Case Report

Epstein–Barr virus-positive ulcer in the oral cavity

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Abstract

Epstein–Barr virus-positive ulcer (EBV + U) is a recently reported B cell lymphoproliferative disorder in the oral cavity, oropharynx, gastrointestinal tract and skin, principally in immunosuppressed patients. A 53-year-old female patient with rheumatoid arthritis treated with methotrexate, presenting ulcers of unknown duration on the dorsum and the lateral left border of the tongue. Excisional biopsy, histopathological analysis and histochemical stains for syphilis (Warthin–Starry), mycotic diseases (Grocott silver methenamine), tuberculosis (Ziehl–Neelsen), immunohistochemistry tests for herpesvirus type 8 (CMV), EBV (LMP-1) and DNA extraction for polymerase chain reaction (PCR) assay to CMV, EBV and herpes simplex virus-1 were performed. Posterior to PCR assay, the final diagnosis was EBV + U in the oral cavity. Acyclovir® was prescribed, showing clinical improvement. A case of EBV + U with clinical characteristics similar to other lesions or conditions has been reported. Special assays are necessary for an accurate diagnosis and treatment.

Keywords: Acyclovir, chronic ulcers, Epstein–Barr virus, methotrexate

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INTRODUCTION

Epstein–Barr virus (EBV) is present in 90% of the population. It has a predilection for B cells, in which it causes proliferation and transformation.[1] Its viral latency is established in memory B cells after primary infection, which occurs mainly during childhood and adolescence.[2] The cell-mediated immune system keeps B cells infected by EBV (B-EBV +) under viral latency. Therefore, drug-related immunosuppression allows B-EBV + to proliferate resulting in a wide range of lymphoproliferative disorders immunodeficiency associated. The above is observed in patients with lymphoproliferative diseases associated with primary immune disorders or posttransplanting (iatrogenic immunodeficiency).[3]

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EBV-positive ulcer (EBV+U) is a newly described entity consisting of well-defined and isolated lesions that occur in the skin, oral cavity or gastrointestinal tract. the morphological characteristics of EBV+U may vary, but usually, an ulcer with mixed lymphoplasmacytic infiltrates on a background of necrotic tissue is found. Cells within the infiltrate consist of small T lymphocytes, large B lymphocytes and often Reed–Sternberg-like cells that coexpress B cell and CD30 antigens.[4] EBV+U occurs predominantly in patients with age-related immunosuppression or iatrogenic immunosuppression by autoimmune disease treatment.[5,6]

EBV+U could be developed in the oral mucosa since the oral cavity has the highest concentration of infected B

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cells in Waldeyer's ring. This alteration was first described in 2010 by Dojcinov *et al*.[3] in a study that included 26 patients that presented ulcers in the oral cavity, skin and colon, related to some type of immunosuppression. They proposed the EBV+U as a new clinicopathological entity with histopathological characteristics similar to Hodgkin's lymphoma but self-limited, which generally responds well to the administration of conservative treatments, such as the regulation of immunosuppressive drugs.

CASE REPORT

A 53-year-old female patient was referred to her general dentist, to our Oral Medicine Clinic of the Postgraduate and Research Division, Faculty of Dentistry National University of Mexico (UNAM). The patient complained of ulcers on the dorsum and left border of the tongue. On interrogation, she mentioned suffering rheumatoid arthritis with 30 months of evolution, treated with methotrexate and prednisone as well as hypertension for 2 years under metoprolol medication. Intraoral examination confirms the presence of an ulcer on the left border of her tongue, with well-defined and indurated borders, about 0.7 cm in length and another ulcer on the dorsum of the tongue also well-defined and indurated borders, measuring 1.8 cm × 0.5 cm × 0.5 cm [Figure 1a and b]. Both ulcers caused painful symptoms. The patient reported 4 months of evolution, increasing in size and symptoms. The patient referred the previous prescription of diverse pharmacotherapy indicated by general dentists such as amoxicillin, ketoconazole and nystatin without improvement.

Our differential clinical diagnosis included mycosis, traumatic ulcerative granuloma with stromal eosinophils or oral squamous cell carcinoma. An excisional biopsy was performed; hemostasis with electrocautery was fulfilled in both lesions. Specimens were fixed in 10% formalin and send to our histopathological diagnostic service in our institution.

