The value of the exfoliative cytology to the diagnosis of oral hairy leukoplakia – case report

Wartość cytologii eksfoliatywnej w diagnostyce leukoplakii włochatej jamy ustnej – studium przypadku

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ABSTRACT: Oral hairy leukoplakia is a lesion that generally occurs within the tongue. It is characterized by a white patch on the side of the tongue with a corrugated or hairy appearance. The OHL affects patients with HIV infection and has characteristics that differ from oral leukoplakia. The etiology of oral hairy leukoplakia is linked to the infection of epithelial cells by Epstein-Barr virus. The objective of this paper was to report a case of atypical oral hairy leukoplakia in a patient with HIV infection. A male patient, 43 years old, was hospitalized complaining of abdominal pain and symptoms suggestive of jaundice. A white plaque was identified on his tongue during a clinical examination. That lesion was painless and not removable by scraping. A liquid-based cytology was performed and cytological analysis revealed structures suggestive of Candida sp. and cytological changes consistent with oral hairy leukoplakia (Cowdry type A inclusion bodies, and ground-glass nuclei). Exfoliative cytology is a reliable test that can be used by a dentist and otolaryngologists to determine the diagnosis of atypical cases of oral hairy leukoplakia.

KEYWORDS: Oral hairy leukoplakia, Epstein-Barr virus infections, Cytology, HIV, AIDS

STRESZCZENIE: Leukoplakia włochata jamy ustnej (OHL) to zmiana z reguły występująca w obrębie języka. Leukoplakia włochata charakteryzuje się obecnością białej plamy o pofałdowanym lub włochatym wyglądzie na bocznej powierzchni języka. OHL występuje u pacjentów zakażonych wirusem HIV i posiada cechy odróżniające ją od leukoplakii jamy ustnej. Etiologia leukoplakii włochatej jamy ustnej powiązana jest z zakażeniem komórek nabłonka wirusem Epsteina-Barr. Celem niniejszego artykułu jest przedstawienie przypadku atypowej leukoplakii włochatej jamy ustnej u pacjenta z zakażeniem HIV. Pacjent płci męskiej, lat 43, został przyjęty do szpitala z bólem brzucha i z objawami wskazującymi na żółtaczkę. Podczas badania klinicznego na języku pacjenta stwierdzono obecność białej plamy. Zmiana była bezbolesna i nie dawała się usunąć poprzez zdrapanie. Wykonano badanie cytologiczne w cieczy; analiza obrazu cytologicznego wykazała obecność struktur charakterystycznych dla rodzaju *Candida* oraz zmian zgodnych z obrazem leukoplakii włochatej jamy ustnej (ciałka inkluzyjne Cowdry'ego typu A oraz obraz matowej szyby w jądrach komórkowych). Technika cytologii eksfoliatywnej jest wiarygodną techniką badawczą, która może być stosowana przez stomatologów i laryngologów w rozpoznawaniu atypowych przypadków leukoplakii włochatej jamy ustnej.

SŁOWA KLUCZOWE: Leukoplakia włochata jamy ustnej; zakażenia wirusem Epsteina-Barr; cytologia; HIV; AIDS.

INTRODUCTION

The acquired immunodeficiency syndrome (AIDS) favors the development of specific oral lesions, such as Kaposi's sarcoma, non-Hodgkin lymphoma, and multiple opportunistic infections such as candidiasis and oral hairy leukoplakia (OHL) [1-4]. OHL was first described by Greenspan et al. in 1984. These authors observed 37 cases of OHL involving homosexuals that were illicit drug users [1,2].

Early studies suggested that a characteristic OHL was found in homosexual patients. However, the occurrence of this lesion in other risk groups showed that it is a marker of HIV infection [5,6]. Its clinical manifestation in children, adolescents, patients undergoing immunosuppressive therapy, and individuals without immune impairment is rare [7-9]. However, some studies have also shown the manifestation of OHL in patients with moderate immunodeficiency and in immunocompetent individuals [6,10].

OHL lesions can develop in the floor of the mouth, tongue, buccal mucosa, and soft palate. However, the most common clinical feature is the presence of a bilateral asymptomatic white patch on the edge of the tongue (not removable by scraping). The surface of the lesion may be flat, corrugated or hairy with projections arranged in the form of vertical lines [1,6,9-13].

