ORAL DISEASE IN FIVE PATIENTS INFECTED WITH HIV AND SCREENED FOR EPSTEIN-BARR VIRUS

Santos, L.S.1; Azevedo, K.M.L.2; Oliveira L.H.S.1

1. Department of Microbiology and Parasitology, Universidade Federal Fluminense (UFF), Niterói, Rio de Janeiro, Brazil, 24210-130.
2. College of Medicine, Infectious Diseases Service, Universidade Federal Fluminense (UFF), Niterói, Rio de Janeiro, Brazil.

ABSTRACT

Oral opportunistic infections can lead to a range of diseases in people who are living with human immunodeficiency virus (HIV). Among them, herpes viruses are responsible for common diseases in HIV positive people. Both benign and malignant oral diseases are associated with the Epstein-Barr virus (EBV). Five cases of oral lesions from a cohort of HIV positive patients were associated with EBV detection in the oral mucosa. Active EBV deoxyribonucleic acid (DNA) was detected in three of the samples. Of the two cases with herpes simplex type 1 (HSV-1)-like ulcers, only one was associated with EBV detection. Similarly, only one candidiasis case was also EBV DNA positive. Severe immunosuppression was found in the patient with candidiasis and hairy leukoplakia. This man, who harbored the EBV-2 strain, was also positive for EBNA-2 ribonucleic acid (RNA) message and showed several symptoms indicative of disease development. This study provides baseline data on the dynamics of opportunistic oral infections in HIV infected individuals in the era of highly active antiretroviral therapy (HAART), as well as the detection of EBV markers and their link with clinical outcome.

Keywords: Epstein-Barr virus infections; HIV; Mouth mucosa; Polymerase chain reaction; RNA messenger

SHORT COMMUNICATION

Oral lesions are associated with HIV status infection and have been used to classify the stages of this infection. Among oral lesions, those promoted by the Epstein-Barr virus (EBV) are frequent in these patients. EBV is largely prevalent worldwide and it is normally acquired by oral transmission, frequently developing as an asymptomatic and persistent infection. The EBV life cycle occurs in two compartments: the peripheral blood and the oral cavity (Slots et al. 2006). Although EBV replication in oral epithelial cells is an infrequent event, the virus is typically shed in and transmitted by saliva (Liu et al. 2012).

In spite of the ubiquitous virus nature, the host-balance in health individuals EBV infection can result in a primary disease known as infectious mononucleosis, which is followed by asymptomatic viral persistence (Rickinson & Fox, 2013). Occasionally host control of this low level persistence breaks, resulting in the development of EBV-associated diseases. In this way the immune system plays an important role in virus-host establishment. Patients with acquired immunosuppression are at high risk for developing lymphoproliferative disorders and oral EBV diseases (Cesarman, 2014). In the era of highly active antiretroviral therapy (HAART), these treatments have reduced the prevalence of oral EBV manifestations in HIV positive patients (Hodgson et al. 2006). Despite these improvements, frequent presence of opportunistic infections in the oral cavity, especially oral candidiasis and oral hairy leukoplakia (OHL) have been observed (Miziara & Weber, 2006). The point prevalence of OHL may be as high as 28.8% (Moura et al. 2006).

Oral hairy leukoplakia (OHL) is one of the most common EBV-induced oral manifestations in HIV-positive individuals. It is a white, shaggy-appearing lesion that typically occurs on the lateral borders of the tongue (Greenspan & Greenspan, 1989). OHL is a unique example of a replicating EBV infection in the tongue epithelium (Webster-Cyriaque & Raab-Traub, 1998). Additionally, it is the only epithelial disease in which the EBV nuclear antigen 2 (EBNA-2) gene is expressed, although the role of EBNA-2 in this condition is unclear (Walling et al. 2004).

Screening for EBV in oral scrapes through molecular diagnostics is a noninvasive procedure to detect a virus that is potentially harmful to the oral cavity. Moreover, detection of markers for EBV is useful
to study the virus pathogenesis in the epithelial tissues. In a previous study we reported the presence of EBV DNA and RNA in a sampling of asymptomatic HIV positive subjects (Santos et al. 2014). Here we searched for EBV markers in five individuals carrying symptomatic oral diseases.

