The Journal of Infectious Diseases

MAJOR ARTICLE

Diagnostic Usefulness of Varicella-Zoster Virus Real-Time Polymerase Chain Reaction Analysis of DNA in Saliva and Plasma Specimens From Patients With Herpes Zoster

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Background. We evaluated the diagnostic usefulness of polymerase chain reaction (PCR) analysis for detecting varicella-zoster virus (VZV) infection and reactivation of VZV, using DNA extracted from saliva and plasma specimens obtained from subjects with suspected herpes zoster and from healthy volunteers during stressful and nonstressful conditions.

Methods. There were 52 patients with a diagnosis of herpes zoster (group 1), 30 with a diagnosis of zoster-mimicking disease (group 2), and 27 healthy volunteers (group 3). Saliva and plasma samples were evaluated for VZV DNA by real-time PCR analysis.

Results. Among patients with suspected herpes zoster (ie, patients in groups 1 and 2), the sensitivity of PCR analysis of salivary DNA for detecting VZV (88%; 95% confidence interval [CI], 74%–95%) was significantly higher than that of PCR analysis of plasma DNA (28%; 95% CI, 16%–44%; $P < .001$), whereas the specificity of PCR analysis of salivary DNA (100%; 95% CI, 88%–100%) was similar to that of PCR analysis of plasma DNA (100%; 95% CI, 78%–100%; $P > .99$). VZV DNA was not detected in saliva and plasma samples from group 3 (0%; 95% CI, 0%–14%).

Conclusions. Real-time PCR analysis of salivary DNA is more sensitive than that of plasma DNA for detecting VZV among patients with suspected herpes zoster. We found no subclinical reactivation of VZV in group 3 following exposure to common stressful conditions.

Keywords. Herpes zoster; varicella-zoster virus (VZV); saliva; plasma.

Primary infection with varicella-zoster virus (VZV) results in chickenpox, and reactivation of latent VZV infection manifests as a painful blistering rash called herpes zoster (HZ) [1]. The HZ rash is sufficiently distinctive that a diagnosis based on clinical findings is usually accurate. However, diagnostic confirmation may occasionally be needed for the following reasons [2]. First, the skin lesions may be atypical, especially in immunocompromised patients. Second, numerous conditions mimic the presentation of HZ, such as herpes simplex virus (HSV) infection and dermatologic disease [3]. In several clinical diagnostic studies, up to 10% of specimens submitted from patients with presumed HZ contained HSV [4]. Although the antiviral treatment is the same, the dose may differ, and herpes simplex can recur over short intervals. Therefore, the need to distinguish between these 2 viruses is important in terms of antiviral use and prognosis. Finally, some individuals experience pain in the absence of any characteristic rash; this condition is termed zoster sine herpete and is difficult to diagnose [5]. A previous study showed that the presence of unexplained dermatomal pain did not predict clinical or subclinical reactivation [6]. Routine antiviral agent use for this purpose is not supported, and there is some concern that such therapy may prevent appropriate investigation of the pain etiology [2]. Therefore, diagnostic laboratory tests are needed for VZV infections with or without cutaneous lesions [6].

Previous studies have shown that active VZV infection can be confirmed by detecting VZV DNA in human saliva and blood specimens [7, 8]. Indeed, in a previous study, salivary VZV was detected in all 54 patients with acute zoster who were studied over a 3-week period [7]. However, VZV was found in saliva specimens from healthy but stressed astronauts because of subclinical VZV reactivation during and after flight [9], while salivary VZV DNA was not detected in healthy individuals without stress [10]. Therefore, if VZV-specific PCR analysis of saliva specimens frequently has positive results under common stressful conditions, the diagnostic usefulness of PCR analysis of saliva specimens will be decreased by false-positive results caused by subclinical VZV reactivation. However, there are limited data on whether subclinical VZV reactivation occurs under normal stressful conditions, such as examinations. We therefore evaluated the diagnostic usefulness of VZV-specific PCR, using

Received 5 July 2017; editorial decision 15 September 2017; accepted 19 September 2017; published online September 26, 2017.

