Quantification of HIV-1 viral load in the fluid of ranulas in HIV-positive patients

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Objective. This study aimed to detect and quantify the HIV-1 viral load in ranula fluid of HIV-positive patients.

Study design. Fourteen HIV-positive patients (13 not on highly active antiretroviral therapy) presenting with ranulas were prospectively evaluated. The viral load in the ranula fluid was quantified, and the results were correlated with CD4⁺ cell count and viral load in the patient’s blood. The NucliSens EasyQ system (NucliSens Easymag; bioMérieux), which is based on nucleic acid sequence–based amplification and real-time detection using molecular beacons, was used to quantitate viral RNA in the fluid.

Results. Concentrations of HIV-1 RNA ranged from 25 to 1,600,000 copies/mL in the fluid. However, no significant statistical correlation could be established with either CD4⁺ cell count or viral load in patients’ blood.

Conclusions. Various concentrations of HIV-1 RNA were found in ranula fluid, which appears to serve as a viral reservoir in HIV-infected patients. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;111:715-719)
HIV-positive patients and to compare this parameter with the viral load and CD4$^+$ cell count.

**MATERIAL AND METHODS**

This study was based on 54 patients with oral mucocceles and/or ranulas referred to a tertiary department of maxillofacial and oral surgery. Patients with ranulas were divided into 2 categories: simple or plunging. HIV-negative patients and those with history of previous and recent operation or fine needle aspiration (FNA) of the lesion were excluded. The HIV status of patients was determined at the first consultation if it was not known, and the history of any highly active antiretroviral treatment (HAART) was recorded.

The following laboratory tests were performed: HIV test (HIV-1 antigen/p24 antibody) combination assay (Abbott Diagnostic Division, Wiesbaden, Germany), CD4$^+$ cell count profile (Beckman-Coulter FC500), measurement of viral load in the blood, and viral load in the cystic fluid. The results for viral load in the ranula fluid were expressed as copies/mL and were correlated with the circulating viral load and CD4$^+$ cell count in the blood. FNA was performed to collect fluid samples. An intravenous injection (IVI) catheter (Jelco; Johnson & Johnson) no. 20 or 22 was used for the procedure. Special attention was paid to not contaminate the fluid with blood during the process of FNA, by selecting the most translucent overlying mucosa on the ranula. The metallic component of the IVI catheter was immediately withdrawn after penetration of the cystic wall. The atraumatic and plastic component of the catheter was left in place and used for aspiration of the ranula fluid (Fig. 1). The fluid thus collected had to display a clear yellow to amber color without any macroscopic trace of blood contamination (Fig. 2). Only 14 fluid samples from HIV-positive patients satisfied the following selection criteria: no recent operation, no recent FNA, and no trace of macroscopic blood contamination. Therefore, only those specimens were finally considered and prospectively enrolled for the purpose of this study. The fluid was then immediately sent to the laboratory of the Department of Medical Virology in a plain tube.

Biopsy of the cystic lesion, which is part of the standard protocol, was also performed to ascertain the extravasation phenomenon or retention nature of the cystic lesion. The biopsy was either incisional or excisional.

**Quantification of viral load**

The Nuclisen EasyQ assay (bioMérieux), which is based on nucleic acid sequence–based amplification (NASBA) and real-time detection using molecular beacons for the quantitative detection of RNA in human samples, was used. The 3 technologies used in the Nuclisen EasyQ system were the following:

- The Boom method for nucleic acid release and isolation.
- NASBA technology for amplification of RNA.
Real-time detection of amplifications using fluorescent molecular beacons.

- These technologies were performed in 2 steps: nuclei acid release and isolation.
  - NASBA amplification and real-time detection.

