Hepatitis C virus quantification in serum and saliva of HCV-infected patients

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The hepatitis C virus (HCV) can be detected in blood and other bodily fluids, such as saliva, semen and gastric juices. The aim of this study was to compare the HCV viral loads in the serum and saliva of infected patients. Twenty-nine patients with detectable HCV RNA in their serum and saliva were included in this study. The HCV viral loads were determined through quantitative real-time polymerase chain reactions. The median viral RNA levels were 5.78 log₁₀ copies in the serum and 3.32 log₁₀ copies in the saliva. We observed that the salivary HCV viral load was significantly lower than the viral load in the serum. Further studies are required to understand the role of saliva in the diagnosis, management and potential transmission of HCV.

Key words: HCV - HCV transmission - serum HCV load - salivary HCV load

The hepatitis C virus (HCV) is an important public health concern, affecting over 130 million individuals worldwide (Alter 2007). It is transmitted mainly through the parenteral route and sexual and vertical transmissions are considered to be rare. However, epidemiological studies suggest that the transmission routes of a large number of HCV infections are unknown (Heintges & Wands 1997, Murphy et al. 2000).

Tests for the diagnosis and management of HCV infection include enzyme-linked immunosorbent assays (EIA) for the detection of anti-HCV antibodies, qualitative and quantitative tests to detect the HCV RNA and methods to determine the HCV genotypes (NHI 2002). A patient’s response to antiviral therapy can be predicted by examining his or her HCV genotype (NHI 2002, Bacon & McHutchison 2007). While the detection of anti-HCV antibodies and HCV RNA in blood is the traditional method of viral surveillance, venipuncture is invasive, often painful and expensive. The use of saliva samples to diagnose HCV offers several potential advantages, such as minimal training requirements for sample collection and the use of a non-invasive collection technique. Such specimens are especially suitable for seroprevalence studies when blood samples are difficult to obtain (e.g., in intravenous drug users, children and haemophilicacs). Furthermore, the analysis of saliva can provide a cost-effective approach for the screening of large populations.


Abe and Inchauspe (1991) demonstrated that chimpanzees that were inoculated with HCV-containing saliva developed an infection and two reports have suggested that HCV is transmissible to humans via bite injuries (Dusheiko et al. 1990, Abe & Inchauspe 1991, Figueiredo et al. 1994). The present study was designed to compare the HCV viral loads in the serum and saliva of HCV-infected patients.

This cross-sectional study was performed from March-August of 2006. A total of 29 patients with chronic hepatitis C were selected among individuals who visited the Central Public Health Laboratory of Bahia, which is the only public laboratory that conducts the HCV-RNA detection test. Individuals with detectable HCV RNA in both their serum and saliva were included in the study and patients who had undergone antiviral therapy or had co-infections were excluded. None of the patients reported xerostomia or oral lesions.

Saliva and serum samples from 20 healthy donors were used as controls. The research protocol was approved by the Institutional Ethical Committee of Oswaldo Cruz Foundation-Bahia. Informed consent was obtained from all subjects who agreed to participate in this study.

The saliva and serum samples were collected from the patients after an eight-hour fasting period. Blood samples were collected in 10 mL Vacutainer tubes lacking anticoagulant (Becton Dickinson, San Jose, CA, USA) and centrifuged for 15 min at 3,000 rpm to separate the sera. Non-stimulated whole saliva was collected through spitting without expectoration. The saliva and serum samples (approximately 3 mL each) were stored at -70°C.
The Amplicor was used to confirm the HCV-RNA loads in the saliva and serum samples of all of the patients that were studied. The presence of HCV RNA in human saliva may indicate that the saliva has been contaminated with blood particles, allowing for the transfer of HCV particles from the circulatory system. However, HCV RNA has been measured in the saliva of infected individuals independent of mucosal lesions and periodontal disease (Liou et al. 1992, Fabris et al. 1999, Hermida et al. 2002, Lins et al. 2001). This mechanism would explain the detection of active HCV replication in the salivary glands (Arrieta et al. 2001). An alternative mechanism for viral entry into saliva is the existence of different viral genotypes in the serum and saliva of individual patients (Roy et al. 1998) and the existence of patients with HCV RNA-negative serum and HCV RNA-positive saliva (Harle et al. 1993).

