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HIV Disease/Oral Medicine

Multiple parotid lymphoepithelial cysts in patients with HIV-infection: report of two cases

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OBJECTIVE: Bilateral and multiple lymphoepithelial cysts (LECs) of major salivary glands, in particular of parotid glands, are quite rare and have been reported in human immunodeficiency virus (HIV) infected patients with an incidence of about 3–6%. These lesions represent an early manifestation of HIV infection and are rarely found in patients with advanced acquired immunodeficiency syndrome.

MATERIALS: Two cases of parotid LECs, the first occurring in a middle-age white woman and the second in a young white boy, both in advanced phases of HIV infection, are reported.

RESULTS: Clinical, cytological, histological and immunohistochemical (cytokeratin AE1/AE3, CD20, CD45RA, CD8, κ and λ immunoglobulin light chains, S-100, MLA and Ki67) features are described.

CONCLUSIONS: Fine needle aspiration (FNA), a relatively non-traumatic procedure, could represent both a diagnostic and a therapeutic tool in parotid LECs. No surgical therapy is usually required for these lesions and aspiration of cystic fluid with FNA is quite resolutive, although evidence of further relapses does exist. Surgical excision may become necessary when pain, because of persistent and progressive swelling of the parotid gland, occurs.
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Introduction

Lymphoepithelial cysts (LECs) are uncommon lesions of the oral cavity, major salivary glands and neck, first described by Bernier and Bhaskar in 1958 and so defined to distinguish them from other cystic lesions derived from embryological branchial remnants (Bernier and Bhaskar, 1958; Bhaskar and Bernier, 1959). Until early 1980s LECs were considered a rare manifestation of Sjögren's syndrome, while later some studies showed a strict predilection of these parotid lesions for human immunodeficiency virus (HIV)-positive patients (Ryan *et al*, 1985; Shaha *et al*, 1993). Nowadays the incidence among HIV-infected patients is estimated at about 3–6% (Allen *et al*, 1999; Vargas *et al*, 2001). The association of LECs with HIV is more frequent during the early phases of viral infection, while cases of LECs in advanced acquired immunodeficiency syndrome (AIDS) phases are rarely described (Fortuno-Mar *et al*, 1999; Vargas, 2003). The simultaneous occurrence of multiple LECs, cytomegalovirus and mycobacterial parotid infections in HIV-patients has been recently reported (Vargas *et al*, 2001).

The histopathogenesis of parotid LECs is still unclear at this point. The parotid swelling may originate from a hyperplastic activity of intraglandular lymphocytes or, as the prevailing hypothesis, from glandular epithelial inclusions in intraparotid lymph nodes during early embryogenic development. In this case the subsequent epithelial proliferation would result in a clinically evident parotid enlargement. Clinical features are not useful alone to distinguish between LECs and other parotid masses (Warthin's Tumor and mucosa associated lymphoid tissue (MALT) Lymphoma) and so fine needle aspiration (FNA) could represent the best mini-invasive diagnostic tool.

Treatment is usually not necessary in LECs in view of the benign nature of the disease, although worrisome histological alterations requiring surgical excision have been reported after diagnostic FNA (Kavishwar *et al*, 1999; Li *et al*, 2000).

Materials and methods

Two cases of parotid LECs, the first occurring in a middle-age white woman and the second in a young white boy, both in advanced phases of HIV infection, were reported. The aspiration specimens were fixed with cytologic fixative spray and stained with Hematoxylin

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and Eosin (H&E) using a system of quick staining. The surgical specimens were fixed in 10% neutral buffered formalin, and embedded in paraffin-wax.

Four-micrometer serial sections from formalin-fixed paraffin-embedded blocks were cut for each case, and one section stained with H&E was used for the histopathological diagnosis.

Immunohistochemistry was performed on the remaining sections mounted on poly-L-lysine coated glass slides after microwave antigen retrieval. Specimens were incubated overnight at 4° with the following monoclonal antibodies: cytokeratin AE1/AE3 (monoclonal anti-human; 1:100, Dako, Milan, Italy), anti-CD20 (monoclonal anti-human; 1:75, Dako, Italy), anti-CD45RA (monoclonal anti-human; 1:50, Dako, Italy) anti-CD8 (monoclonal anti-human; 1:20, Dako, Italy), anti-κ and anti-λ immunoglobulin ligth chains (monoclonal anti-human; 1:100, Dako, Italy), anti S-100 protein (monoclonal anti-rabbit; 1:300, Dako, Italy), anti-mucosal lymphocytes antigen, or MLA (monoclonal anti-human; 1:50, Dako, Italy) and anti-Ki67 (monoclonal anti-human; 1:100, Immunotech, Marseille, France).

