliva after pain resolved. There was a significant positive correlation between pain and the presence of VZV DNA in saliva ($P < .0005$) as well as between pain and the VZV DNA burden ($P < .0005$). Overall, reported pain levels were highest when the VZV copy numbers were high. Furthermore, as VZV DNA disappeared, pain scores decreased.

In the 6 patients with chronic nondermatomal distribution pain, VZV DNA was not detected in any of 18 saliva samples (3 from each patient) obtained over a 2-week period. During a 6-month follow-up period, none of these patients developed herpes zoster or exhibited an increase in VZV-specific IgG levels (Quest Laboratory). In 14 other healthy control subjects, VZV DNA was not detected in any of 42 saliva samples (3 from each individual) obtained over a 2-week period (data not shown).

Saliva samples from 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 cultures of saliva from patient 11. Both PCR and immunohistochemistry revealed that the CPE was VZV specific (data not shown).

One 21-year-old patient with herpes zoster whose pain preceded rash (patient 48 in table 1) was studied extensively (table 2). 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; 3 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 (1 g 3 times daily for 7 days). Two days after antiviral treatment, the pain level decreased to 7, and VZV was detected in saliva and peripheral blood mononuclear cells (PBMCs). Seven days after antiviral treatment, her pain level was still 7, but VZV DNA was no longer detected in saliva, although it was found in her PBMCs. Three weeks after the 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 and pain. When rash developed, every patient was treated immediately with valacyclovir and then studied for 2 weeks. VZV DNA was found in the saliva of all 54 patients. During the 2-week study period, the VZV DNA copy number declined in nearly all patients and disappeared in 82% of the patients. In 2 patients with herpes zoster, 1 of whom (patient 16) was being treated with immunosuppressive drugs for cancer, both salivary virus DNA copy number and pain increased throughout the 14-day study period. In 2 other patients (patients 31 and 45), salivary VZV DNA copy number also increased from day 1 to 8, but both virus and pain disappeared by day 15. In addition to the detection of VZV DNA in saliva from all patients with herpes zoster, infectious VZV was isolated from 1 of 2 patients with herpes zoster whose saliva was cultivated in tissue culture. In contrast, PCR revealed no VZV DNA in saliva sampled identically 3 times over a 2-week period from 6 control subjects with chronic pain or in any of 14 healthy adults. The observed decline in salivary VZV DNA copy number in patients with herpes zoster, matched by a reduction in pain in nearly all patients and the ultimate disappearance of pain in 74% of patients by the end of the 2-week study period, most likely reflects a boost in cell-mediated immune responsiveness to VZV that occurs in adults with herpes zoster [7] combined with oral antiviral treatment.

VZV DNA has been detected in the saliva of patients with Ramsay Hunt syndrome (zoster oticus and peripheral facial palsy) [8,9]. Because this syndrome results from virus reactivation in the geniculate ganglion, the detection of VZV DNA in the 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 the saliva of our patients with zoster in trigeminal, cervical, thoracic, and lumbar dermatomes remote from geniculate ganglia. One possibility might rest in VZV viremia. Infectious VZV has been recovered from blood mononuclear cells (MNCs) of immunosuppressed patients with cancer 2–6 days after zoster [10], VZV has been isolated from the blood of an immunocompetent patient with zoster [11], and VZV DNA can be found in blood MNCs 1–23 days after zoster [12,13]. Another possibility is that VZV reactivated from geniculate ganglia simultaneously with VZV reactivation from ganglia in the dermatome where zoster occurred. VZV is latent in ganglia at all levels along the human neuraxis. The notion of simultaneous VZV reactivation from multiple ganglia is provided by the classic work of Lewis [14], 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 [15].

Our findings demonstrate the usefulness of saliva for the detection of virus in patients with herpes zoster. VZV DNA was present in the saliva of every patient with zoster early during disease. Interestingly, there were a few instances when VZV DNA was found in the saliva of patients with herpes zoster after pain had disappeared, and once when radicular pain preceded rash. Given that there have been multiple reports of virologically confirmed VZV-induced neurological disease without any history of zoster rash—including myelitis, cerebellar ataxia, meningoencephalitis, VZV vasculopathy, and zoster sine herpete—it will be important to determine whether VZV DNA can be detected in the saliva of such patients. To date, definitive virological confirmation has required blood and cerebrospinal fluid examination for VZV DNA and anti-VZV IgG.