The demographic and virologic characteristics of the 29 subjects included in the study are shown in Table. The study group was composed of 17 males (58.6%) and 12 females (41.3%) with a median age of 50 (interquartile range: 40-57 years). In the saliva and serum samples from each patient, the viral loads were determined through reverse transcription followed by a quantitative RT-PCR. The primers and probes that were labelled with the 5' reporter dye 6-carboxyfluorescein (FAM) and the 3'-quencher dye 6-carboxy-N, N', N'-tetramethyl-rhodamine (TAMRA) were purchased from Applied Biosystems (Foster City, CA, USA). A reaction mixture containing 10 µM of each primer (forward: 5'-CGGGACGAC CATA GTCG TGCAGTACCC-TAMRA; reverse: 5'-CGCGACCCAACACTACTC) and 5 µM of the fluorogenic probe (FAM-TGCGGAACCG GTGAGTACACC-TAMRA) was subjected to RT-PCR using the TaqMan system (Applied Biosystems, Foster City, CA, USA). A standard curve was generated using known concentrations of purified recombinant plasmid containing the HCV UTR region. Serial dilutions of this control plasmid ranging from 2.5 copies/mL to 250,000 copies/mL were prepared. RT-PCR was performed using the ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The HCV viral load levels for all of the unknown samples were calculated through extrapolation of the standard curve. The Amplicor HCV Monitor Test (Amplicor HCV Monitor, Roche, NJ, USA) was conducted according to the manufacturer’s guidelines. The saliva samples were treated with proteinase K (20 mg/mL for 1 h at 56°C) before the HCV RNA was quantified. This test was used to confirm the HCV-RNA loads in the saliva and serum samples of all of the patients that were studied.

Statistical analyses were performed using Epi Info 2000 (CDC, Atlanta, GA, USA) and GraphPad Prism version 5.1 (GraphPad Software Incorporated, San Diego CA, 120 USA). Standard parametric and non-parametric statistical testing were applied as warranted. All of the tests were two-tailed and p-values < 0.05 were considered to be significant.

The demographic and virologic characteristics of the 29 subjects are shown in Table. The study group was composed of 17 males (58.6%) and 12 females (41.3%) with a median age of 50 (interquartile range: 40-57 years). In the saliva and serum samples from the healthy donors, HCV-RNA was undetectable.

Among the 29 HCV-infected patients, 22 (75.8%) were infected with genotype 1, three (10.3%) with genotype 2 and four (13.7%) with genotype 3. The saliva and serum samples from each patient were from the same genotypes. The data analysis did not demonstrate any significant differences between the HCV viral loads in the saliva and serum for the different HCV genotypes (Table).
In this work, a quantitative PCR assay was used to determine HCV viral load levels in the saliva and serum of patients. The median viral RNA levels were 3.32 log_{10} copies (2.1 x 10^3 copies/mL) in the saliva and 5.78 log_{10} copies (1.21 x 10^6 copies/mL) in the serum (p < 0.0001) (A in Figure). We did not use internal controls in the RT-PCR, which would have detected the possible presence of PCR inhibitors. However, to reduce the probability of inhibition occurring during the RNA extractions, proteinase K (20 mg/mL for 1 h at 56ºC) was used. Additionally, we used the same volumes of saliva and serum samples in all of the reactions to effectively demonstrate their contrasting viral loads.

No significant correlation was found between the HCV viral loads in the saliva and serum, most likely due to sample size (Pearson’s r = -0.081, p = 0.67) (B in Figure). All of the saliva and serum samples were tested using RT-PCR (Amplicor® HCV Monitor, Roche, NJ, USA) and the results were similar to the results obtained (Pearson’s r = 0.018, p = 0.934). These results are likely due to the small number of available samples.

Our study indicates that the viral loads in saliva are 100 times lower than those in serum. Katayama et al. (2004) investigated the minimal number of HCV RNA copies that is able to transmit HCV infection in chimpanzees using pre-acute-phase serum. They observed that serum containing 20 HCV RNA copies was able to produce infection, suggesting that HCV can be transmitted by low virus levels. Abe and Inchauspe (1991) demonstrated the transmission of HCV through human saliva, but the number of viral copies was not determined. Epidemiological studies suggest that the infective capacity of HCV RNA virions in saliva is low (De Cock et al. 2004, Eirea et al. 2005). Further studies are required to investigate the infective potential of HCV-positive saliva.

It is possible that a defence mechanism in the saliva may attenuate or abolish the infective potential of HCV RNA virions. Belec et al. (2003) observed that the saliva of HCV-infected individuals contains specific IgG and IgA-neutralising antibodies that are directed against the E1 and E2 surface glycoproteins and may block viral adhesion to the host cell.

Although this study has some limitations due to the small number of available samples, we were able to provide information regarding the relationship between HCV viral loads in serum and saliva samples. However, further studies using larger sample sizes should be performed to confirm these results.

Other studies are also required to investigate the biological factors that are associated with HCV transmission. The elucidation of the mechanisms that are used by hosts to block HCV entry into the cell may contribute to the understanding of the role of saliva in HCV transmission.

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REFERENCES


