Effect of Non-Surgical Periodontal Therapy on Hepatitis C Virus Levels in Gingival Crevicular Fluid and Saliva

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Abstract: Objectives: The aim of this study was to determine the prevalence of HCV in gingival crevicular fluid (GCF) and saliva of hepatitis C virus (HCV) viremic patients and to evaluate the effect of periodontal therapy (scaling/root planing) on their virus level in a representative sample of the Egyptian population. Materials and Methods: 30 patients with chronic HCV infection suffering from moderate to severe chronic periodontitis were diagnosed based on their criteria of the American Academy of Periodontology (AAP). Detection of HCV RNA in saliva and GCF samples using commercial automated polymerase chain reaction was carried out. Gingival index (GI), probing depth (PD) and clinical attachment level (CAL) were recorded for all subjects before and after mechanical periodontal therapy to be correlated with laboratory findings. Results: There was a statistically significant reduction in PD, CAL, GI, Salivary PCR and GCF PCR values after treatment. The % reduction of salivary HCV was 11.6% while that of GCF was 12.1 % after periodontal therapy. There was a significant positive correlation between % reduction in salivary HCV levels, GCF HCV levels and GI as well as a significant positive correlation between % reduction in GCF HCV levels and GI. There was no significant correlation between salivary HCV and GCF HCV levels with either PD or CAL. Conclusion: Regular SRP in periodontitis aected HCV-seropositive patients is highly recommended in an attempt to limit viral transmission among spouses and family members.


Keywords: Hepatitis C virus; Gingival crevicular fluid/virology; Saliva/virology.

1. Introduction

Hepatitis C is a worldwide public health problem as the world health organization (WHO) estimated that about 3% of the world’s population has been infected with hepatitis C virus (HCV) and that approximately 20% of Egyptian blood donors were chronic HCV carriers.2

Although HCV is considered essentially hepatotropic, some studies have suggested that viral replication occurs also in peripheral blood mononuclear cells3 and in the submaxillary glands.4 However, possible sources of HCV in saliva may include serum exudates, i.e. gingival crevicular fluid (GCF), and migration of HCV containing mononuclear cells from inflamed periodontium into the salivary pool.5

Mononuclear cells as leukocytes constitute about 47% of the somatic cells in the gingival sulcus and continuously migrate from the dentogingival vessels into the crevice of both healthy and chronically inflamed gingiva.3 Monocytes represent about 2-3% and lymphocytes 1-2% of the inflammatory cells in the GCF.7

As it is well documented that periodontal disease increases mononuclear cell excretion in GCF,7,8 it is also generally accepted that mechanical removal of contaminants by scaling and root planing (SRP) are the conventional treatment modality for chronic periodontitis with subsequent reduction in inflammatory cellular infiltrate and GCF transudation.9 Accordingly, and in view of the aforementioned findings, this clinical-biochemical study was undertaken to evaluate the effect of non-surgical periodontal therapy (SRP and oral hygiene instructions) on the Hepatitis C viral load in saliva and GCF of HCV sero-positive chronic periodontitis patients by comparing HCV RNA levels before and after conventional periodontal therapy.

2. Subjects and methods

Thirty patients with definitive diagnosis of chronic HCV infection, and no-HCV-treatment concurrent to the study, were enrolled in this study selected from the outpatients’ clinic of Oral Medicine and periodontology department, Faculty of Oral and
Dental Medicine, Cairo University. The HCV patients consisted of 14 women and 16 men with a mean age of 51.1±4.54 years, ranging from 43 to 59 years. All patients signed a consent form after being advised of the nature of the study. They underwent a full-mouth clinical examination of periodontal tissues using manual, calibrated periodontal probe to assess the following parameters: probing depth (PD), clinical attachment level (CAL) and gingival index (GI). Periodontitis was diagnosed when the patient had at least 9 teeth with 5 mm probing depth and ≥ 4mm attachment loss.10

The diagnosis of chronic HCV infection was based on the history provided by the patient and confirmed by collecting and screening a blood sample for anti-HCV antibodies for each patient prior to treatment.

**Saliva collection**

Whole un-stimulated saliva (WUS) was collected from all participants prior to treatment and at the end of the therapeutic intervention. All subjects were refrained from eating, drinking or using chewing gum for at least one hour before sampling. Samples were obtained by asking the patient to first swallow, tilt their head forward, and expectorate all saliva into 50-ml sterile centrifuge tubes for 5 min without swallowing. Saliva was centrifuged for 2 min at 10,000 g and the clarified supernatant was filtered through a 0.45 µm low protein binding membrane, separated into 0.5 ml aliquots and frozen at -80°C for later analysis of HCV RNA level by PCR.