The Boom method is a solid phase–based nucleic acid extraction method involving silica particles as the solid phase component. This method can be used to isolate nucleic acids from a variety of human fluid specimens other than conventional cerebrospinal and blood samples. The Boom method also has a broad range of specimen input ranges and can therefore detect nucleic acid material within normal-, high-, and low-volume human samples. The NASBA technology is based on primer extension using the coordinated activities of 3 enzymes. Real-time detection using molecular beacons is based on the measurement of the time-related increase in fluorescent signal and only occurs after specific binding of the molecular beacons to the amplicon (amplified portion of the nucleic acid). Measurement and interpretation of the signals was performed using the EasyQ analyzer. Detection of the signal (value) was considered to be positive and non-detection of the signal to be negative. Discordant samples, with viral load in the fluid higher than the viral load in the blood, were analyzed in duplicate. Negative control subjects were not used, because HIV-negative patients were excluded from the study.

### Statistical analysis

As a preliminary analysis, description of the 54 patients were summarized using frequency and percentages for age group and HIV status. Paired t test or its nonparametric equivalent was used to test for equality of viral load or CD4\(^+\) cell count in ranula fluid and blood. Pairwise association between viral load sources and CD4\(^+\) cell count was estimated using Pearson or Spearman correlation coefficients depending on the shape of the distribution. A P value of \(< 0.05\) was used to indicate significant results.

### Ethical considerations

This study was conducted in accordance with all regulations and guidelines governing research and HIV/AIDS management in South Africa. The protocol for this study and the informed consent documents were reviewed and approved by the Faculty of Health Sciences Research Ethics Committee, University of Pretoria.

### RESULTS

The distribution of patients with ranulas according to HIV status and age was determined (Table 1). The majority of patients with ranulas, 36 out of 54 (66.7%),

### Table I. Distribution of patients with ranula according to HIV status and age groups

<table>
<thead>
<tr>
<th>HIV status</th>
<th>0-10</th>
<th>11-20</th>
<th>21-30</th>
<th>31-40</th>
<th>41-50</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV (+)</td>
<td>11</td>
<td>7</td>
<td>8</td>
<td>8</td>
<td>2</td>
<td>36 (66.7%)</td>
</tr>
<tr>
<td>HIV (-)</td>
<td>6</td>
<td>11</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>18 (33.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>18</td>
<td>9</td>
<td>8</td>
<td>2</td>
<td>54</td>
</tr>
</tbody>
</table>

### Table II. Epidemiologic and clinical main variables of 14 HIV-positive patients

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age</th>
<th>Gender</th>
<th>Viral load in blood (copies/mL)</th>
<th>Viral load in fluid (copies/mL)</th>
<th>CD4(^+) cells in blood ((\times 10^6/\text{L}))</th>
<th>Ranula: simple or plunging</th>
<th>Biopsy results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>F</td>
<td>&lt;25</td>
<td>74,000</td>
<td>521</td>
<td>Simple</td>
<td>MEP</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>F</td>
<td>190</td>
<td>&lt;25</td>
<td>821</td>
<td>Simple</td>
<td>MEP</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>M</td>
<td>290</td>
<td>&lt;25</td>
<td>400</td>
<td>Simple</td>
<td>MEP</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>F</td>
<td>950</td>
<td>6,300</td>
<td>518</td>
<td>Simple</td>
<td>MEP</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>F</td>
<td>1,900</td>
<td>26,000</td>
<td>121</td>
<td>Simple</td>
<td>MEP</td>
</tr>
<tr>
<td>6</td>
<td>27</td>
<td>F</td>
<td>5,500</td>
<td>640</td>
<td>302</td>
<td>Plunging</td>
<td>MEP</td>
</tr>
<tr>
<td>7</td>
<td>42</td>
<td>F</td>
<td>8,300</td>
<td>1,002</td>
<td>339</td>
<td>Plunging</td>
<td>MEP</td>
</tr>
<tr>
<td>8</td>
<td>39</td>
<td>F</td>
<td>17,000</td>
<td>1,600,000</td>
<td>54</td>
<td>Simple</td>
<td>MEP</td>
</tr>
<tr>
<td>9</td>
<td>29</td>
<td>F</td>
<td>20,000</td>
<td>110,000</td>
<td>207</td>
<td>Plunging</td>
<td>MEP</td>
</tr>
<tr>
<td>10</td>
<td>31</td>
<td>F</td>
<td>24,000</td>
<td>81</td>
<td>92</td>
<td>Plunging</td>
<td>MEP</td>
</tr>
<tr>
<td>11</td>
<td>39</td>
<td>M</td>
<td>46,000</td>
<td>230,000</td>
<td>121</td>
<td>Plunging</td>
<td>MEP</td>
</tr>
<tr>
<td>12</td>
<td>13</td>
<td>M</td>
<td>73,000</td>
<td>2900</td>
<td>34</td>
<td>Plunging</td>
<td>MEP</td>
</tr>
<tr>
<td>13</td>
<td>23</td>
<td>F</td>
<td>130,000</td>
<td>34,000</td>
<td>324</td>
<td>Simple</td>
<td>MEP</td>
</tr>
<tr>
<td>14</td>
<td>26</td>
<td>F</td>
<td>190,000</td>
<td>26,000</td>
<td>231</td>
<td>Plunging</td>
<td>MEP</td>
</tr>
</tbody>
</table>

