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Early and Reliable Detection of Herpes Simplex Virus Type 1 and Varicella Zoster Virus DNAs in Oral Fluid of Patients With Idiopathic Peripheral Facial Nerve Palsy: Decision Support Regarding Antiviral Treatment?

Andreas Lackner,[1] Harald H. Kessler,[2] Christian Walch,[1] Stefan Quasthoff,[3] and Reinhard B. Raggam[4]*

[1]*Department of Neurotology, Medical University of Graz, Graz, Austria*
[2]*Research Unit Molecular Diagnostics, IHMEM, Medical University of Graz, Graz, Austria*
[3]*Department of Neurology, Medical University of Graz, Graz, Austria*
[4]*Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria*

Idiopathic peripheral facial nerve palsy has been associated with the reactivation of herpes simplex virus type 1 (HSV-1) or varicella zoster virus (VZV). In recent studies, detection rates were found to vary strongly which may be caused by the use of different oral fluid collection devices in combination with molecular assays lacking standardization. In this single-center pilot study, liquid phase-based and absorption-based oral fluid collection was compared. Samples were collected with both systems from 10 patients with acute idiopathic peripheral facial nerve palsy, 10 with herpes labialis or with Ramsay Hunt syndrome, and 10 healthy controls. Commercially available IVD/CE-labeled molecular assays based on fully automated DNA extraction and real-time PCR were employed. With the liquid phase-based oral fluid collection system, three patients with idiopathic peripheral facial nerve palsy tested positive for HSV-1 DNA and another two tested positive for VZV DNA. All patients with herpes labialis tested positive for HSV-1 DNA and all patients with Ramsay Hunt syndrome tested positive for VZV DNA. With the absorption-based oral fluid collection system, detections rates and viral loads were found to be significantly lower when compared to those obtained with the liquid phase-based collection system. Collection of oral fluid with a liquid phase-based system and the use of automated and standardized molecular methods allow early and reliable detection of HSV-1 and VZV DNAs in patients with acute idiopathic peripheral facial nerve palsy and may provide a valuable decision support regarding start of antiviral treatment at the first clinical visit. ***J. Med. Virol. 82:1582–1585, 2010.*** © 2010 Wiley-Liss, Inc.

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INTRODUCTION

The idiopathic peripheral facial nerve palsy also referred as "Bell's Palsy" is a diagnosis of exclusion [Finsterer, 2008]. The reactivation of herpes simplex virus type 1 (HSV-1) or varicella zoster virus (VZV) has been suggested to be among the etiologies of idiopathic peripheral facial nerve palsy [Burgess et al., 1994; Furuta et al., 2001, 2004; Abiko et al., 2002; Lazarini et al., 2006; Yamakawa et al., 2006; Lockhart et al., 2009]. Antiviral treatment for patients with idiopathic peripheral facial nerve palsy has been discussed controversially [Adour, 1994; Ahmed, 2005; Hato et al., 2007, 2008; Kawaguchi et al., 2007; Lockhart et al., 2009]. Reported detection rates for HSV-1 and VZV differ significantly. This may be due to the use of non-standardized oral fluid collection or absorption-based oral fluid collection devices in conjunction with home-brew molecular assays lacking internal controls in those studies.

Andreas Lackner and Reinhard B. Raggam contributed equally to this work.

*Correspondence to: Reinhard B. Raggam, Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, 8036 Graz, Austria.
E-mail: reinhard.raggam@medunigraz.at

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In this pilot study, liquid phase-based and absorption-based oral fluid collection was compared. Oral fluid samples were collected from patients with acute idiopathic peripheral facial nerve palsy, those with herpes labialis, those with Ramsay Hunt syndrome, and healthy controls with both oral fluid collection systems. HSV-1 DNA and VZV DNAs were detected by in vitro diagnosis (IVD/Conformité Européenne (CE)) labeled molecular assays based on fully automated sample preparation and real-time PCR. Detection rates and viral loads were compared in order of the oral fluid collection systems used.

METHODS

Study Population and Collection of Oral Fluid Samples

A total of 10 patients (3 females, 7 males; mean age, 44 years; age range, 29–77 years) suffering from acute idiopathic peripheral facial nerve palsy were enrolled and assigned as study group. Five patients with visible herpes labialis (three females, two males; mean age, 28 years; age range, 18–51 years) and five patients with acute Ramsay Hunt syndrome (three females, two males; mean age, 58 years; age range, 37–76 years) served as positive control groups. Ten healthy subjects (six females, four males; mean age, 28 years; age range, 23–39) years lacking IgM antibodies against both HSV and VZV and without clinical presentation compatible to infection produced by HSV and VZV served as negative controls. None of the patients and healthy controls showed any immunodeficiency. No corticosteroids and/or antiviral treatment were administered within 4 weeks before enrolment. This pilot study was approved by the ethical committee of the Medical University of Graz. All study participants gave written informed consent.