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DOI: 10.1093/infdis/jix508

DNA extracted from saliva and plasma specimens from subjects with suspected HZ, and investigated the use of PCR to detect VZV reactivation, using DNA in saliva and plasma specimens obtained from healthy volunteers exposed to stress.

METHODS

Study Population

Patients With HZ or Zoster-Mimicking Disease

Patients aged ≥18 years with suspicion of HZ were enrolled between May 2016 and April 2017. HZ was diagnosed by the attending physicians on the basis of the presence of a typical dermatomal distribution rash and/or pain or molecular confirmation of VZV in skin lesion or cerebrospinal fluid specimens (group 1) while blinded to results of VZV DNA–specific PCR analysis of saliva or plasma specimens. Patients who had HZ-mimicking disease (group 2) were defined as those with (1) zoster-mimicking skin lesions and (2) zoster sine herpete–mimicking disease, such as atypical pain without skin lesions and aseptic meningitis with initial differential diagnosis of VZV meningitis. The final diagnosis was made by attending physicians on the basis of routine molecular, pathologic, and microbiologic confirmation of VZV absence from skin lesion or cerebrospinal fluid specimens, as well as clinical manifestations, with physicians blinded to saliva or plasma VZV DNA–specific PCR results. Patients without clear evidence of noninfection with VZV or whose diagnosis was indeterminate were excluded from group 2. When patients with HZ or zoster-mimicking disease visited the outpatient clinic or during admission, saliva samples, with or without concurrent collection of blood samples, were collected upon receipt of the primary diagnosis of HZ or zoster-mimicking disease. Samples were collected before or after antiviral treatment. The median time from symptom onset and rash onset to sample collection was 6 days (interquartile range [IQR], 3–7 days) and 3 days (IQR, 2–5 days), respectively.

Healthy Volunteers During and After Stressful Conditions

Healthy university students during and after midterm examinations were enrolled as volunteers (group 3). To evaluate VZV subclinical reactivation, matched saliva and plasma samples were collected under stressful conditions (during midterm examinations) and nonstressful conditions (1 month after midterm examinations). Stress was defined on a 10-point scale based on volunteer self-reporting. Zero on the stress scale corresponded to no stress, and 10 on the scale corresponded to the most severe stress encountered. The local institutional review board approved this study, and informed consent was obtained from all subjects (groups 1–3).

VZV DNA–Specific PCR Analysis of Salivary and Plasma DNA

Plasma samples were obtained from heparin-treated peripheral blood specimens and immediately frozen at −80°C. Saliva samples (at least 1 mL) were collected with an Omnigene-Oral kit (DNA Genotek, Ottawa, Canada) at any time of day, at least 1 hour after a meal. The samples were shaken vigorously for at least 10 seconds and incubated in a water bath at 50°C for 1 hour. DNA was extracted with a Qia-Amp DNA mini-kit (Qiagen, Chatsworth, CA) as described by the manufacturer. VZV was quantified with a VZV-specific real-time PCR kit (GeneProof, Brno, Czech Republic), using a LightCycler 480 System (Roche, Basel, Switzerland). VZV DNA copy numbers were determined by comparing the cycle thresholds of the test samples to the cycle threshold of the reference VZV DNA supplied with the PCR kit. The limit of quantitative PCR detection was 1 copy of VZV DNA per PCR reaction or 10 copies/mL, which is the lowest concentration in 95% of positive samples during 20 repeated test samples, as defined in Minimum Information for Publication of Quantitative Real-Time PCR Experiments guidelines [11].

Statistical Analysis

Statistical analyses were performed with SPSS, version 20 (IBM, Armonk, NY). Continuous variables are expressed as means ± standard deviations. Continuous variables were compared by the Mann-Whitney *U* test or the Student *t* test, as appropriate. Categorical variables were compared by the Pearson χ^2 test or the Fisher exact test. Paired data were compared by the Wilcox test. The Spearman correlation coefficient was used to compare viral loads between saliva and plasma samples, and the McNemar test was used to compare the diagnostic accuracy of saliva and plasma VZV DNA–specific PCR. A 2-tailed *P* value of $< .05$ was considered to be statistically significant.