**GCF sampling:**
The sample site was gently air dried and all supragingival plaque was removed. The area was carefully isolated to prevent samples from being contaminated by saliva. Filter paper strips (2x5mm) were inserted into the gingival crevice until mild resistance was felt and left in place for 30 seconds. Strips contaminated by blood were discarded. Strips were then placed into coded sealed plastic microcentrifuge tubes containing transfer media and stored at -80 till further processing.

**Periodontal treatment procedures**

Patients received periodontal therapy including thorough full-mouth supra and subgingival scaling and root planing (SRP) using standard periodontal curettes and ultrasonic device followed by oral hygiene instructions. The patients were reviewed after 3 weeks for periodontal reassessment (PD, GI, CAL) and post-treatment saliva and GCF sample collection.

**RT-PCR for HCV RNA Quantitation**

Total RNA was extracted from saliva specimens and from paper strips with collected GCF using a QIAamp® viral RNA mini kit (QIAGEN, Valencia, CA). To determine the quantity of HCV RNA, real-time polymerase chain reaction (RT-PCR) involving single-tube reactions was performed using TaqMan® EZ RT-PCR Core reagents (Applied Biosystems, Foster City, CA). The reaction mixture contained 1X TaqMan EZ buffer, 500nM concentrations of each primer from the HCV 5' noncoding region (5'-AACACTGTCTTTACGGCAGAAAGC-3' and 5'-CCCCACACTCTCGGCTAG-3'), a 200nM concentration of fluorogenic probe [5'-(FAM) TGGCGTTAGTAGGTCGTGCAG (TAMRA)-3'] described by Morris et al., primers and probe were obtained from Metabion International AG (Martinsried, Germany), 200µM concentrations of each deoxynucleoside triphosphate, 3mM Mn(OAc), 5U of rTth DNA polymerase, 0.5U of AmpErase uracil N-glycosylase (UNG), and template RNA. The primers and probe were designed on the basis of the conserved sequences among HCV genotypes. The RT step was started with 2min incubation at 50°C, followed by 50min at 65°C. Thermal cycling conditions were as follows: a preincubation period of 5 min at 95°C for deactivation of UNG, followed by 50 cycles of denaturation at 94°C for 15 seconds and annealing at 55°C for 10 seconds and extension at 69°C for 1 min. All reactions and analyses of the amplification plots were performed on an Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). Standard curves of the assays were obtained from serial dilutions of HCV RNA linearity panel (Cat No PHW804-0.5) (Sera Care life science, BBI diagnostics Milford, MA). Using standard curve, the Applied Biosystems 7500 Real-Time PCR System software calculated automatically the concentration of RNA copies in the experimental samples.

**Statistical analysis**

Data were presented as mean and standard deviation (SD) values. A logarithmic transformation (log10 transformation) of PCR values (number of copies/ml) was performed because of the high range of these values. Paired t-test was used to study the changes after treatment. Wilcoxon signed-rank test was used to study the changes in GI scores.

Spearman’s correlation coefficient was used to determine significant correlations between % reductions in different parameters.

The significance level was set at $P \leq 0.05$.

Statistical analysis was performed with IBM(IBM Corporation, NY, USA) SPSS(SPSS, Inc., an IBM Company) Statistics Version 20 for Windows.

**3. Result**

As shown in table (1), there was a statistically significant reduction in PD, CAL, GI, salivary and GCF PCR values after treatment.
There was a statistically significant positive (direct) correlation between % reduction in salivary HCV level (Log_{10} copies/ml), GI and GCF HCV level (Log_{10} copies/ml). Also, there was a statistically significant positive (direct) correlation between % reduction in GCF HCV levels (Log_{10} copies/ml) and GI. On the other hand, there was no statistically significant correlation between salivary or GCF PCR records with PD or CAL. These data are illustrated in table (2) and figures (1&2).

### Table 1: The mean, mean log_{10} of (Copies/ml), standard deviation (SD), % reduction values and results of comparison between pre- and post-operative measurements.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pre-operative</th>
<th>Post-operative</th>
<th>Mean reduction</th>
<th>Mean % reduction</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD (mm)</td>
<td>5.9± 1.3</td>
<td>4.3± 1.4</td>
<td>1.6</td>
<td>28.1</td>
<td>0.002*</td>
</tr>
<tr>
<td>CAL (mm)</td>
<td>6.6± 1.6</td>
<td>5± 1.8</td>
<td>1.6</td>
<td>25.5</td>
<td>0.005*</td>
</tr>
<tr>
<td>GI</td>
<td>2.1 ± 0.5</td>
<td>1.6 ± 0.6</td>
<td>0.5</td>
<td>25.2</td>
<td>0.007*</td>
</tr>
<tr>
<td>Saliva PCR (Mean log_{10})</td>
<td>3.3 ± 0.7</td>
<td>2.9 ± 0.8</td>
<td>0.4</td>
<td>11.6</td>
<td>0.037*</td>
</tr>
<tr>
<td>GCF PCR (Mean log_{10})</td>
<td>4.2 ± 0.8</td>
<td>3.7 ± 1</td>
<td>0.5</td>
<td>12.1</td>
<td>0.047*</td>
</tr>
</tbody>
</table>