Idiopathic peripheral facial nerve palsy was diagnosed according to general investigation guidelines and the grade of palsy was determined according to the a grading scale described by House and Brackmann [1985] and Ahmed [2005].

Whole blood, cerebrospinal fluid (CSF), and oral fluid samples were collected within the first 4 days of onset of clinical symptoms. For collecting oral fluid, two different collection systems, the liquid phase-based Saliva Collection System (SCS; Greiner Bio-One GmbH, Kremsmünster, Austria) and the absorption-based Salivette® (Sarstedt GmbH, Nümbrecht, Germany) were used according to the manufacturers' instructions. In all patients, oral fluid was collected with the absorption-based system first, followed by the use of the liquid phase-based system.

Antibody Testing

Serum and CSF testing for IgM and IgG antibodies against neurotropic viruses including HSV, VZV, Coxsackie A and B, and *Borrelia burgdorferi* was done by using commercially available ELISA tests (Institut Virion/Serion GmbH, Würzburg, Germany) on the BEP III instrument (Siemens Healthcare Diagnostics GmbH, Eschborn, Germany).

Molecular Assays

Molecular assays for detection of HSV-1 DNA and VZV DNA in oral fluid were based on automated DNA extraction and real-time PCR as reported recently [Raggam et al., 2008]. For automated DNA extraction on the MagNA Pure Compact instrument (Roche Applied Science, Penzberg, Germany), the MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche) was employed. For quantitative real-time PCR on the IVD-LightCycler 2.0 instrument (Roche), commercially available CE-labeled assays, the artus® HSV-1/2 LC PCR Kit (Qiagen, Hilden, Germany) and the artus® VZV LC PCR Kit (Qiagen) were employed according to the instructions of the manufacturer. The lower limit of detection of both the HSV-1/2 and the VZV assay is 250 copies/ml.

RESULTS

In this pilot study, a total of 30 study participants were included. None of the 10 idiopathic peripheral facial nerve palsy patients included showed IgM antibodies for neurotropic viruses and *Borrelia burgdorferi* in serum and in CSF. When collected with the liquid phase-based SCS, oral fluid samples of three idiopathic peripheral facial nerve palsy patients tested positive for HSV-1 DNA and that of another two idiopathic peripheral facial nerve palsy patients tested positive for VZV DNA. For the corresponding oral fluid samples collected with the absorption-based Salivette®, positive results for HSV-1 DNA were identical but showing distinctly lower viral copy numbers when compared to oral fluid samples having been collected with the liquid phase-based system. VZV DNA was not detectable in any of the 10 investigated oral fluid samples having been collected with the absorption-based system (Table I).

When oral fluid samples were collected with the liquid phase-based SCS from five patients with herpes labialis, all were positive for HSV-1 DNA; when collected with the absorption-based Salivette® only three of five patients were positive with distinctly lower viral copy numbers (Table II). When oral fluid samples were collected from patients with Ramsay Hunt syndrome, all of them tested positive for VZV DNA with the liquid phase-based SCS and four of five patients were positive when collected with the absorption-based Salivette® showing also distinctly lower viral copy numbers (Table II). All 10 healthy controls were negative for both viral DNAs tested.

DISCUSSION

The etiopathogenesis of idiopathic peripheral facial nerve palsy is uncertain; acute immune demyelination triggered by a viral infection may be responsible since

TABLE I. Detection Rates and Viral Loads of HSV-1 DNA and VZV DNA in Oral Fluids Collected From Idiopathic Peripheral Facial Nerve Palsy Patients Using Two Different Collection Systems, the Liquid Phase-Based SCS and the Absorption-Based Salivette®

	Oral fluid samples collected with			
	Liquid phase-based SCS		Absorption-based Salivette®	
Patient no.	No. of HSV-1 DNA (copies/ml)	No. of VZV DNA (copies/ml)	No. of HSV-1 DNA (copies/ml)	No. of VZV DNA (copies/ml)
1	TND	2.5×10^3	TND	TND
2	TND	TND	TND	TND
3	3.5×10^5	TND	2.9×10^2	TND
4	TND	TND	TND	TND
5	2.0×10^5	TND	6.6×10^3	TND
6	TND	TND	TND	TND
7	TND	TND	TND	TND
8	TND	2.6×10^2	TND	TND
9	1.4×10^5	TND	2.5×10^3	TND
10	TND	TND	TND	TND

TND, target not detected.

viral shedding into the oral cavity was reported recently [Furuta et al., 1998; Singhi and Jain, 2003].

