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Detection of Crimean-Congo hemorrhagic fever virus genome in saliva and urine

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ABSTRACT

Background: The Crimean-Congo hemorrhagic fever (CCHF) virus is transmitted by tick bites and by contact with the blood or tissues of infected patients and livestock. This study was designed to investigate the genome of CCHF virus in saliva and urine samples of patients with CCHF.
Methods: Eight patients with laboratory-confirmed CCHF were included in the study. The diagnosis was made by detection of viral RNA in blood by real-time reverse transcriptase-polymerase chain reaction (real-time RT-PCR). Samples of saliva from six patients and samples of urine from three patients were collected at the same time as the blood samples and analyzed for viral RNA.
Results: The genome of CCHF virus was detected in the saliva from five of the six patients and in the urine from two of the three patients. The levels of viral load in the saliva and urine samples were similar to those in the blood samples in all but one patient, in whom higher levels were detected in blood compared to saliva or urine.
Conclusions: This study shows that during human infection with CCHF virus, viral genomes are present in the saliva and urine. Further studies to isolate infectious viruses from these fluids and to study whether they represent an infectious risk are underway.

1. Introduction

Crimean-Congo hemorrhagic fever (CCHF) is a potentially fatal and acute infection caused by the CCHF virus, which belongs to the *Nairovirus* genus of the family *Bunyaviridae*.[1] CCHF is transmitted by tick bites or by close contact with blood or tissues of infected humans or livestock with viremia.[1] CCHF is a public health problem in more than 30 countries in Africa, Asia, Southeast Europe, and the Middle East.[2] Since 2002, CCHF has also occurred in the central, northern, and eastern regions of Turkey.[3–5] Fever, headache, myalgia, malaise, diarrhea, nausea, and vomiting are common clinical signs of the disease. The outcome can be fatal in severe cases, with bleeding and organ failure.[3–6]

To our knowledge, there is no study reported in the literature that has investigated the viral genome of CCHF in samples of saliva and urine. This study was designed to fill the void in the literature and to initiate further studies on this subject.

2. Materials and methods

2.1. Patients

This prospective study was conducted at Ankara Numune Education and Research Hospital during the spring and summer seasons of 2008. The viral genome of CCHF was measured in blood, saliva, and urine from the patients admitted to the hospital with a possible diagnosis of CCHF. Specifically, samples were collected aseptically upon admission and were immediately transported to the national reference laboratories for detection of viral genome of CCHF and viral loads. A TaqMan-based 1-step reverse transcriptase-polymerase chain reaction (real-time RT-PCR) was used for the detection and quantification of CCHF viral RNA in the blood, saliva, and urine.[7]

Patients with confirmed CCHF, in whom CCHF viral RNA was detected in blood samples, were included in the study. Patients with hematuria, hemoglobinuria, epistaxis, gingival

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Table 1
Results of CCHF real-time RT-PCR tests and viral loads

Patient number	Duration of symptoms[a] (days)	Blood viral load (copies/ml)	Saliva viral load (copies/ml)	Urine viral load (copies/ml)
1	2	7×10^4	2×10^5	NT
2	6	3×10^3	6×10^4	NT
3	10	5×10^5	1×10^4	NT
4	6	8×10^3	6×10^5	NT
5	4	2×10^7	Negative	7×10^3
6	7	4×10^4	NT	1×10^4
7	7	8×10^4	NT	Negative
8	6	1×10^4	8×10^5	NT

CCHF, Crimean–Congo hemorrhagic fever; RT-PCR, reverse transcriptase polymerase chain reaction; NT, not tested.
[a] Time between the beginning of symptoms and collection of specimens.

bleeding, hematemesis, or hemophthisis were excluded from the study.