Five descriptive case studies were conducted in the city of Niterói, Rio de Janeiro. Between 2009 and 2010 oral cavity scrapes were taken from 150 HIV-infected adults. Samples were collected by scraping the epithelium of the lateral tongue borders with a cytobrush after the clinical examination. Demographic, behavioral, and HIV infection-related data were obtained via a structured questionnaire. The Ethics Committee of the College of Medicine at the university approved the protocols for sample collection and informed consent. CD4+ T cell counts were determined by flow cytometric immunophenotyping using standard protocols (Health Ministry, 2012). EBV detection and typing were performed using standard and nested PCRs, respectively (Durmaj et al. 1998; Telenti, 1993). Ribonucleic acid (RNA) was investigated by reverse transcription assay. Among 150 HIV-seropositive patients, 5 had oral lesions. A clinical examination of their oral cavities demonstrated two cases of candidiasis, two cases of possible herpes simplex virus serotype 1 (HSV-1) ulcers, and a case of candidiasis associated with oral hairy leukoplakia. All individuals were male, white, and ranged from 41 to 50 years old. Regarding educational status, one attended college and four had been to primary or high school. Four had smoked at least six years in their lifetime, from ten to twenty cigars a day. Three claimed to have non-stable sexual partners. With respect to HIV infection status, all reported being diagnosed more than four years prior. These individuals also had a detectable HIV viral load and CD4+ T cell counts below 500 cells/mm3. Four patients were on antiviral treatment. Active EBV DNA was detected in three samples, one with EBV type 1 and two with EBV type 2. EBNA-2 messenger RNA (mRNA) was also detected in all positive EBV samples. Of the two cases with HSV-1-like ulcers, only one was associated with EBV DNA detection. Similarly, only one candidiasis case was also EBV DNA positive. These individuals had CD4+ T counts below 200 cells/mm3. Severe immunosuppression was also found in the patient with candidiasis and oral hairy leukoplakia. This man, who harbored the EBV-2 strain, was also positive for EBNA-2 mRNA. In addition to being male, homosexual, white, and diagnosed more than four years prior (similar to the other four patients), he was a former smoker and was 49 years old. He had been receiving antiretroviral treatment for less than one year (Table 1).

In a cohort study of 150 HIV positive participants, where 85.3% has received antiretroviral therapy, we detected five patients with oral lesions, which have been seen as indicators of HIV clinical status, and more recently, it has been suggested, as an indicator of antiviral therapy failure (Moodley & Wood, 2012). In addition to the well-known standard symptoms associated with oral opportunistic infections, such as the decline of CD4+ T cell counts and detectable viral load, we found other common characteristics among the five patients. All were male. Gender differences associated with oral lesions have been previously shown (Dongo et al. 2013), most likely due to the higher counts of CD4+ T cells and lower viral load detected in females. White ethnicity was also a common trait among the group. In spite of the majority of our patients regarding themselves as whites (67.3%), the miscegenation of the Brazilian people makes the inference of any conclusions regarding this finding very difficult.

It is known that antiviral therapy reduces the development of oral opportunistic diseases (Palella et al 1998). In our study, the presence of lesions was independent of long-term therapy, which ranged from 10-15 years with no treatment. Ramírez-Amador et al.(2007) considered the presence of these oral lesions, especially candidiasis, as a marker of therapy failure. Here, two individuals carrying oral candidiasis had been treated for at least five years. However, it is important to consider that we have found five symptomatic patients out of 150 participants with 85.3% therapy coverage.