There is no consensus regarding treatment options. Clinical trials investigating the efficacy of specific antiviral treatment did not show a significant benefit in comparison to alternative treatment [Adour, 1994; Ahmed, 2005; Hato et al., 2007, 2008; Kawaguchi et al., 2007; Lockhart et al., 2009]. However, these treatment-based trials did not consider the possible presence of HSV-1 and VZV DNAs in oral fluid of idiopathic peripheral facial nerve palsy patients as inclusion criterion; furthermore, non-automated homebrew assays for detection of viral DNAs lacking internal controls were used. In addition, neuroborreliosis as a possible cause of facial paralysis was not excluded in recent studies [Ahmed, 2005; Hato et al., 2007, 2008; Kawaguchi et al., 2007; Lockhart et al., 2009].

In this pilot study, IVD/CE-labeled molecular assays based on an automated DNA extraction and real-time PCR were employed. A possible systemic neurotropic virus infection and the existence of Lyme neuroborreliosis were excluded.

All patients included in this study suffered from acute idiopathic peripheral facial nerve palsy and had not received corticosteroids and/or antiviral treatment within 4 weeks prior to admission to hospital. Oral fluid samples were obtained within the first 4 days of onset of clinical symptoms. This is of importance because the prevalence of viral DNAs has been reported to be highest during the acute phase of the disease due to extensive viral shedding into the oral cavity [Singhi and Jain, 2003]. Oral fluid samples from five patients with herpes labialis and from five patients with Ramsay Hunt syndrome served as positive controls for HSV-1 DNA and VZV DNA detection in collected oral fluid samples, respectively.

When the liquid phase-based oral fluid collection system was used, viral DNAs were detected in five

TABLE II. Detection Rates and Viral Loads of HSV-1 DNA and VZV DNA in Oral Fluids Collected From Patients With Herpes Labialis and From Patients With Ramsay Hunt Syndrome Using Two Different Collection Systems, the Liquid Phase-Based SCS and the Absorption-Based Salivette®

Oral fluid samples collected with			
Liquid phase-based SCS		Absorption-based Salivette®	
No. of HSV-1 DNA (copies/ml)	No. of VZV DNA (copies/ml)	No. of HSV-1 DNA (copies/ml)	No. of VZV DNA (copies/ml)
Patients with herpes labialis			
9.2×10^2	TND	TND	TND
8.8×10^4	TND	1.5×10^3	TND
2.6×10^4	TND	1.7×10^3	TND
5.3×10^4	TND	3.8×10^3	TND
7.4×10^4	TND	TND	TND
Patients with Ramsay Hunt syndrome			
TND	6.5×10^3	TND	TND
TND	7.0×10^6	TND	8.8×10^4
TND	1.7×10^5	TND	2.1×10^3
TND	2.5×10^5	TND	2.5×10^3
TND	3.8×10^5	TND	4.0×10^3

TND, target not detected.

patients with acute idiopathic peripheral facial nerve palsy. Considering the small sample size of this study, the detection rate (50%) of viral DNAs in oral fluid obtained from idiopathic peripheral facial nerve palsy patients was found to be higher compared to those found in other studies [Burgess et al., 1994; Furuta et al., 2001, 2004; Abiko et al., 2002; Lazarini et al., 2006; Yamakawa et al., 2006; Hato et al., 2007, 2008; Kawaguchi et al., 2007; Lockhart et al., 2009]. When the oral fluid collection systems were compared in the idiopathic peripheral facial nerve palsy group, positive results for HSV-1 DNA were identical but oral fluid samples collected with the absorption-based system showed up to 3 log lower viral copy numbers. Detection of VZV DNA in oral fluid samples of these patients was only possible with the liquid phase-based oral fluid collection system. The obtained results for VZV DNA showed viral copy numbers close to the lower limit of detection of the molecular assay, suggesting that liquid phase-based oral fluid collection may be superior when only a small amount of viral DNA is present. This may indicate a matrix-dependent lower yield of viral nucleic acids when absorption-based oral fluid collection is used. Similar effects were also observed in the two control groups where oral fluid samples collected with the absorption-based collection system gave negative results for both viral DNAs while low-positive results could be observed in corresponding oral fluid samples collected with the liquid phase-based oral fluid collection system.

In conclusion, both collection of oral fluid with a liquid phase-based system and the use of automated molecular methods allow early and reliable detection of HSV-1 and VZV DNAs in oral fluid samples collected from patients with acute idiopathic peripheral facial nerve palsy. This information may offer a valuable decision support for clinicians regarding start of antiviral treatment at the first clinical visit since sampling is non-invasive, quick and easy to handle, and automated standardized molecular assays have a short turnaround time. A major limitation of this pilot study is the small samples size. To verify these findings, a multicenter study including a greater number of patients with idiopathic peripheral facial palsy should be performed.

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