The specimens were labeled as follows: (A) single oval-shaped specimen of soft tissue, measuring 2.2 cm × 1.5 cm × 1 cm, irregular surface with a poorly delimited ulcer, irregular edges, firm consistency, dark and light brown color toward the center, edges were inked and representative sections were included. (B) A single specimen measuring 1 cm × 0.7 cm × 0.5 cm, with an oval shape, ulcerated, firm consistency, dark brown color and the edges were inked [Figure 1c].

The histological sections showed loss of continuity of the epithelium replaced by fibrin, the connective tissue presented abundant mixed inflammatory infiltrate predominantly lymphoplasmacytic, some cells exhibited pleomorphism. In some areas, biological material that resembled a foreign body reaction with the presence of epithelioid cells, histiocytes and multinucleated giant cells was also observed, as well as cytopathic effect in the periphery of the ulcerated epithelium [Figure 1d-f].

The patient's rheumatoid arthritis condition impaired to assist to her postoperative checkup and removal of suture appointment. She attended to her private general dentist, which was contacted and reported that the postsurgical zone was observed in unfavorable conditions for both lesions.

According to microscopic findings, a diagnosis of a nonspecific chronic ulcer with granulomatous foreign body reaction was emitted; however, the special stains

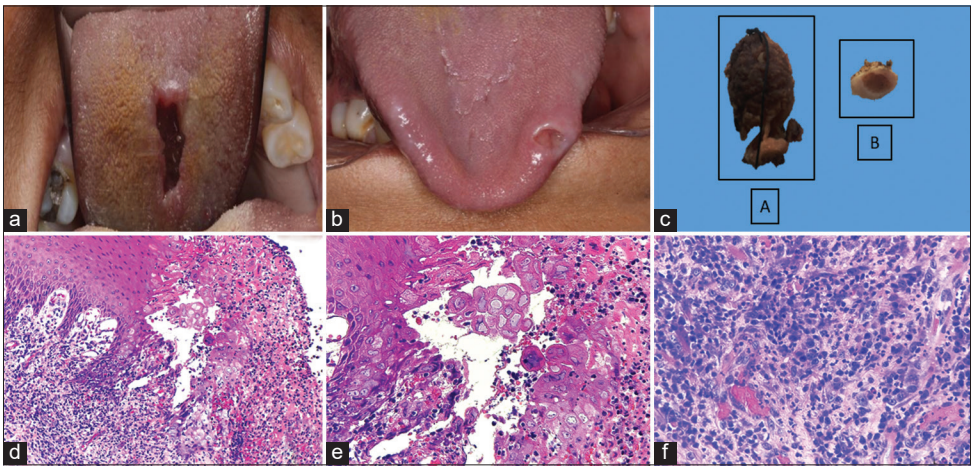

Figure 1: (a) Ulcer in the tongue dorsum (b) Ulcer in the left border of the tongue, (c) Macroscopic analysis of both specimen labeled as A and B, (d) photomicrography at ×100 magnifications that show the discontinuity of epithelium with inflammatory infiltrate, (e) photomicrography at ×400 magnifications showing cytopathic changes suggestive of viral infection in epithelial cells and (f) photomicrography at ×400 magnifications showing the high presence of lymphoplasmacytic infiltrate underlying conjunctive tissue

to detect the presence of fungi (Grocott methenamine silver), for *Mycobacterium tuberculosis* (Ziehl–Neelsen) and for *Treponema pallidum* (Warthin–Starry), were negative [Figure 2].

In relation to the cytopathic changes, treatment with 200 mg acyclovir 5 times daily for 3 weeks and clinical evaluation at 2 weeks was scheduled. The patient again failed to her evaluation appointment, but she provided photographs of her clinical progress treatment, showing resolution of the ulcer located on the lateral left border of the tongue and improvement in ulcer located on the dorsum of the tongue, with a decrease in size and depth.

To validate the response to herpes virus treatment, endpoint polymerase chain reaction (PCR) assay was performed, for which DNA extraction was done from 50 μm thick slice, using the ReliaPrep™ FFPE gDNA Miniprep system (Promega, MA, USA); the quality and amounts of DNA were determinate through NanoDrop 2000 spectrophotometer (Thermo Fisher, Wilmington, DE, USA).