**MEP:** Mucus extravasation phenomenon.
were in the group of HIV-positive patients. Table II displays the results for 14 HIV-positive patients, including the following variables: viral load in the blood, viral load in the ranula fluid, and the CD4+ cell count in the blood.

Thirteen out of 14 patients were not informed about their HIV status and were diagnosed as HIV positive after consulting for ranula as their main complaint. Therefore, these patients were not receiving any HAART medication. Patient 9 was the only one known to be HIV positive, and she was on HAART (d4T + 3TC + EFV) at the time of first consultation. Patient 10 was 7 months’ pregnant. No other significant oral or systemic comorbidity was reported.

All fluid samples tested positive and demonstrated different titer values ranging from undetectable (<25 copies/mL) to high concentration values (1,600,000 copies/mL). Six out of 14 ranula fluid specimens had a higher titer of HIV-1 RNA than the corresponding patient blood. All of the lesions were diagnosed as “mucus extravasation phenomenon pseudocyst.”

There was a positive linear correlation between the viral load in the blood and viral load in the fluid (Spearman test: \( r = 0.440 \), and between the CD4+ cell count and the viral load in the fluid (Spearman test: \( r = 0.421 \)), but both were not statistically significant. There were no statistical differences between simple and plunging ranulas regarding the level of viral load in the cystic lesion.

DISCUSSION

HIV-related salivary gland disease terminology describes a group of intra- and extraoral manifestations that are frequently observed in association with the HIV infection. This association does not imply a specific etiologic link between HIV and those clinical manifestations. The majority of HIV-SGD, i.e., enlargement of the parotid salivary gland, had been described long before the advent of HIV in immunologically competent patients. 12,13 Sjögren syndrome and lymphoepithelial cyst of the parotid gland in HIV-negative patients are associated with parotid enlargement and/or xerostomia. However, the frequent observation of these conditions in association with HIV infection has led to the concept of HIV-SGD. The presence of the HIV-1 virus in parotid tissue and the detection of high levels of viral particles in the fluid from parotid cysts in HIV-positive patients has contributed to the concept of HIV-SGD. 10,11 Finally, description of the diffuse infiltrative lymphocytosis syndrome (DILS) in various organs, including parotid and minor salivary glands, in HIV-positive patients, was a cornerstone in defining HIV-SGD. 14-16 DILS is characterized by a persistent increase of circulating and visceral CD8+ lymphocytic infiltration. It has been reported to play an important role in the physiopathogenesis of benign lymphoepithelial cyst (BLEC). 14,15 The HIV-related parotid cyst, commonly described as “benign lymphoepithelial cyst” of the parotid gland has, therefore, been accepted as the typical example of HIV-SGD.

