Saliva Polymerase Chain Reaction Assay for Detection and Follow-up of Herpesvirus Reactivation in Patients With Drug Reaction With Eosinophilia and Systemic Symptoms (DRESS)

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**Importance:** Reactivations of human herpesviruses (HHVs) contribute to the development of drug reaction with eosinophilia and systemic symptoms (DRESS). Diagnosis of HHV reactivation is conventionally based on quantitative real-time polymerase chain reaction (PCR) analysis of blood samples, but these viruses are present in the oropharynx and are shed in saliva.

**Objective:** To evaluate the use of a saliva PCR assay for demonstrating HHV shedding in patients with DRESS.

**Design:** Shedding of HHV in saliva was prospectively studied in patients with DRESS. Reactivations of HHV, including HHV-6, HHV-7, cytomegalovirus (CMV), and Epstein-Barr virus (EBV), were studied by performing quantitative real-time PCR analysis of blood samples obtained at admission) and serial samples of saliva obtained within the first 2 weeks of DRESS; saliva samples from controls were compared.

**Participants:** The study included 5 patients who met definite criteria for DRESS and 15 controls (5 immunosuppressed patients and 10 healthy adults).

**Main Outcome Measures:** DNA viral loads of HHV, including HHV-6, HHV-7, CMV, and EBV as measured with real-time PCR in blood and saliva samples from patients with DRESS and saliva samples from immunosuppressed and healthy controls.

**Results:** The PCR assay demonstrated shedding of HHV-7, EBV, HHV-6, and CMV, listed by order of magnitude. The DNA viral loads in blood and saliva samples, also measured with real-time PCR, were found to be close. In 1 patient, reactivations in saliva preceded clinical manifestations of CMV disease. Significant production of HHV-7 and EBV was demonstrated in saliva samples from the controls, but neither HHV-6 nor CMV were detected.

**Conclusions and Relevance:** The saliva PCR assay is a useful tool for demonstration and follow-up of HHV reactivation. The interpretation of HHV viral loads in saliva differs according to HHV type.

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DRUG REACTION WITH EOSINOPHILIA AND SYSTEMIC SYMPTOMS (DRESS) is a severe and possibly life-threatening drug reaction. The role of human herpesvirus (HHV) reactivation in the development of this adverse drug reaction is well recognized. HHV-6 reactivation is among the criteria proposed by a Japanese consensus group for the diagnosis of drug-induced hypersensitivity syndrome or DRESS. Other herpesviruses, including HHV-7, cytomegalovirus (CMV), Epstein-Barr virus (EBV), and human herpes simplex virus, participate in this syndrome. We recently demonstrated that the cutaneous and visceral manifestations of DRESS are mediated by activated CD8⁺ T lymphocytes, which are directed against these viruses. Viral reactivations are induced in DRESS by a limited number of drugs, and how these drugs induce HHV reactivation is not yet understood. It was recently demonstrated that DRESS-associated drugs may increase in vitro HHV-6 and EBV replication.

Demonstration of HHV reactivation and monitoring of HHV load are of interest in DRESS, especially in severe cases. Management of DRESS is not well codified and monitoring of HHV load together with studying clinical and biological manifestations may help in the choice of the treatment (corticosteroids, intravenous immunoglobulins, and/or antiviral drugs). Today, diagnosis of HHV reactivation is...
based on findings of quantitative real-time polymerase chain reaction (PCR) in total blood. The hallmark of these HHVs is their location in the oropharynx as a latent infection. The first manifestations of DRESS, including pharyngitis, cervical lymphadenopathy, and facial edema, may demonstrate that oropharynx is the first site of herpesvirus reactivation in DRESS. It is useful to look for HHV shedding in saliva, especially as new devices for DNA collection are available. We used real-time quantitative PCR analysis of saliva samples to study HHV reactivation in patients with DRESS.

**RESULTS**

**DRESS PATIENT POPULATION**

Complete series of specimens were obtained from 5 of the 10 patients identified with DRESS, including 4 women and 1 man. Their mean age was 50.8 years; 3 were white, 1 was Arab, and 1 was black African. Most of the culprit drugs were already known to be trigger factors for DRESS; these included allopurinol (3 patients), sulfasalazine (1 patient), and olanzapine (1 patient). Severe manifestations included hepatitis (5 patients), renal failure (1 patient), and hemophagocytic syndrome (1 patient). All the patients had severe DRESS and were treated with corticosteroids within the first week, and their clinical conditions improved within the first 2 weeks.