RESULTS

Baseline Characteristics

During the study period, 117 subjects were prospectively identified, and 109 subjects were enrolled in the final analysis (Figure 1). Of these, 52 were classified as having HZ. Saliva specimens were collected from 52 patients with HZ, of whom 46 (88%) provided blood specimens concurrently. Skin lesion specimens from 16 patients (31%) were subjected to VZV DNA–specific PCR analysis. Of these patients, VZV DNA was detected in 15 (94%). PCR analysis of skin lesion specimens was performed more frequently for immunocompromised patients than for immunocompetent patients (60% vs 19%; $P = .006$). The most common sites of zoster were V1 (31%) and the thoracic nerve area (21%). Disseminated zoster was present in 4 patients (8%).

Group 2 patients included those with zoster-mimicking skin lesions and zoster sine herpete–mimicking conditions (Table 1). A total of 38 had zoster-mimicking conditions, and 8 patients were excluded because of indeterminate diagnosis (Figure 1). Finally, 30 patients were included in group 2. Zoster-mimicking skin lesions were due to HSV infection (n = 16), contact dermatitis (n = 2), insect bite (n = 1), and Sweet syndrome (n = 1). Pain syndrome not related to VZV was due to spinal stenosis

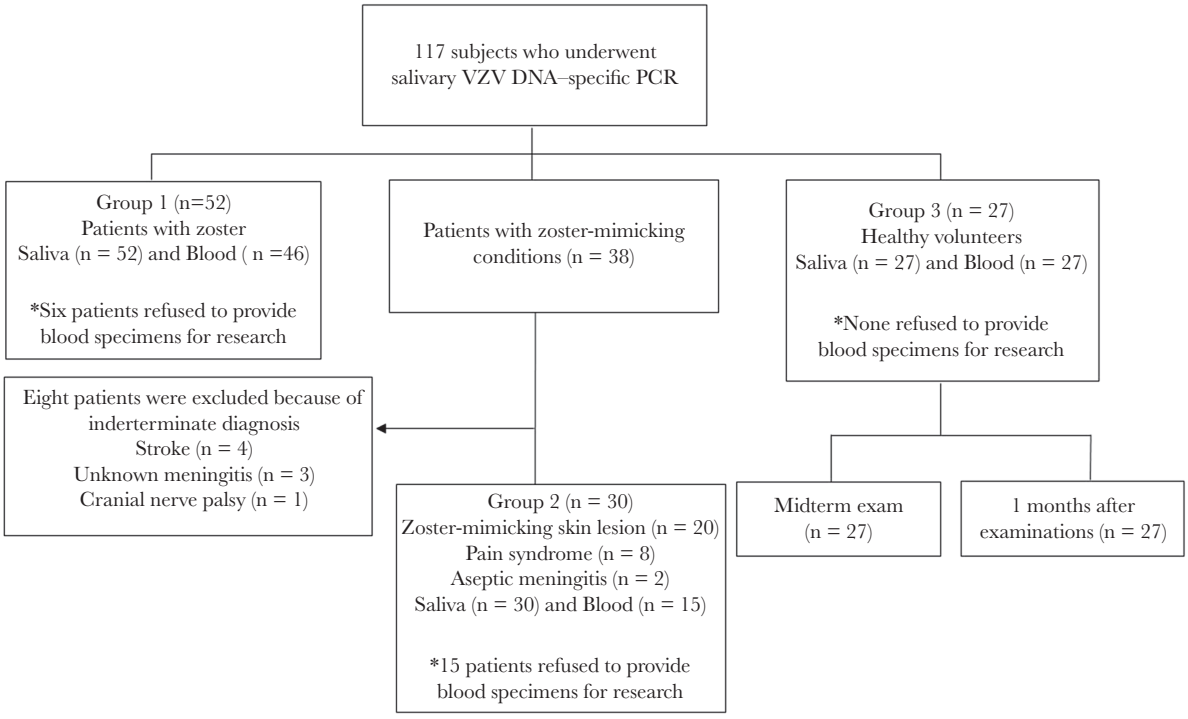

Figure 1. Flow chart of the study. VZV, varicella-zoster virus.