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Table 1

Characteristics of patients with herpes zoster, pain scores, and salivary varicella-zoster virus (VZV) DNA burden during antiviral treatment.

Patient (age in years/sex)	Underlying disease	Zoster site[a]	Days of pain before antiviral treatment,	Days of rash before antiviral treatment	Day 1 (start of treatment)		Day 8		Day 15	
					Pain	VZV[b]	Pain	VZV[b]	Pain	VZV[b]
1 (64/F)	None	V1	2	1	3	1.4×10^7	ND	ND	0	0
2 (52/M)	Stomach cancer	V1	4	2	3	3.2×10^4	0	0	0	0
3 (44/M)	None	V1	18	12	7	1.0×10^5	3	9.3×10^3	0	0
4 (48/F)	None	V1	1	1	2	1.8×10^5	1	1.8×10^3	1	1.8×10^3
5 (59/F)	None	V1	1	1	1	6.3×10^2	0	0	0	0
6 (62/M)	Cancer	V1	14	7	5	2.5×10^4	5	6.8×10^4	7	4.7×10^4
7 (41/M)	None	V1	6	2	6	1.0×10^3	0	6.3×10^2	0	0
8 (82/F)	Infected finger	V1	7	2	4	5.5×10^3	2	2.2×10^3	0	0
9 (75/M)	Hypertension	C2	3	1	1	7.2×10^4	0	3.4×10^4	2	1.3×10^3
10 (65/M)	Hypertension, hypothyroidism	C2	7	3	8	5.0×10^5	5	4.9×10^2	3	0
11 (79/M)	None	C2–C3	6	2	6	6.5×10^3	ND	ND	ND	ND
12 (49/F)	None	C3	1	1	7	1.4×10^7	2	7.5×10^2	0	0
13 (40/M)	Colon cancer	C3	3	7	3	1.2×10^6	2	3.8×10^3	2	3.5×10^2
14 (53/F)	Hepatitis	C3	6	1	6	8.0×10^0	ND	ND	0	0
15 (44/F)	None	C3	3	1	7	5.8×10^5	2	0	0	0
16 (48/M)	Malignant brain tumor	C3	3	1	2	4.5×10^1	4	3.0×10^2	6	1.0×10^5
17 (63/M)	Back surgery	C3–C4	14	10	4	6.5×10^4	0	0	0	0
18 (26/F)	None	C4	21	3	6	8.2×10^4	2	0	0	0
19 (26/F)	None	C4	12	5	4	4.5×10^4	ND	ND	ND	ND
20 (47/F)	Cancer	T1	2	1	1	8.0×10^1	0	0	0	0
21 (58/F)	Lumbar disk disease	T2	14	4	5	2.0×10^4	2	2.1×10^2	0	0
22 (53/F)	Asthma, anemia	T2–T3	13	3	6	6.6×10^3	ND	ND	ND	ND
23 (66/F)	None	T3	5	1	3	6.9×10^1	0	0	0	0
24 (51/M)	None	T3–T4	7	6	2	4.2×10^1	0	0	0	0

