Detection of Hepatitis C Virus in Saliva Before and After Scaling of Dental Calculus

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Abstract

Dentists and dental health care workers are at risk of contracting hepatitis C virus (HCV) through dental treatment, since HCV RNA was reported to be easily detectable in the saliva of patients with chronic HCV liver disease. We tested for the presence of HCV RNA in saliva before and after removal of dental calculus, and in splashes on the chin-length face shields of dentists following treatment of six patients with HCV chronic liver diseases. We used a sensitive reverse transcription polymerase chain reaction (RT-PCR) method, to estimate exposure to HCV. All patients were anti-HCV and HCV RNA seropositive. HCV RNAs in saliva before or after scaling treatment were detected in three of the six, but none of the face shields showed positive samples.

In conclusion, dentists and dental health care workers should be aware of the possibility of HCV infection via contact with serum and saliva during dental practice.


Introduction

Hepatitis C virus (HCV) is a major causative factor of chronic hepatitis C, cirrhosis, and hepatocellular carcinoma (HCC) worldwide. The principal route of HCV infection is through blood products. On the other hand, HCV RNA is well known to be detectable in the saliva of patients with HCV infections⁵⁻⁷. Dentists are at risk of contracting HCV via blood and contaminated saliva during dental treatment, espe-
cially in Fukuoka prefecture where the risk for HCC is one of the highest in Japan\(^8\). We tested for the presence of HCV in saliva before and after dental treatment, and in splash samples on dentists’ face shields used during ultrasonic scaling, to evaluate exposure to HCV in dental practice.

**Materials and Methods**

**Patients**

The subjects were six patients with chronic HCV infections (5 men and 1 woman), who visited the Department of Oral Surgery, Kurume University School of Medicine, for periodontal treatment. Peripheral blood samples were obtained on their first visit to the hospital. Informed consent was obtained from the patients. All sera were assayed for anti-HCV antibody (PHA, Dinabot, Tokyo, Japan); for serum HCV RNA by the reverse transcription (RT)-nested PCR method; and for serum hepatitis B virus surface antigen (HBsAg) (EIA, Mizuho Medy, Tosu, Japan).

HCV RNA in saliva and splash samples by RT-PCR

Saliva and splash samples from patients with HCV infections were collected. Saliva samples were obtained from the submandibular glands before and after scaling of dental calculus, and splash samples from sterile chin-length face shields worn by dentists after using an ultrasonic scaler for 10 minutes. HCV RNA in saliva and splash samples were extracted using a modification of the method described by Numata et al\(^4\). In brief, 50 μL of saliva were added to 150 μL of deionized water and 180 μL of lysis buffer [100 mM Tris-HCl, pH 8.0, 20 mM ethylenediaminetetraacetic acid (EDTA), 400 mM NaCl, 4% sodium dodecyl sulfate (SDS)], which was then mixed with 20 μL of proteinase K (20 mg/mL; Merk Corp., Darmstadt, Germany). The mixture was incubated at 60°C for 60 min. Splash samples were removed with sterile cotton sticks, dipped in 200 μL of deionized water and 180 μL of lysis buffer, mixed with 20 μL of proteinase K, and incubated as above. The mixture was then twice extracted with an equal volume of phenol/chloroform 1:1 (vol/vol), and once with chloroform. Glycogen (5 μL, 4 mg/mL) was added to the RNA solution, followed by precipitation with 3 volumes of ethanol. After chilling for 30 min at -80°C, the RNA was recovered by centrifugation at 1.5 × 10^4 r.p.m. at 4°C for 15 min. The pellet was rinsed with 1 mL of 95% ethanol and dried in air for 20 min. The RNA was suspended in 10 μL deionized water and reverse transcribed. cDNA was subjected to PCR amplification using a set of 5’-non-coding region primers, 5’-GGCGACACTCCACCAGATCACT-3’ (sense primer), and 5’-CACGAATTCAGTCTTCTTTGTCCGCAGCACCCCAAC-3’ (antisense primer) for the first round of PCR, and 5’-ATAGGATCCACTCCCTCTG-GAGAACACTACTGTC-3’ sense, 5’-ATGAGTTCT-ATGGTCACGCTCTACGAGACCTCCCG3’ (antisense primer) for the second round of PCR\(^6\). The conditions for the first round of PCR amplification were 20 cycles (94°C/1 min., 45°C/1 min., 72°C/1 min.) followed by 20 cycles (94°C/1 min., 65°C/1 min., 72°C/1 min.). Those for the second round of PCR amplification were 35 cycles (94°C/1 min., 60°C/1 min., 72°C/1 min.). The PCR products were analyzed by agarose gel electrophoresis and ethidium bromide