(n = 4), cancer (n = 2), trigeminal neuralgia (n = 1), and renal calculi (n = 1). Causes of aseptic meningitis included leptomeningeal seeding (n = 1) and Epstein-Barr virus infection (n = 1). Of these 30 patients, saliva specimens were collected from all 30 patients, and blood specimens were collected concurrently from 15 patients. A total of 16 group 2 patients (31%) underwent VZV DNA–specific PCR analysis of skin lesions, but all samples showed negative results. The demographic characteristics of the patients with HZ and those with zoster-mimicking conditions are given in Table 1.

Of the 27 healthy volunteers (group 3), saliva and blood specimens were collected concurrently from all subjects under stressful conditions (during midterm examinations) and nonstressful conditions (1 month after midterm examinations).

VZV DNA–Specific PCR Analysis of Saliva and Plasma Specimens From Study Subjects

VZV DNA Results for Subjects With HZ

Salivary DNA was extracted from the 52 patients with HZ, and VZV DNA was detected in saliva specimens from 46 patients (88%). The mean salivary VZV DNA load (±SD) was 2.37 ± 1.47 log copies/mL. The baseline characteristics and DNA loads in saliva and plasma specimens from the 52 patients with HZ are shown in Supplementary Table 1.

VZV was detected in plasma specimens from 13 of 46 patients (28%) with zoster from whom saliva and blood specimens were collected concurrently. Viremia was detected more often in the immunocompromised patients than in the immunocompetent ones (50% vs 19%; P = .04). VZV DNA loads in plasma specimens were higher in immunocompromised patients than in immunocompetent patients (1.09 vs 0.31 log copies/mL; P = .008). However, saliva DNA loads did not differ between immunocompromised patients and immunocompetent patients (2.29 vs 2.40 log copies/mL; P = .83). Of the 13 patients with plasma specimens that tested positive by VZV PCR, 9 also had saliva specimens with positive VZV PCR results. The remaining 3 patients with positive VZV PCR results did not have VZV DNA in their saliva. The mean VZV DNA load (±SD) in plasma specimens was 0.55 ± 0.95 log copies/mL. There was no correlation between salivary VZV DNA load and plasma VZV DNA load (Spearman r^2 = 0.09; P = .55; Figure 2).

The mean VZV DNA load in saliva was higher than that in plasma (2.37 vs 0.55 log copies/mL; P < .001). VZV DNA was present in saliva or plasma specimens from 44 of 46 patients (96%) from whom we concurrently collected saliva and blood samples.

We analyzed the differences according to site of zoster in case of localized HZ (cranial nerve vs other dermatomes; Supplementary Table 2). Interestingly, the salivary VZV DNA load was higher in patients with cranial nerve involvement than in patients without cranial nerve involvement (2.81 vs 1.88 log copies/mL; P = .02). Plasma VZV DNA was detected less frequently in patients with cranial nerve involvement than in