2.2. Real-time RT-PCR assay

A TaqMan-based 1-step reverse transcriptase PCR (real-time RT-PCR) was used for detection and quantification of CCHF viral RNA. Real-time RT-PCR was performed as described by Yapar et al.[7] Briefly, 5 μl of viral RNA was added to 20 μl of the mixture containing 5 pmol of each primer, 4 pmol of TaqMan probe, 0.2 mM of each dNTP (containing dUTP), and 6 mM magnesium chloride. The cycle conditions were carried out as follows: 42 °C for 40 min, 95 °C for 10 min; and then 40 PCR cycles as follows: 15 s at 95 °C, 1 min at 60 °C. The assay was run on a Perkin–Elmer 7700 Sequence Detection System by using the combination of reverse transcriptase (MBI Fermentas) and Hot Start Taq DNA polymerase (Bioron GmBH, Germany). Detailed data analyses for copies/ml were as per Yapar et al.[7]

3. Results

A diagnosis of CCHF was confirmed in eight patients who had a positive blood real-time RT-PCR test for CCHF virus. Saliva samples of six patients and urine samples of three patients were available for testing for the presence of viral genomes of CCHF. Three patients were female. All of the patients had a history of tick bite, fever, myalgia, malaise, nausea, and vomiting. Two patients had a maculopapular rash. There were no bleeding manifestations in any of the patients. Thrombocytopenia ($59–110 \times 10^9$ cells/l) and leukopenia ($1–3 \times 10^9$ cells/l) were detected in all patients except one. The activated partial thromboplastin time (aPTT) was slightly elevated in only one patient. All patients had high serum levels of aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatine phosphokinase (CPK). The mean duration from the beginning of symptoms until the collection of specimens was 6 ± 2.3 days. CCHF RNA was detected in the saliva from five of six patients (83.3%) and in the urine from two of three patients (66.7%) (Table 1). All samples were cell free during microscopic examination. Viral loads in blood, saliva, and urine are shown in Table 1. Symptomatic treatment was given to the patients and all survived.

4. Discussion

To our knowledge, this study is the first showing that CCHF viral RNA can be detected in saliva and urine from CCHF-infected patients. The study also demonstrates that the viral loads in saliva and urine samples are similar to those in blood. These findings are consistent with other viral hemorrhagic fevers, such as Lassa fever, dengue fever, and hantavirus renal syndrome. In fact, there are documented cases of other viral hemorrhagic fever viruses being detected in saliva or in urine by PCR methods, and it has been reported that these samples could be used for diagnostic purposes.[8–10] Additionally, it has been suggested that specific IgM antibodies in saliva are useful markers for the detection of dengue infection.[11]

In this brief work, we have only studied the detection of viral genomes of CCHF in saliva and urine and made comparisons to the viral RNA load in blood samples. We have not carried out virus isolation from these samples and thus it has not been possible for us to determine that viruses are present in these body fluids. Similarly we have not studied whether saliva and urine are infectious or not, and it remains unclear if these body fluids play a role in human-to-human transmission.

According to reports from the Turkish Ministry of Health, about 60% of patients with CCHF have a history of tick bite.[12] However, the source of infection in the other 40% of patients is often unknown. In addition, seroepidemiological studies have shown that in endemic areas, some individuals had CCHF IgG antibodies in their serum samples even though they did not have symptomatic disease.[13,14] Therefore, the role of other body fluids like saliva and urine in the transmission of the CCHF virus should be investigated in further studies.

In conclusion, the study has provided scientific evidence that viral genomes of CCHF virus are present in saliva and urine during a clinical infection with the disease. At the moment it is not possible to determine if these body fluids present infectious hazards; however this is the subject of further research, and work to isolate infectious viruses from urine and saliva is underway. In addition, as with other viral hemorrhagic fevers, detection of the genome of CCHF in urine or saliva may serve as a noninvasive alternative diagnostic method, particularly when blood collection is difficult. Further studies with larger patient populations are needed to confirm these findings and interpretations.

Conflict of interest

No conflict of interest to declare.

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