The etiological agent responsible for OHL is the Epstein-Barr virus (EBV), also called HHV-4 (Human herpesvirus 4). This virus is transmitted by close contact with oral secretions [6,9,10,12,14]. EBV has been associated with other diseases such as infectious mononucleosis, nasopharyngeal carcinoma, T-cell lymphomas and non-Hodgkin lymphoma. However, the only clinical manifestations recognized by replicative EBV infection are OHL and infectious mononucleosis [15,16]. The differential diagnosis of OHL includes oral leukoplakia, lichen planus, pseudomembranous candidiasis, chronic hyperplastic candidiasis and geographic tongue [5].

A detailed clinical examination associated with histopathological and/or cytological test are essential to establish the definitive diagnosis of OHL, because they detect the replicative virus infection. Some methods can be used to confirm the presence of EBV during the latency period: electronic microscopy, *in situ* hybridization, immunohistochemistry, and polymerase chain reaction (PCR) ascertaining the presence of the agent [1,8,17,18].

The histopathological characteristics of OHL include: acanthosis, papillomatosis, basal layer hyperplasia, hyperparakeratosis, parakeratosis, clear cells, nuclear changes in keratinocytes (Cowdry type A inclusion bodies, and ground-glass nuclei), and ballooning degeneration [1,6,8,9]. The purpose of this paper was to report on an atypical case of OHL the diagnosis of which was confirmed by exfoliative cytology.

CASE REPORT

A male patient, 43 years old was referred to Hospital Oswaldo Cruz (Curitiba-PR) complaining of widespread pain, abdominal bloating and anorexia. Medical history revealed that the patient was a smoker and a drinker. Moreover, he had hepatitis C and HIV infection. During the anamnesis, he reported that his HIV and HCV infections had not been treated or controlled. A list of laboratory tests was requested to assess his general health. The results of some of those tests are shown in Table I.

Table 1. Results of the laboratorial exams

TESTS	RESULTS	REFERENCE VALUES
Erythrocyte sedimentation rate	125 mm	0–10 mm
Erythrocytes	3.20 M/µL	4,5–6.0 M/µL
Hemoglobin	10.0 g/dL	14.0–16.0 k/µL
Leucocytes	4.60 k/µL	4.0–11.0 k/µL
Platelets	69 k/µL	150–450 k/µL
Bastonetes (Rods)	15.0%	0–4%

The intra-oral clinical examination revealed the presence of caries, advanced periodontitis, dental calculus, atrophic glossitis, and cheek biting (oral frictional hyperkeratosis). Furthermore, a painless white plaque was observed on the right side of the tongue (Fig. 1a). That lesion had a square shape and well-defined limits. The patient did not report any discomfort and did not know that there was a lesion on his tongue. Initially, we tried to establish its relationship with a local traumatic injury. However, there was no crown or fractured tooth malocclusion which could justify the presence of the lesion. Thus, the diagnosis of oral leukoplakia was established as the patient was a smoker and an alcoholic.

Before the biopsy, the lesion was subjected to oral exfoliative cytology. The cytological examination was performed with a cytology kit (Liqui-PREP™ Cellular base solution). The collected material was centrifuged and prepared as a slide, which was followed by Papanicolaou staining. The evaluation of the smear was performed under a light microscope (Eclipse E200, Nikon, Japan) with a 10x and 40x objective lens.

Four days later the patient was reexamined and other oral lesions appeared. Lesions in the form of vertical white lines were observed bilaterally on the edge of the tongue and were suggestive OHL (Figure 1b). Moreover, several pseudomembranes easily removed by scraping, involved the palate, buccal mucosa, floor of the mouth and gums, showing a characteristic picture of pseudomembranous candidiasis (Fig. 2).

The cytological evaluation revealed a degree of cellularity satisfactory for classification of the smears. The presence of keratinocytes showing cytological changes consistent with those induced by EBV was detected. Some cells showed nuclear changes: cowdry type A inclusion bodies (Fig. 3), and ground-glass nuclei with margination of chromatin-shaped collar (Fig. 4). Furthermore, it was also possible to observe the material filamentous structures compatible with *Candida sp.* characteristics, indicating the presence of oral candidiasis. Other cytological findings were the presence

Figure 1. Lesions of oral hairy leukoplakia in the tongue: (a) = plaque; (b) = vertical lines.