The inclusion of ranula in the HIV-SGD category remains controversial. The exact prevalence of ranula in HIV-positive patients is not known. However, there are several reports of ranula observed in association with HIV. 6-9 Chidzonga and Mabomva 9 specifically reported a ranula incidence of 88.5% in HIV-positive patients: 38 cases of ranula. In another small sample, Butt et al. 8 found an incidence of 67.9% in HIV-positive patients: 28 cases of ranula. The present study found ranula in 66.7% of HIV-positive patients: 54 cases. This result is in line with other reports concerning the occurrence of ranula in HIV-positive patients.

This study demonstrated the presence and quantified various titer of HIV-1 RNA in the fluid from ranulas. All ranula fluid samples from HIV-positive patients tested positive. The viral load quantified in ranulas fluid in this study ranged from undetectable (<25 copies/mL) to very high concentration (160 million copies/mL). The relevance of this latter finding remains unclear and is the subject of ongoing research. HIV-1 has, indeed, been identified in several other human fluids aside from the blood, e.g., saliva, breast milk, urine, and semen. 17,18 In the present study, special steps were taken to minimize blood contamination during sample collection through FNA. The discordance between the concomitant HIV-1 RNA values in the blood and in the ranula fluid remains to be elucidated. Various studies that have compared HIV-1 RNA values in blood and other body fluids have reported different results, depending on the method used. 10 Uccini et al. 10,11 has detected 3 times more (3-15 ng/mL) HIV-1 p24 protein in the parotid BLEC fluid, whereas levels were undetectable in the peripheral blood of the same patients. The author postulated that the metaplastic squamous epithelium of benign lymphoepithelial cystic lesions of the parotid gland recruits HIV-1–infected cells from the underlying lymphoid tissue and favors their discharge into the cystic cavity. It was also suggested that the BLEC of parotid gland was a viral reservoir in HIV-1–infected patients.

The presence of HIV-1 in the normal saliva could also explain the presence of the virus in the extravasated mucus from the ranula. However, one would expect reduced viral load in the ranula fluid than in the concomitant patient’s blood. Indeed, the viral load in normal saliva is reported to be minimal compared with the corresponding viremia. 20,21 In the present study, it was found that 6 of the 14 specimens had HIV-1 RNA
copies higher than their respective blood samples. This latter finding is of utmost importance, because it might imply one of the following: There is a genuine high concentration of HIV-1 virus in some cases of ranula (viral reservoir), or it is a case of blood contamination during FNA. The contamination theory seems unlikely, considering the strict selection criteria implemented (clear fluid only). Furthermore, it is difficult to explain the very high concentrations observed in some ranula fluid samples (1,600,000 copies/mL). Even if the fluid aspirated was hemorrhagic, the viral load values in the fluid should remain similar to that of viral load in the blood of the same patients. Therefore, the theory that a ranula might, like the BLEC, be an HIV-1 viral reservoir is strongly supported.

Based on the observed prevalence of ranula in HIV-positive patients (66.7%), combined with the possibility that ranula may be a viral reservoir, the present authors believe that ranula should, like the BLEC, be considered as a HIV-SGD.

Notwithstanding the limitation due to the small sample in this study, there was no statistically significant correlation between the level of viral load in the blood and that of viral load in the cystic fluid for the 14 tested patients. The clinical presentation of the lesion, simple or plunging ranula, does not seem to statistically affect the viral content. A larger-sample study might provide a more conclusive outcome in relation to study of this rare pathologic entity.

CONCLUSIONS

It was demonstrated that all 14 ranula fluid samples studied contained HIV-1 RNA at various concentrations, ranging from <25 to 1,600,000 copies/mL. In 6 of the 14 specimens, the level of HIV-1 RNA copies/mL was higher than in the corresponding blood samples. Therefore, the ranula might represent an HIV-1 viral reservoir.

REFERENCES


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