Patient (age in years/sex)	Underlying disease	Zoster site[a]	Days of pain before antiviral treatment,	Days of rash before antiviral treatment	Day 1 (start of treatment)		Day 8		Day 15	
					Pain	VZV[b]	Pain	VZV[b]	Pain	VZV[b]
25 (75/M)	None	T3–T4	4	2	2	4.3×10^2	0	0	0	ND
26 (39/F)	None	T4	5	3	4	2.3×10^1	0	0	0	0
27 (48/M)	None	T4–T5	3	2	5	8.8×10^4	0	3.1×10^3	0	0
28 (30/F)	None	T5	4	3	6	4.4×10^1	ND	ND	0	0
29 (56/F)	Hyperlipidemia, kidney stone, diabetes	T5	5	3	3	6.5×10^1	0	0	0	0
30 (60/M)	None	T5	8	7	2	9.0×10^0	0	ND	0	0
31 (65/M)	None	T5	3	1	8	1.9×10^4	6	1.7×10^5	0	0
32 (59/M)	None	T5	3	2	3	1.6×10^1	0	0	0	0
33 (62/M)	None	T5	12	5	1	1.6×10^2	4	0	2	0
34 (62/M)	None	T5–T6	3	2	5	1.2×10^2	6	4.8×10^2	6	4.5×10^4
35 (58/F)	None	T6	4	2	2	1.3×10^2	ND	ND	ND	ND
36 (43/F)	None	T6	5	3	4	2.1×10^1	0	0	ND	ND
37 (61/M)	Hypertension, chronic back pain	T6	6	3	4	5.8×10^2	0	0	0	0
38 (40/M)	None	T6	2	1	2	1.9×10^3	0	0	0	0
39 (39/M)	None	T6	6	5	5	2.7×10^7	2	3.5×10^5	0	0
40 (35/F)	Hypertension	T6–T7	9	4	8	7.6×10^3	3	6.1×10^3	0	0
41 (59/F)	None	T7	3	2	2	2.7×10^1	0	0	1	0
42 (23/F)	None	T7–T8	6	6	3	2.6×10^4	0	ND	0	0
43 (50/M)	None	T8	5	3	6	1.5×10^3	3	0	0	0
44 (35/M)	Heart disease	T8	14	3	2	6.5×10^1	0	0	0	0
45 (72/F)	None	T11	5	2	2	2.2×10^2	3	2.6×10^3	0	0
46 (69/F)	None	T11	3	2	3	3.5×10^1	ND	ND	0	0
47 (65/F)	None	T12	2	1	6	5.7×10^3	0	0	0	0
48 (21/F)	None	T12	3	0	9	5.5×10^3	ND	ND	ND	ND
49 (51/F)	None	L1	2	1	2	2.9×10^1	0	0	0	0
50 (58/F)	None	L2–L3	4	2	3	4.3×10^1	0	0	2	0
51 (72/M)	None	L3	1	1	3	1.5×10^2	ND	ND	1	0

Patient (age in years/sex)	Underlying disease	Zoster site[a]	Days of pain before antiviral treatment,	Days of rash before antiviral treatment	Day 1 (start of treatment)		Day 8		Day 15	
					Pain	VZV[b]	Pain	VZV[b]	Pain	VZV[b]
52 (67/F)	None	L3	5	1	3	1.4×10^2	0	0	0	0
53 (44/F)	None	L4–L5	21	3	4	4.5×10^4	0	1.1×10^2	0	0
54 (74/M)	None	L5	2	1	2	1.4×10^2	ND	ND	0	0

NOTE. ND, not determined because of failure of patient to return to the clinic for follow-up of pain/salivary VZV DNA determinations.

[a] Site of zoster: C, cervical; L, lumbar; T, thoracic; V1, ophthalmic division of trigeminal nerve.

[b] VZV DNA copies/mL of saliva.

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Table 2

Clinical and laboratory profile of a 21-year-old patient (patient 48 in table 1) with thoracic-distribution preherpetic neuralgia followed by zoster.

			Days after antiviral treatment		
Parameter	**3 days before rash[a]**	**Onset of rash[b]**	**2**	**7**	**21**
Pain score[c]	8	9	7	7	0
VZV DNA					
In saliva[d]	1.6×10^1	5.5×10^3	6.5×10^1	0	0
In vesicle fluid	NA	−	+	+	ND
In PBMCs	−	−	+	+	ND
In plasma	+	+	−	−	ND

NOTE. NA, not applicable; ND, not determined; PBMCs, peripheral blood mononuclear cells; VZV, varicella-zoster virus.

[a] Initial emergency department visit, no rash.

[b] Sample taken before oral valacyclovir treatment.

[c] Pain rating on a 10-point scale.

[d] VZV DNA copies/mL of saliva, determined by real-time polymerase chain reaction.