Patient 1 is of interest because of the contribution of herpesvirus reactivation to the development of DRESS. This 42-year-old man was hospitalized for an allopurinol-induced DRESS with severe manifestations, including hemophagocytic syndrome and hepatitis. He was treated with corticosteroids, and his condition dramatically improved. At the beginning of the third week, however, a few days after the last saliva sampling, CMV disease with colitis developed. Blood PCR confirmed CMV and HHV-6 reactivation at that time. The patient was successfully treated with valganciclovir hydrochloride.

**HHV REACTIVATION IN BLOOD AND HHV SHEDDING IN SALIVA**

The HHV loads were measured in every patient by performing quantitative real-time PCR analysis of a blood sample at hospital admission and 5 to 7 serial saliva samples at admission and within the next 2 weeks (Figure 1).

This study confirmed the early reactivation of HHV followed by sequential reactivations in DRESS. Reactivations included hepatitis (5 patients), renal failure (1 patient), and hemophagocytic syndrome (1 patient). All the patients had severe DRESS and were treated with corticosteroids within the first week, and their clinical conditions improved within the first 2 weeks.

**METHODS**

Patients with DRESS were selected by members of the French Study Group of Cutaneous Drug Adverse Reactions of the French Society of Dermatology. Inclusion criteria were as follows: (1) definite case of DRESS according to the criteria of Kardaun et al, (2) inclusion within the first week after the first manifestations of DRESS, (3) possible follow-up of 3 weeks, and (4) signed patient consent form. Exclusion criteria were age younger than 18 years and prior antiviral treatment. Analyzed items included age, ethnic origin (white, Asian, black African, or Arab), associated disorders, culprit drug, systemic involvement, and treatment. Real-time quantitative PCR analysis for HHV, including HHV-6, HHV-7, CMV, and EBV, was performed in total blood samples (obtained at admission) and in saliva samples (obtained at admission and 3 times a week for the next 2 weeks), as reported elsewhere.

Levels of HHV DNA were also quantified in saliva samples from 15 unmatched controls, including 3 immunosuppressed patients (transplant recipients) consecutively recruited from a day unit (for follow-up evaluation) of the departments of pulmonology and nephrology in Bichat Hospital, Paris, and 10 healthy adults were recruited among colleagues.

DNA collection in saliva was performed with a new device (OMNIgene DISCOVER; DNA Genotek); this was done by autosalvoring 1 mL of saliva. The HHV loads were expressed as DNA copies/µg DNA. Results were considered significant at levels of more than 100 DNA copies/µg DNA. The Institutional Review Board of Paris North Hospitals approved this study (IRB00006477, No. 10-077).
vations of HHV were observed in every patient. In blood samples, EBV and HHV-7 reactivations were observed in all the patients with DRESS, whereas HHV-6 reactivation was demonstrated in only 2. No blood sample was positive for CMV. In saliva samples, EBV, HHV-6, and HHV-7 shedding was demonstrated in all the patients, but CMV shedding in only 1 (patient 1). The most frequently shed HHV was HHV-7, following by EBV and HHV-6. DNA viral loads were highest for EBV (patient 5), and viral loads in total blood samples were close to the measurements in the first samples of saliva.

Human herpesvirus shedding was also studied in saliva samples from 15 controls (10 healthy adults and 5 transplant recipients; Figure 2). Shedding of HHV-7 and EBV was observed in most immunosuppressed patients (5 of 5 and 4 of 5, respectively) and in some healthy subjects (9 of 10 and 2 of 10, respectively). Production of HHV-6 and CMV was absent or low (≤100 DNA copies/µg DNA).

During this 2-week period, the intensity of viral shedding did not correlate with clinical improvement. Human herpesvirus shedding increased in patients 1 and 4 and demonstrated a flare in patient 5. In patient 1 HHV-6 and HHV-7 reactivation was demonstrated in both blood and the first saliva samples, and an increase in HHV-7 shedding was observed in the following sequential saliva samples. At the end of the second week (saliva samples 5 and 6) shedding of HHV-6 and CMV was detected. Interestingly, the reactivation of CMV in saliva (samples 5 and 6) preceded the clinical manifestations of CMV disease.