Table 1. Characteristics of Patients With Suspected Herpes Zoster

Variable	Herpes Zoster (n = 52)	Zoster-Mimicking Conditions (n = 30)	*P*
Male sex	26 (50)	16 (53)	.48
Age, y, mean ± SD	52.6 ± 14.3	50.0 ± 15.7	.36
No underlying disease	26 (50)	16 (53)	.48
Underlying disease			
Malignancy	13 (25)	6 (20)	.41
Diabetes mellitus	7 (13)	3 (10)	.47
Transplant recipient	9 (17)	2 (5)	.08
Immunocompromised	15 (29)	5 (17)	.17
Zoster site			
V1	16 (31)	…	
Thoracic nerve	11 (21)	…	
Lumbosacral nerve	10 (19)	…	
Cervical nerve	8 (15)	…	
VII	2 (4)	…	
V2	1 (2)	…	
Disseminated	4 (8)	…	
Diagnosis		…	
Zoster-mimicking skin lesion			
HSV	…	16 (53)	
Other[a]	…	4 (13)	
ZSH-mimicking disease			
Pain syndrome[b]	…	8 (27)	
Aseptic meningitis[c]	…	2 (7)	

Data are no. (%) of patients, unless otherwise indicated.

Abbreviations: HSV, herpes simplex virus; ZSH, zoster sine herpete.

[a]Contact dermatitis (n = 2), insect bite (n = 1), and Sweet syndrome (n = 1).

[b]Pain syndrome not related to varicella-zoster virus consisted of pain due to spinal stenosis (n = 4), cancer (n = 2), trigeminal neuralgia (n = 1), and renal calculi (n = 1).

[c]Due to leptomeningeal seeding (n = 1) and Epstein-Barr virus infection (n = 1).

patients with involvement at other sites (6% vs 37%; $P = .02$). Plasma VZV DNA load was also lower in patients with cranial nerve involvement than in patients with other dermatomes (0.07 vs 0.68 log copies/mL; $P = .004$). Analysis of saliva and plasma specimens collected ≤4 days versus ≥5 days after rash onset revealed that VZV DNA positivity (90% [34 of 38 patients] vs 86% [12 of 14] in saliva specimens [$P = .52$] and 30% [10 of 33 patients] vs 23% [3 of 13] in plasma specimens [$P = .45$]) and VZV DNA loads (2.38 vs 2.57 log copies/mL in saliva specimens [$P = .72$] and 0.56 vs 0.51 log copies/mL in plasma specimens [$P = .88$]) did not differ according to sample collection time.

VZV DNA Results for Subjects With Zoster-Mimicking Conditions

No VZV DNA was detected in saliva specimens from the 30 patients in group 2. Plasma samples were collected from 15 patients (50%; Figure 1), and no specimens yielded VZV DNA. Among the 15 patients from whom saliva and blood specimens were collected concurrently, samples were collected from 6 (40%) after antiviral agent administration.

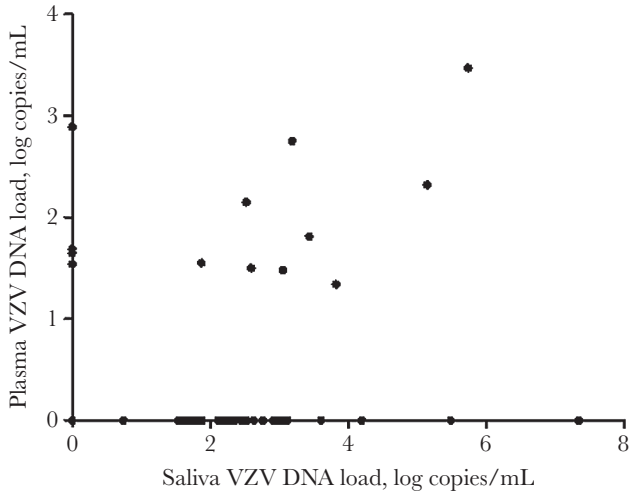

Figure 2. Relationship between saliva varicella-zoster virus (VZV) DNA load and plasma VZV DNA load. There was no correlation between VZV DNA loads in saliva and plasma specimens (Spearman $r^2 = 0.09$; $P = .55$)

Results for Healthy Students Under Stressful and Nonstressful Conditions

Group 3 patients consisted of 27 healthy university students. Their mean age (±SD) was 23.0 ± 2.5 years, and 21 (78%) were male. All had histories of VZV vaccination. The 10-point stress score decreased significantly from the time of a midterm examination to 1 month after the examination (mean scores [±SD], 7.3 ± 0.3 and 3.2 ± 0.4, respectively; $P < .001$). No saliva or blood samples (0%; 95% confidence interval [CI], 0%–14%) obtained during stressful or nonstressful conditions were positive for VZV DNA.