Table 1. Baseline demographic, behavioral and HIV related results from five patients demonstrating oral lesions

<table>
<thead>
<tr>
<th>Age</th>
<th>Gender</th>
<th>Sexual partners</th>
<th>Ethnicity</th>
<th>Smoking time (years)</th>
<th>HIV diagnosis (years)</th>
<th>HAART time (years)</th>
<th>CD4 cell/ml counts</th>
<th>Viral load/ml</th>
<th>Oral lesions</th>
<th>EBV1</th>
<th>EBV2</th>
<th>mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>M</td>
<td>Stable</td>
<td>W</td>
<td>10-20</td>
<td>10 - 15</td>
<td>87</td>
<td>1323</td>
<td>HSV-1</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>49</td>
<td>M</td>
<td>Non stable</td>
<td>W</td>
<td>6-10</td>
<td>&gt; 15</td>
<td>&lt; 1</td>
<td>34</td>
<td>46842</td>
<td>OHL, candidiasis,</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>48</td>
<td>M</td>
<td>Non stable</td>
<td>W</td>
<td>10-20</td>
<td>5 - 9</td>
<td>5 - 9</td>
<td>270</td>
<td>62</td>
<td>Candidiasis</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>57</td>
<td>M</td>
<td>Non stable</td>
<td>W</td>
<td>10-20</td>
<td>10 - 15</td>
<td>10 - 15</td>
<td>253</td>
<td>8684</td>
<td>Candidiasis</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>48</td>
<td>M</td>
<td>Stable</td>
<td>W</td>
<td>N</td>
<td>&gt; 15</td>
<td>N</td>
<td>99</td>
<td>35306</td>
<td>HSV-1</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
</tr>
</tbody>
</table>

Legends: M – male; W – white; N – No; Y – yes.
Two patients had clinical symptoms of alpha herpesvirus and three had indications of EBV, a gamma herpesvirus. HIV infection is associated with an increased risk for herpesvirus disease. Chakraborty et al. (2010), demonstrated a high prevalence of herpesvirus signals among 200 Indian patients, which were dominant in the male subgroup. In an attempt to characterize the dynamics of opportunistic diseases, we have seen that out of two cases with HSV-1-like ulcers, only one showed EBV oral co-infection; however the sores between the two cases did not present notable differences. On the contrary, only one case of candidiasis was EBV positive, and here the patient had OHL disease. EBV is involved, at least in primary infection of healthy individuals, in a lytic oral infection. In immunosuppressed patients, later EBV lytic replication in the oral mucosa can result in OHL. The pathogenesis of OHL is clearly complex, requiring a convergence of several factors. Beyond the most relevant factors, such as active EBV-2 infection, EBNA-2 gene expression, a detectable HIV viral load and a weakened immune system, other epidemiologic determinants have been associated with this disease, which was observed in the patient reported here. Male gender specificity for OHL has been noted (Rao et al. 2012). Tobacco use has also been associated with oral lesions in HIV-positive individuals (Boulter et al. 1996). In a South African study, it was also shown that white ethnicity was a predictive factor for the development of OHL and oral candidiasis (Badri et al. 2001). The disease is common in the 50-70-year age group. It also appears to be associated with male-to-male HIV transmission (Patton et al. 2002). Therefore, in HIV-seropositive individuals without good treatment compliance, determinants such as active, severe immunosuppression, male gender, white ethnicity, former smoking, middle age, and lifestyle can act synergistically to promote the emergence of this disease. Notably, the patient had been receiving antiretroviral treatment for less than one year, and his immune system had most likely not yet recovered.

Like most oral lesions that are often clearly visible, OHL is usually presumptively diagnosed. According to Greenspan et al. (1998), the definitive diagnosis of OHL requires demonstration of EBV infection. OHL has been diagnosed by cytology, immunohemochromy, and in situ hybridization (Dias et al. 2000, Dias et al 2012). Here, EBV was diagnosed by clinical exam followed by detection of viral DNA and mRNA through PCR from tongue scraping without the need for surgical biopsy. Therefore, we have determined not only the presence of EBV DNA in the oral mucosa but also its activity. We do not consider the expression of the EBNA-2 gene to be an exclusive marker for OHL, but together with the others factors specified here, these diagnostic components have a high degree of specificity.

The dynamics of oral opportunistic infections in immunocompromised people deserves attention, as well as the risk for EBV infection in these patients. To that end, this work provides evidence for further discussion regarding the relevance of oral manifestations in the HAART era and the association of EBV disease with clinical outcome. It also highlights the search for less invasive methods for the detection of oral opportunistic infections.

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