Figure 2. Oral pseudomembranous candidiasis in the buccal mucosa.

of leukocytes and rarely a high frequency of binucleated cell. The sample was found negative for dysplastic or neoplastic changes.

The patient was treated with diuretics (100 mg of Spironolactone and 40 mg of Furosemide), antimicrobials (1 g of Ceftriaxone and 600 mg of Clindamycin), anxiolytics (5 mg of Diazepam), opioid analgesics (100 mg of Tramadol), anticonvulsants (100 mg of Topiramate) and anti-inflammatory drugs (500 mg of sodium dipyrone). Treatment of oral candidiasis was conducted by administering 200 mg of Fluconazole for seven days.

The patient suffered a fall in the hospital corridor during one of the attempted escapes. Due to a trauma in the head, the patient was transferred to a hospital specializing in the treatment of trauma. The systemic involvement at the time of transfer included pancreatitis, cholestatic hepatitis, cirrhosis, pulmonary infection, abscess of the chest wall and cranial trauma. Monitoring and treatment of oral lesions was not performed because the patient died due to complications of his systemic condition.

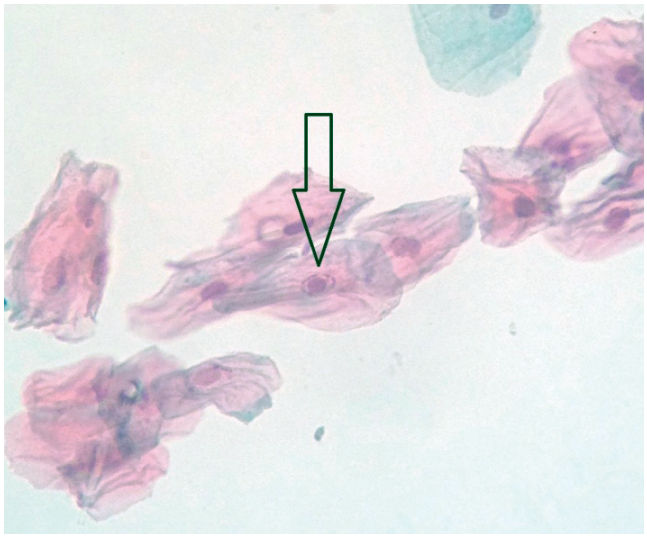

Figure 3. Oral exfoliative cytology: (a) = keratinocyte nuclear changes Cowdry type A (Papanicolaou x40).

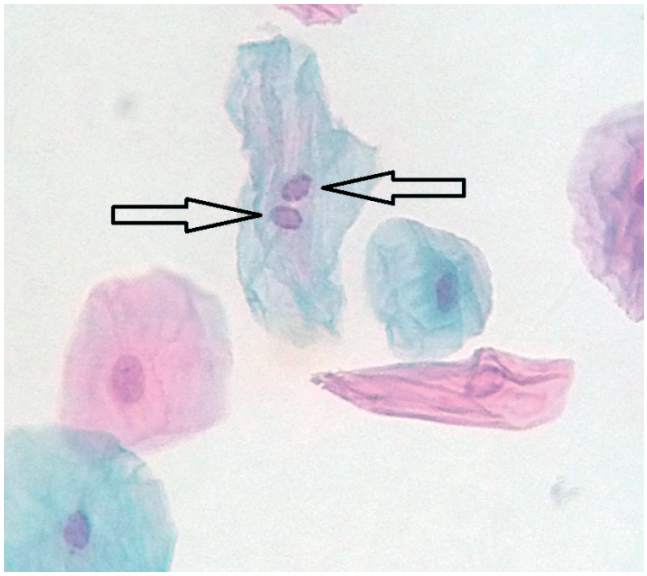

Figure 4. Oral exfoliative cytology. Margination of collar-shaped chromatin (Papanicolaou x100).

DISCUSSION

OHL has been described as the most common injury associated with EBV infection in patients in advanced stages of HIV / AIDS [5,6,9,10,17]. Often, OHL is the first sign of an HIV infection [17]. OHL is a clinical entity whose diagnosis can be easily established by a dentist aware of the clinical presentation. Clinically, the characteristics found are represented in the form of a painless white lesion. In general, the lesions are resistant to scraping [1,2,6,11-13,17]. In most cases, vertical white lines appear on the lateral margins of the tongue in a bilateral pattern [1,2,8,12].