Diagnostic Performance of VZV DNA–PCR Analysis of Saliva and Plasma Specimens

The diagnostic performances of the salivary and plasma VZV DNA–specific PCR analyses are presented in Table 2. The sensitivity, specificity, positive predictive value, and negative predictive value of salivary VZV DNA–specific PCR analysis for HZ were 88%, 100%, 100%, and 83%, respectively. The sensitivity, specificity, positive predictive value, and negative predictive value of plasma VZV PCR for HZ were 28%, 100%, 100%, and 31%, respectively. The sensitivity of saliva VZV DNA–specific PCR analysis for HZ (88%) was significantly higher than that of plasma VZV DNA–specific PCR analysis (28%; $P < .001$), while the specificity of salivary VZV DNA–specific PCR analysis for HZ (100%) was similar to that of plasma VZV DNA–specific PCR analysis (100%; $P > .99$). Sensitivity and specificity of skin lesion VZV DNA–specific PCR analysis were 94% (95% CI, 70%–100%) and 100% (95% CI, 85%–100%), respectively.

Of the 61 patients (46 in group 1 and 15 in group 2) from whom we concurrently collected saliva and blood samples, 25 (41%; 19 from group 1 and 6 from group 2) were collected after administration of an antiviral agent. Subgroup analysis was performed

Table 2. Accuracy of Varicella-Zoster Virus (VZV) DNA–Specific Polymerase Chain Reaction Analysis for Diagnosing Herpes Zoster in Patients With Suspected Herpes Zoster

	Sensitivity		Specificity		Predictive Value (95% CI)		Likelihood Ratio (95% CI)	
Group, Specimen(s)	Proportion[a]	Percentage (95% CI)	Proportion[b]	Percentage (95% CI)	Positive	Negative	Positive	Negative
Overall (n = 82)								
Saliva	46/52	88 (74–95)	30/30	100 (88–100)	100 (92–100)	83 (67–94)	NA	0.12 (0.05–0.26)
Plasma	13/46	28 (16–44)	15/15	100 (78–100)	100 (75–100)	31 (19–46)	NA	0.72 (0.60–0.86)
Saliva or plasma	50/52	96 (87–100)	30/30	100 (88–100)	100 (93–100)	94 (79–99)	NA	0.04 (0.01–0.15)
Underwent concurrent testing for salivary and plasma VZV DNA before antiviral treatment (n = 36)								
Saliva	24/27	89 (71–98)	9/9	100 (66–100)	100 (86–100)	75 (43–95)	NA	0.11 (0.04–0.32)
Plasma	9/27	33 (17–54)	9/9	100 (66–100)	100 (67–100)	33 (17–54)	NA	0.67 (0.51–0.87)
Saliva or plasma	27/27	100 (87–100)	9/9	100 (66–100)	100 (87–100)	100 (66–100)	NA	NA
Underwent concurrent testing for salivary and plasma VZV DNA after antiviral treatment (n = 25)								
Saliva	16/19	84 (60–97)	6/6	100 (54–100)	100 (79–100)	67 (30–93)	NA	0.16 (0.06–0.45)
Plasma	4/19	21 (6–46)	6/6	100 (54–100)	100 (40–100)	29 (11–52)	NA	0.79 (0.63–1.0)
Saliva or plasma	17/19	90 (67–99)	6/6	100 (54–100)	100 (80–100)	75 (35–97)	NA	0.11 (0.03–0.39)
Immunocompetent (n = 62)								
Saliva	34/37	92 (78–98)	25/25	100 (86–100)	100 (90–99)	89 (72–98)	NA	0.08 (0.03–0.24)
Plasma	6/32	19 (7–36)	11/11	100 (72–100)	100 (54–100)	29 (16–47)	NA	0.81 (0.69–0.96)
Saliva or plasma	36/37	97 (86–100)	25/25	100 (86–100)	100 (90–100)	96 (80–100)	NA	0.03 (0.004–0.19)
Immunocompromised (n = 20)								
Saliva	12/15	80 (52–96)	5/5	100 (48–100)	100 (62–100)	63 (25–91)	NA	0.20 (0.07–0.55)
Plasma	7/14	50 (23–77)	4/4	100 (40–100)	100 (50–100)	36 (11–69)	NA	0.50 (0.30–0.84)
Saliva or plasma	14/15	93 (68–100)	5/5	100 (48–100)	100 (77–100)	83 (36–100)	NA	0.07 (0.01–0.44)