A total of 100 ng of DNA was amplified with the PCR master mix kit (Promega, Madison, WI). Primers used to identify LMP1 EBV, CMVUL44 and HVS UL56; listed in Table 1, obtained from Eurofin mwg/operon (Louisville, KY). Glyceraldehyde-3-phosphate dehydrogenase was employed as internal control and normalization reference. Amplifications were carried out in a MaxyGene II thermal cycler (Axygen, NY) carrying out 40 cycles. Amplification products were resolved on 2% agarose gels in TAE buffer (Amresco, Solon, OH) at 100 mV. The gels were stained with 0.05 μg/ml ethidium bromide (Promega, Madison, WI) and visualized in a gel documentation system (Axygen, NY), to determine their expression by optical density in GelQuantNet (BiochemLab Solutions, UCSF, CA, USA). Amplification assay was performed in triplicate obtaining predominant positivity for EBV, with the presence of CMV and herpes simplex virus (HSV) [Figure 3].

Two weeks later, the patient attended to our clinic, commenting that she did not finish the treatment as indicated since she felt improvement during the 1st day. The lesion in the dorsum tongue was still present as well as painful symptoms. It was decided to resume the therapy with acyclovir as it was previously prescribed.

The patient failed again to her appointment because of a hip fracture, but she provided clinical photographs showing the resolution of the other lesion, without symptomatology [Figure 4].

DISCUSSION

EBV+U is a lymphoproliferative disease described for the first time in 2010 by Dojcinov *et al*.,[3] in which a series of 26 cases were reported showing mucocutaneous ulcers that slowly developed induration, involving the oropharyngeal mucosa, gastrointestinal tract or skin, with characteristic histological and immunophenotypic characteristics of lymphoproliferative disease associated with EBV, related to different forms of immunosuppression.[7-11]

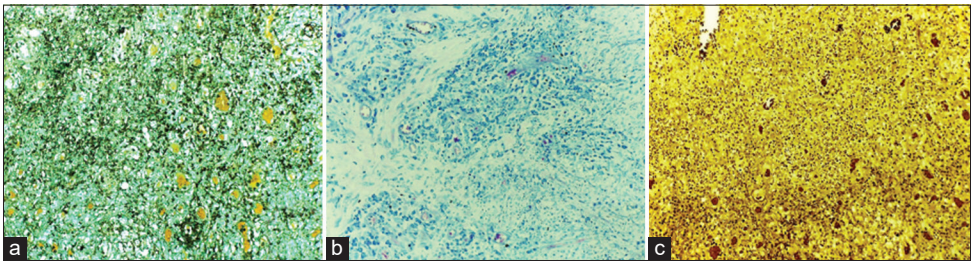

Figure 2: Photomicrographs at ×100 magnifications of special stains, (a) Grocott methenamine silver, (b) Staining with Ziehl–Neelsen and, (c) Staining with Warthin–Starry. Negative results were obtained

Table 1: Primers' sequence for polymerase chain reaction

Marker	Probe sequence	Estimated product size (bp)	Accession number GEO (http://ncbi.nlm.nih.gov/geo)
LMP1 EBV	us: 5'-TGAACACCACCACGATGACT-3' ds: 5'-GTGCGCCTAGGTTTTGAGAG-3'	155	NC_007605.1
CMVUL44	us: 5'-ATCTAGATTTCGGCGTGGTG-3' ds: 5'-GTGGAAACTGACGCGGTTAT-3'	157	NC_006273.2
HVS	us: 5'-GAGCACGTCCTCCTGTTTTC-3' ds: 5'-GGCCAGTCGAAGTTGATGAT-3'	160	MH102298.1
GAPDH	us 5´-ACCACAGTCCATGCCATCAC-3´ ds 5´-TCCACCACCCTGTTGCTGTA-3´	151	NM_001289745.1