<table>
<thead>
<tr>
<th>No. of patients (n = 6)</th>
<th>HCV RNA in saliva before/after scaling</th>
<th>HCV RNA in splash samples</th>
<th>HCV RNA in serum</th>
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<tbody>
<tr>
<td>1</td>
<td>+ / +</td>
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<td>2</td>
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<td>5</td>
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<td>6</td>
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<td>+</td>
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</tbody>
</table>
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Results

All patients were positive for both anti-HCV and serum HCV RNA, and were negative for HBsAg. Of the six patients, HCV RNA in saliva was detected in three (Table 1). Of the three, HCV RNA was detected in the saliva of one patient before and in one patient after scaling. HCV RNA was not detected in any splash samples.

Discussion

Dentists are exposed to many possible routes of transmission for various infections such as hepatitis B virus (HBV), HCV, and human immunodeficiency virus type 1 (HIV-1) through blood, saliva, and teeth fragments cut by high-speed dental turbines. The high frequency of injuries from sharp objects occurring in the dental setting places the dental health care worker at risk of contracting HCV. However, the low prevalence of HCV infection in dentists and dental health care workers has been reported to be similar to that in healthy blood donors (control). However, Klein et al reported that the prevalence of anti-HCV in dentists (1.75%, 8/456) was higher than in volunteer blood donors (control) (0.14%, 1/723). Moreover, it is reported that the prevalence of anti-HCV in oral surgeons (9.3%, 4/43) was higher than in other dentists (0.97%, 4/413).

HCV RNA is reported to be detectable in the saliva of HCV infected persons. Recently, HCV RNA was detected in urine, seminal fluid, ascites, gastric juices, and various cells as well as in serum and saliva. In the present study, of the six patients diagnosed with chronic HCV disease, three had HCV RNA in saliva. In splash samples from the six patients, HCV RNAs were not detected. The lesser prevalence in detection of HCV RNA after scaling suggests that there was less bleeding from the gums following an ultrasonic scaler than by a hand scaler. Bleeding and pain in the gums using an ultrasonic scaler are reduced, since an ultrasonic scaler is more useful in removing supragingival dental calculus than subgingival calculus. HCV RNA was not detected in the splash samples containing small amounts of blood. However, contamination of saliva and teeth fragments with blood during and after dental treatment and oral surgery may result in an increased risk of viral exposure. Abe et al reported that HCV RNA was detected in the saliva of chimpanzees infected with hepatitis C and in serum from a recipient chimpanzee inoculated with HCV RNA-positive saliva. This finding strongly suggests that the saliva of seropositive HCV patients is infectious.

In conclusion, dentists and dental health care workers should be aware of the possibility of HCV infection via contact with serum and saliva during dental treatment extra precaution is advised for Fukuoka prefecture where the prevalence of anti-HCV in the general population is 3.3% (73/2237 persons), and increases with advancing age from 0.6% in the 20~29 age group to 10.1% in the 70 and over age group. The use of disposable gloves, masks, protective eyewear, face shields, clinic coats, hair coverings, and automated decontamination devices should be used in daily dental practice, especially while performing intraoral procedures with the potential for bleeding caused by hand instruments.

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References

HCV感染者の歯石除去前後の唾液におけるHCV RNAの検出

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C型肝炎ウイルス（HCV）の感染者では、唾液中にHCV RNAが検出されることが知られている。従って歯科医療従事者は、歯科治療を通じてHCVに感染するリスクが高いことが予想される。そこで歯科医療従事者がどの程度の感染リスクに曝露されているのかを明らかにするために、6人のHCV持続感染者に対して，口腔内への出血の誘因となる歯石除去前の唾液並びに超音波スケーラー使用時のフェイスシールド（頭面全体を覆うプラスチック製ガード）への飛沫サンプルからHCV RNAの検出を試みた。

歯石除去前後を通じて唾液中からHCV RNAが検出された患者は、6人中3人であった。除去前後の両方の唾液にHCV RNAが検出された者は1人。除去前のみに検出された者が1人、除去後ののみの検出は1人であった。フェイスシールドへの飛沫液からは検出されなかった。

歯科医療従事者は、歯科治療時の血液や唾液の接触を介してHCVに感染する可能性があることを認識すべきであろう。

平成12年11月20日