No clinical response is observed when atypical lesions of OHL are confused with other white lesions and treated with topical antifungal agents or corticosteroids. In such cases, biopsy should be performed to determine the correct diagnosis. [10]. In this case report, the patient developed initially a white plaque with well-defined margins on the lateral surface of the tongue. The clinical appearance of the lesion and the patient's history of smoking and alcoholism led the examiners to formulate initial clinical hypothesis of oral leukoplakia. Unlike in OHL, in oral leukoplakia, epithelial changes are seen in the form of a white board not removable by scraping, without any apparent cause and with potential for malignancy [19].

Laboratory tests showed anemia, increased erythrocyte sedimentation rate, and thrombocytopenia. Those laboratory findings did not allow performing the biopsy. Thus, the general health of the patient was critical for planning oral exfoliative cytology. Oral exfoliative cytology is a minimally-invasive technique which leads to a minor trauma. This diagnostic method does not compromise the integrity of the epithelium and allows for exfoliation at more than one site [17,20].

Oral cytology revealed keratinocytes with nuclear changes. Those cellular changes were consistent with the diagnosis of OHL. In addition, four days after exfoliative cytology, the patient developed other white lesions on the tongue that were characteristic for OHL. The cytological findings were similar to those cited by Dias et al. [8]. Those authors analyzed 11 cases of OHL and observed cowdry type A inclusion bodies and ground-glass nuclei with margination of collar-shaped chromatin [8]. Cowdry type A inclusion bodies are also frequent in herpes virus infections. The infected cells show eosinophilic inclusions surrounded by a lighter halo. The ground-glass nuclei present chromatin in the marginal region and the appearance of a chromatin-shaped collar is the result of nodular chromatin condensation along the nuclear membrane [8,17].

Exfoliative cytology is a technique widely used in medicine, especially in the prevention and diagnostics of cervical cancer. It was developed by a Greek doctor, Georgios Papanicolaou, in 1928. By definition, cytology studies the morphological and morphometric aspects of desquamated mucosal cells using optical microscopy [21]. However, this technique has been little used by dentists and otolaryngologists in clinical practice. Exfoliative cytology is a complementary test that can aid in the early diagnosis of several oral lesions [17,20]. Dias et al. [17] investigated three patients who were not aware they had been HIV-positive and had lesions suggestive of OHL on the tongue. That work highlighted the importance of the technique of exfoliative cytology in the early diagnosis of HIV/AIDS.

Cytology, histopathology, immunohistochemistry, *in situ* hybridization and PCR (polymerase chain reaction) can be used to detect OHL at subclinical levels. These methods can be used jointly to obtain a more conclusive result [8,16,22,23]. Some authors claim that cytology should be used as a routine procedure and may be the method of choice for the diagnosis of clinical and subclinical OHL [17]. However, molecular and immunological tests can be used for cases where the patient has classic OHL lesions but the result was negative by cytology [8,18].

The fact of the patient being a smoker may correlate with the appearance of OHL because previous studies have suggested the association of smoking with a clinical manifestation of a lesion [6].

Opportunistic infections declined and consequently there was an improvement in life quality of patients that apply antiretroviral therapy regularly. However, some authors report that there is no influence of antiretroviral therapy on the disappearance or appearance of OHL lesions[6]. There are a few double-blind, placebo-controlled randomized clinical trials on topical treatment of OHL [4].

Topical application of podophyllin resin 25% has been described as one of the treatment options for OHL lesions. However, well-designed randomized clinical trials to evaluate safety and efficacy of topical and systemic treatments in the oral mucosa and perioral regions of HIV-positive patients with oral lesions are necessary [4]. Actually, a combined topical therapy with 25% podophyllin and 5% acyclovir cream has been effective, demonstrating fast healing without recurrence.

CONCLUSION

OHL is an important marker of progression of AIDS associated with immunological impairment. Furthermore, it can manifest in an atypical form in some patients with severe immunosuppression. Thus, exfoliative cytology is very useful for diagnosing atypical cases of OHL.

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