Patients were from groups 1 and 2 and are described in Methods.

Abbreviations: CI, confidence interval; NA, not available.

[a]No. with positive test results/no. tested.

[b]No. with negative test results/no. tested.

according to the initiation of antiviral treatment (Table 2). Of the 36 patients whose samples were collected before antiviral treatment, VZV DNA was present in saliva specimens from 24 (89%) and in plasma specimens from 9 (33%), including 3 patients whose saliva specimens did not contain VZV DNA. The mean salivary VZV DNA load (±SD; 2.58 ± 0.29 log copies/mL) was significantly higher than that of plasma (0.64 ± 0.19 log copies/mL; $P < .001$). Salivary VZV DNA was detected in 16 of 19 patients whose samples were collected after antiviral treatment (84%), and plasma VZV DNA was detected in 4 (21%). VZV DNA was present in saliva or plasma specimens from 17 of 19 patients (90%). The mean salivary VZV DNA load (±SD; 2.06 ± 0.32 log copies/mL) was significantly higher than the mean plasma VZV DNA load (±SD; 0.41 ± 0.21 log copies/mL; $P < .001$).

DISCUSSION

In this study, VZV DNA was detected in saliva specimens from 88% of patients (46 of 52) with zoster, while viremia was concurrently documented in 28% (13 of 46). No VZV DNA was detected in the control patients or in healthy volunteers exposed to stress.

Mehta et al reported that VZV DNA was detected in saliva specimens from all 54 patients with HZ [7]. Gershon et al also reported that detection of salivary VZV DNA in patients with abdominal pain helped to identify putative enteric zoster [12]. The reason why VZV DNA is frequently detected in saliva is not clear [7]. One possible explanation is that VZV viremia often occurs concurrently in patients with localized HZ. Another possible explanation is the geniculate ganglion hypothesis, which suggests that VZV is reactivated in geniculate ganglia at the same time as it is reactivated in ganglia of the dermatome where the zoster occurred. Some may question that the difference of the fitness of VZV between saliva and plasma can explain this phenomenon. However, when we added various concentrations of VZV directly into the saliva and plasma, we found no differences in viral load between saliva and plasma (data not shown). Therefore, we assume that the fitness of VZV in saliva and plasma is not the main reason for our observation. Although further studies are

needed to determine the pathophysiologic mechanisms, our data indicate that saliva samples are more useful for VZV DNA–specific PCR than plasma samples for documenting VZV infection.