**COMMENT**

Human herpesvirus reactivation is the major characteristic of DRESS. To our knowledge, no serial analysis of HHV shedding in saliva has been previously reported in patients with DRESS. This first study demonstrates the usefulness of saliva specimens for PCR detection and follow-up of HHV reactivation. The values for DNA viral loads detected with real-time PCR were found to be close in blood and saliva samples. Salivary shedding of HHV correlated with HHV reactivation in blood. We hypothesized that HHV reactivation in DRESS could begin in the oropharynx. Our study did not demonstrate higher HHV loads in the first saliva sample. It is possible that HHV reactivation in the oropharynx precedes clinical manifestations, by which time the strong antiviral immune response has already decreased HHV shedding in saliva.

One major limitation of HHV salivary detection in DRESS is the demonstration of HHV (HHV-7 and EBV) shedding in our control group, including immunosuppressed patients and healthy adults, but significant levels of HHV-6 and CMV reactivation were observed only in patients with DRESS. Information about HHV shedding in saliva is limited. Human herpesviruses (HHV-6, HHV-7, CMV, and EBV) establish a latent infection in the oropharynx and salivary glands, and their reactivation may be demonstrated by shedding in oropharyngeal secretions and saliva. For example, the saliva PCR assay was recently used to screen newborns for CMV infection. Three longitudinal studies demonstrated that HHV-7 shedding is common, and healthy adults may excrete high levels of this virus. Although they belong to the same herpesvirus family, the frequency and levels of shedding are lower for EBV, HHV-6, and CMV. Viral loads in saliva must therefore be carefully interpreted. The thresholds of significance may differ considerably among HHVs. We confirmed in our study that HHV-7 shedding is frequent in both immunosuppressed patients and healthy adults; EBV shedding is also common in immunosuppressed patients (transplant recipients in our study).

Follow-up of HHV reactivations is a major component in the management of DRESS. Viral loads for HHVs may change dramatically in a short period, for example, doubling within 49 to 56 hours for EBV. Our study demonstrated the variability of HHV shedding within a 2-week period. In vivo, HHV may cooperate with reactivation of...
other HHV. These reactivations are known to be followed by clinical flares. Monitoring patients with PCR saliva assays could enable rapid diagnosis of DRESS and perhaps prediction of clinical flares, which are often unpredictable, as observed in our patient 1. This follow-up is particularly important in patients treated with corticosteroids. Corticosteroids improve clinical condition in patients with DRESS because they decrease antiviral immune response, but they may have a deleterious effect on HHV infection. We observed an increase in HHV shedding in 2 patients (patients 1 and 4) receiving corticosteroids. This finding could explain the frequent flares of DRESS after abrupt discontinuation of corticosteroids. It can be difficult to monitor HHV viral loads with total blood PCR because of the challenges of obtaining and transporting blood samples and finding an available virologist. This new saliva PCR assay enables easy autosampling and simple storage of samples at room temperature for many days.

It can be difficult to interpret HHV reactivations and decide whether to use antiviral treatment. Reactivations may occur sporadically without evidence of associated manifestations. Antiviral treatment needs to be proposed only when high viral loads are associated with clinical or biological manifestations, as observed in our patient 1, in whom HHV-6, HHV-7, and CMV reactivation preceded the clinical manifestations that prompted valganciclovir treatment. One problem with using antiviral drugs in DRESS is that no well-tolerated drug is effective against all HHVs.

Assessment of HHV loads will rapidly emerge as an important tool in the management of patients with DRESS. Strong evidence is accumulating for the importance of these findings in immunosuppressed patients. The saliva PCR assay seems to be an easy and helpful way to monitor HHV loads. To ensure the accuracy and reliability of these results, it is important to perform serial repeated saliva analyses and compare some of these results with those of blood analyses. The interpretation of saliva PCR assays is dependent on HHV type and requires parallel analysis of clinical and biological manifestations. The usefulness of this assay for the diagnosis, monitoring, and follow-up of DRESS will soon be assessed in a large prospective therapeutic study of patients with DRESS in France.

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REFERENCES