GEO: Gene Expression Omnibus

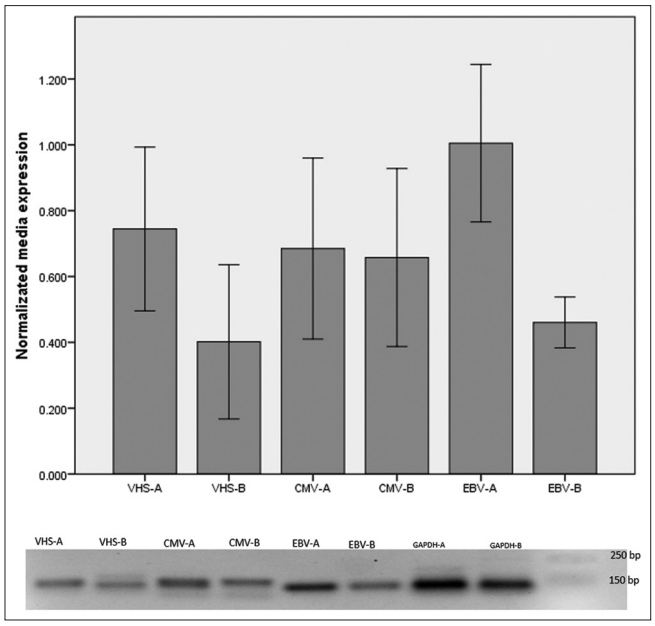

Figure 3: Viral detection by polymerase chain reaction analysis. (a) Media expression of VHS, CMV and Epstein–Barr virus; Glyceraldehyde-3-phosphate dehydrogenase-normalized; and (b) polymerase chain reaction amplification products resolved on agarose 2% gel. Predominant expression in Epstein–Barr virus-A sample was observed

Ulcers in the oral cavity may occur in oral mucosa, gingiva or tongue, as in our case; it can be related to chronic inflammation by local trauma, resulting in the formation of an ulcer and localized proliferation of B cells infected with EBV.[12]

The actual incidence of EBV+U is unknown. In a recent literature review, Roberts *et al*., in 2016 include 51 cases, in which the most frequent causes were immunosuppression and the most common drugs used were as follows: methotrexate, azathioprine and cyclosporine in 56% of the cases; combined with advanced age in 40%. Clinically, EBV+U usually manifests as a single lesion in 84% of cases as mucosal or cutaneous ulcer, well-circumscribed, without lymph node involvement. The oropharynx was the most common site affected, followed by skin.[6]

The main histological features include a polymorphic inflammatory infiltrate with lymphocytes, histiocytes, eosinophils and plasma cells mixed with scattered atypical B cells, some with Reed–Sternberg-type morphology. These atypical lymphocytes Reed–Sternberg type are positive for CD30, CD15, MUM1, PAX5 and OCT-2 and mostly negative for CD20, CD79a, CD45, CD3 and Bcl-6. In relation to auxiliary assays for diagnosis, the *in situ* hybridization for EBV-encoded small RNA (EBER) oligonucleotides is positive by definition. In some cases, large B cells that infiltrated the arteries are associated with

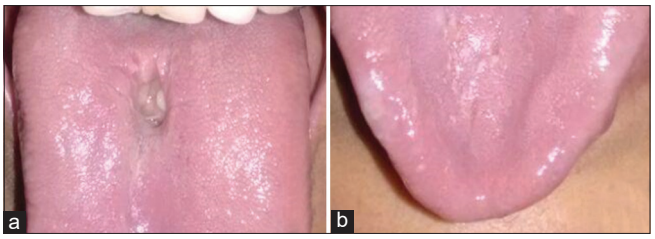

Figure 4: Follow-up 7 days after starting treatment. (a) Partial healing of the dorsum tongue ulcer and (b) total healing of the left lateral ulcer of the tongue

thrombosis and necrosis.[5] In addition, heavy chains of monoclonal immunoglobulin and T cell receptor (TCR) rearrangement have been observed in up to 40% of cases, suggesting a clonal proliferation of B cells driven by EBV.[3]

This is the first time that PCR assay was used for EBV + U diagnosis and this assay proved results as an extremely useful strategy to determine the specific genotype of the virus.

Their applications to lymphoproliferative disorders associated with EBV today have been restricted to determine the clonality of the TCR and immunoglobulins, nevertheless, with this finding, their scope could be extended.[13]

The EBV + U differential diagnosis includes other lymphoproliferative disorders and neoplasms, especially Hodgkin's lymphoma and diffuse B-cell lymphoma.[5] In our case, the clinical diagnosis was unclear, since we considered a squamous cell carcinoma, syphilis, tuberculosis, fungal infection or lymphoma; however, the anatomical location of the lesion, the histopathological findings and the PCR assay favored the final diagnosis of EBV+U.

Several personal problems of the patient, such as her general systemic health complications by arthritis and hypertension and her lack of attachment to guidelines indicated hampered the diagnosis, treatment and follow-up of this case. The therapy used was acyclovir, an antiviral HSV drug, which promoted clinical improvement, validated by our PCR results. Acyclovir can inhibit lytic infection by the herpes virus when it is phosphorylated by viral kinases and incorporated into viral DNA.[14,15] To date, there are no reports of this therapy or management of these alterations, so considering the above, this could be an additional measure for EBV+U cases that occur during pharmacological immunosuppression.[16-18] In our case, an important limiting was the patient lack to therapeutic guidelines suggested and despite that improvement was observed; however, more clinical studies are needed to corroborate our findings.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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