The frequency of viremia in the current study (28%) was lower than in previous studies (60%–95%) [8, 13, 14]. This might be for one of two reasons. First, we performed the VZV DNA–specific PCR assay on plasma specimens, and several laboratories have reported that VZV DNA can be detected as, if not more, efficiently in whole-blood specimens as in plasma specimens during VZV infections and that plasma contains approximately 40% of the VZV DNA present in whole blood [14, 15]. Therefore, it is possible that the VZV DNA content was lower in plasma specimens than in whole-blood specimens and thus could not be detected by the current PCR method. However, in the case of cytomegalovirus, which is one of the herpesviruses, the sensitivity of detection was the same in plasma specimens as in whole-blood specimens [16]. In addition, a positive PCR result for a whole-blood sample may reflect noninfective remnants of the viral genome or latent virus among the blood components, which likely has unknown clinical significance [17, 18]. Thus, further studies are needed to examine this possibility. Second, because blood and saliva specimens were collected after antiviral treatment in nearly half of the patients (41%), it is possible that antiviral therapy affected the sensitivity of VZV DNA–specific PCR analysis more in plasma specimens than in saliva specimens. Despite administration of antiviral therapy to all patients, 45% had salivary VZV DNA detected 8 days after starting treatment [7]. Nagel et al reported that VZV DNA was present in saliva specimens from 66% of patients for a long interval after recovery from HZ [19]. However, the number of VZV DNA copies in the plasma decreased sharply with treatment and resolution of cutaneous eruption [14, 18]. Therefore, it is possible that VZV DNA in plasma is more susceptible to antiviral therapy than in saliva. However, when we performed a subgroup analysis differentiating patients whose samples were collected before from those whose samples were collected after antiviral therapy receipt, the diagnostic performances of the salivary VZV DNA–specific PCR analyses were similar (Table 2). Thus, saliva samples are more useful for VZV DNA–specific PCR analysis than plasma samples for documenting VZV infection.

Although vesicular PCR is the most sensitive and specific test for diagnosing HZ, it cannot be used in patients without cutaneous lesions. Therefore, salivary VZV DNA testing is particularly useful for diagnosing VZV infections that are not accompanied by cutaneous lesions, such as zoster sine herpete or enteric zoster [12, 20], but further studies of the accuracy and sensitivity of identifying VZV DNA in saliva specimens as a routine diagnostic test are necessary.

The presence of VZV DNA in saliva specimens from patients with HZ points to the usefulness of saliva for diagnosis of HZ. It appears that asymptomatic shedding of VZV DNA rarely occurs (in only 0.39% of individuals) [21]. However, severe stress alone may be associated with the appearance of VZV DNA in saliva [9, 22, 23]. Mehta et al found VZV DNA in saliva specimens from 30% of asymptomatic astronauts, during and after space flights [9]. A transient decrease in normal immune function following space travel was demonstrated by scientists at the National Aeronautics and Space Administration [24], and VZV DNA was detected in saliva specimens from medical residents who were experiencing stress [22]. The frequency of shedding of VZV DNA and copy numbers were not reported, but the likelihood of shedding increased with the extent of sleep deprivation. In addition, subclinical VZV reactivation in the saliva of patients with HZ (5% [2 of 39]) was observed in children hospitalized in an intensive care unit [23]. To evaluate VZV reactivation under stressful conditions such as those experienced in normal life, matched saliva and plasma samples were collected under stressful and nonstressful conditions, and no VZV DNA was detected in any specimens. It is possible that the level of stress caused by a midterm examination is lower than that due to hospitalization in an intensive care unit, to space flight, or to sleep deprivation. Additionally, if the group 3 patient population had been larger, we may have detected VZV DNA. Therefore, further larger studies are warranted to evaluate the usefulness of salivary VZV DNA–specific PCR analysis for diagnosing subclinical reactivation of VZV under various stressful conditions.

Our study has several limitations. First, the time points of sample collection during the course of acute HZ were not homogenously distributed. Therefore, the sensitivity of salivary or plasma VZV DNA–specific PCR analysis in the clinical setting of acute presentation of HZ may have been underestimated. Second, approximately 30% of the study patients were immunocompromised, and the resulting heterogeneity of the study population makes the interpretation of performance data and generalization of the data difficult.

In conclusion, our findings demonstrate that saliva is useful for detecting VZV DNA in patients with suspected HZ. No evidence was obtained for subclinical reactivation of VZV in saliva and plasma under relatively normal stressful conditions, such as college examinations, although the sample size was too small for drawing firm conclusions.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Financial support. This work was supported by the Korea Health Technology R&D Project through the Korea Health Industry Development Institute, which is funded by the Ministry of Health and Welfare, Republic of Korea (grant HI15C1763).

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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