Values are mean ± SD, *: Significant at P ≤ 0.05

### Table 2: Results of Spearman’s correlation coefficient for the correlation between % changes in PCR and different variables

<table>
<thead>
<tr>
<th></th>
<th>PD</th>
<th>CAL</th>
<th>GI</th>
<th>GCF PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saliva</td>
<td>Spearman Correlation</td>
<td>0.250</td>
<td>0.054</td>
<td>0.652</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.589</td>
<td>0.908</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>GCF PCR</td>
<td>Spearman Correlation</td>
<td>0.143</td>
<td>0.018</td>
<td>0.730</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.760</td>
<td>0.969</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*: Significant at P ≤ 0.05

Figure (1): Scatter diagram representing positive correlation between % reduction in Log_{10} copies/ml in saliva and % reduction in GI
4. Discussion:

Although the presence of HCV RNA in saliva has been reported by several research groups\(^{12-30}\), few studies have investigated the occurrence of hepatitis C virus in GCF.\(^{5,31,32}\) As compared to blood withdrawing, saliva and GCF may serve as a substitute for serum collection for the detection of HCV copies as they are easier to obtain and non-invasive.

In the current study, quantitative HCV loads within the saliva and GCF of anti-HCV antibody-positive patients was detected using real-time RT-PCR and these levels were compared before and after conventional periodontal therapy to evaluate the effect of SRP on the virus level in both saliva and GCF. The results of the present study revealed that HCV levels in GCF were higher than in saliva of HCV-seropositive patients. Viral loads of $10^2$ to $10^3$ copies/ml were detected in saliva, while about $10^2$ to $10^5$ copies/ml were usually found in GCF. These findings aligned with previous studies\(^{5,31,32}\) and may be explained on the basis of HCV lymphotropism detected by several laboratories in blood mononuclear cells.\(^{33-38}\) HCV-infected cells might allow HCV to infiltrate the GCF through dentogingival vessels into gingival crevices. Also there might be transudation of HCV-containing serum into the GCF. Nevertheless, a
posibility of HCV existence within mucosal epithelial cells as well as salivary glands explains the likelihood that several possible sources are involved in HCV penetration into the saliva and GCF.\textsuperscript{30,40}

Generally, periodontal inflammation increases the excretion of mononuclear cells-rich GCF.\textsuperscript{31} In the present study, there was a significant reduction in salivary HCV levels as well as GCF PCR values after periodontal therapy. Moreover, There was a significant positive correlation between % reduction in salivary and GCF viral loads and GI, which can be explained by the fact that resolution of periodontal inflammation as reflected by a significant reduction in PD, CAL and GI is accompanied by reduction in the excretion of GCF rich in HCV infected mononuclear cells consequently reducing viral load in saliva. In accordance, Matić et al. results implied that HCV viremic patients with a higher degree of periodontal disease at the sites of fluid collection may harbor more potentially infected HCV mononuclear cells in GCF.\textsuperscript{5}

On the other hand, Roy et al. found no correlation between periodontal disease severity and the presence of HCV in saliva of HCV viremic patients.\textsuperscript{24}

In this study, the presence of HCV RNA in saliva was highly dependent on its presence in GCF as observed by previous studies.\textsuperscript{5,31,32} This suggests that besides blood, the other most probable significant source of HCV in saliva is the GCF. However, the difference in the prevalence of HCV RNA between saliva and GCF samples in our study population and a relatively lower prevalence of HCV RNA in saliva may reflect a potential existence of endogenous HCV inhibitory factors in saliva. This may explain the low transmission efficiency of saliva in non-parenteral transmission routes of HCV reported in several seroepidemiologic studies.\textsuperscript{41}

However, transmission of HCV in experimental animals by subcutaneous injection of HCV-infected human saliva has been documented,\textsuperscript{42,43} and there exists at least one report on human HCV transmission by a bite.\textsuperscript{45} Moreover a seroepidemiologic study of general dentists and oral surgeons reported a higher prevalence of HCV infection compared to controls.\textsuperscript{44} Another study suggested that the more years in practice, the more prevalent anti-HCV was among dental personnel, suggesting that exposure to oral fluids could be involved in the transmission of HCV.\textsuperscript{45}

Although the sample size in the current study was limited, HCV was successfully determined in oral fluids (GCF and saliva) from dental patients. Nevertheless, further large-scale epidemiological studies employing RT-PCR assays are required to clarify the clinical significance of HCV detection in saliva and GCF, including the potential for viral transmission through exposure to these fluids.

Finally, we can conclude that reduction in hepatitis C viral load in both GCF and saliva after non-surgical periodontal therapy suggest that it is highly recommended for HCV seropositive patients to undergo SRP on regular basis in an attempt to limit viral transmission via oral fluids.

References


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