Case 1

In July 2002 a 57-year-old white woman was sent to our Department with a 5-year history of recurrent bilateral painless and soft swelling of parotid glands (Figure 1a). She was HIV positive for 10 years and was infected by her husband, a sailor who died of a not well-defined respiratory failure. At the time of hospitalization her CD4+ lymphocytes count was 318 mm^{-3} and the viral load was 5400 copies ml^{-1} plasma. Antiretroviral therapy with Retrovir plus Epivir was then started. Magnetic Resonance Imaging showed bilateral enlargement of the parotid glands, completely affaced by multiple expansive multiloculated cystic lesions, with a diameter ranging from 1 to 3 cm (Figure 1b), interesting only involving both the parenchyma and the intraparotid lymphnodes. Because of the frequent recurrence of the lesions, FNA was performed and revealed a yellow, opalescent fluid, rapidly refilling of the cyst cavity. Aspirate preparations were submitted to cytological analysis which revealed the cytologic findings of LECs. One year later, when parotid swelling became persistent and progressive, superficial parotidectomy was performed and the surgical specimen was submitted to histological analysis which confirmed the diagnosis of LEC. No follow-up data were available for the patient.

Case 2

The second case occurred in an 18-year-old young white boy, who received periodical blood transfusions since birth for Thalassemia Major and who subsequently developed concurrent HCV and HIV infections since 10 years of age. Associated with the typical HIV-related oral lesions such as hairy leucoplakia and candidosis, he presented indolent parotid enlargement, firstly monolateral (Figure 1c), which became bilateral 8 months after. At the time of hospitalization his CD4+ T lymphocytes count was 350 mm^{-3} but the viral load had not been measured. He did not receive any antiretroviral therapy and he received prophylaxis against *Pneumocistis carinii*.

A diagnosis of LECs was made on FNA but, because of the patient's immunocompromission, no surgical excision of the lesion was performed. The patient returned for observation 1 year later when parotid swellings became bilateral and persistent. No treatment was suggested and he died 2 years later of *Pneumocistis carinii* pneumonia.

Results

Macroscopic findings

Macroscopic examination of surgical specimen in case 1 showed an irregular multicystic profile of superficial parotid lobes; dense fibrous septa and nodular structures, resembling intrasalivary lymph nodes.

Microscopic findings

Microscopic cytological features were typical and similar in both reported cases. The cytology of LECs consisted of anucleate squames and squamous cells in a reactive lymphoid background with scattered histiocytes. Histologically multiple cysts were located not only in the parotideal parenchyma where they showed a tendency to replace preexisting salivary ducts and acini, but also in intrasalivary lymphnodes, where they appeared smaller in size (Figure 1d). The cysts showed a predominantly squamous epithelium, with focal cuboidal cells. Lymphocytes were variable in number and ranged in size from small centrocyte-like cells to larger centroblastic cells; plasma cells were rare, focally located in the subepithelial regions or diffuse (Figure 1e). The surrounding parenchyma of the parotid gland did not show any relevant alteration; some ducts and acini were filled with centrocyte-like cells with associated atrophy and only occasional epimyoepithelial islands.

Immunohistochemical analysis revealed the typical immunostaining of LEC (Chetty, 1998; Maiorano *et al*, 1998) with a peculiar positivity for cytokeratins AE1/AE3 along the epithelial lining of the cysts (Figure 1f). Most of the intraepithelial lymphocytes were of B phenotype (CD20+ and CD45RA+) (Figure 1g) while a prevalent CD8 T immunoreactivity was detected within the follicle centers (Figure 1h). κ and λ immunoglobulin light chains staining showed a polyclonal pattern of expression in plasma cells. Immunoreactivity for Ki67 was detected in lymphoid elements and also in epithelial cells of the cyst lining. Lymphocytes of intraparenchymal cysts but not of cysts located in intrasalivary lymph nodes, were positive for MLA. Moreover S-100 positivity was observed in the follicular dendritic cells of the follicle centers.

Discussion

The LECs are important manifestation of HIV-infection and AIDS-related complex and, even if they represent a separate entity in the last World Health Organization classification of salivary glands tumors (Seifert, 1992), they show similar histological and immunohistochemical features to LECs occurring in HIV-negative patients.

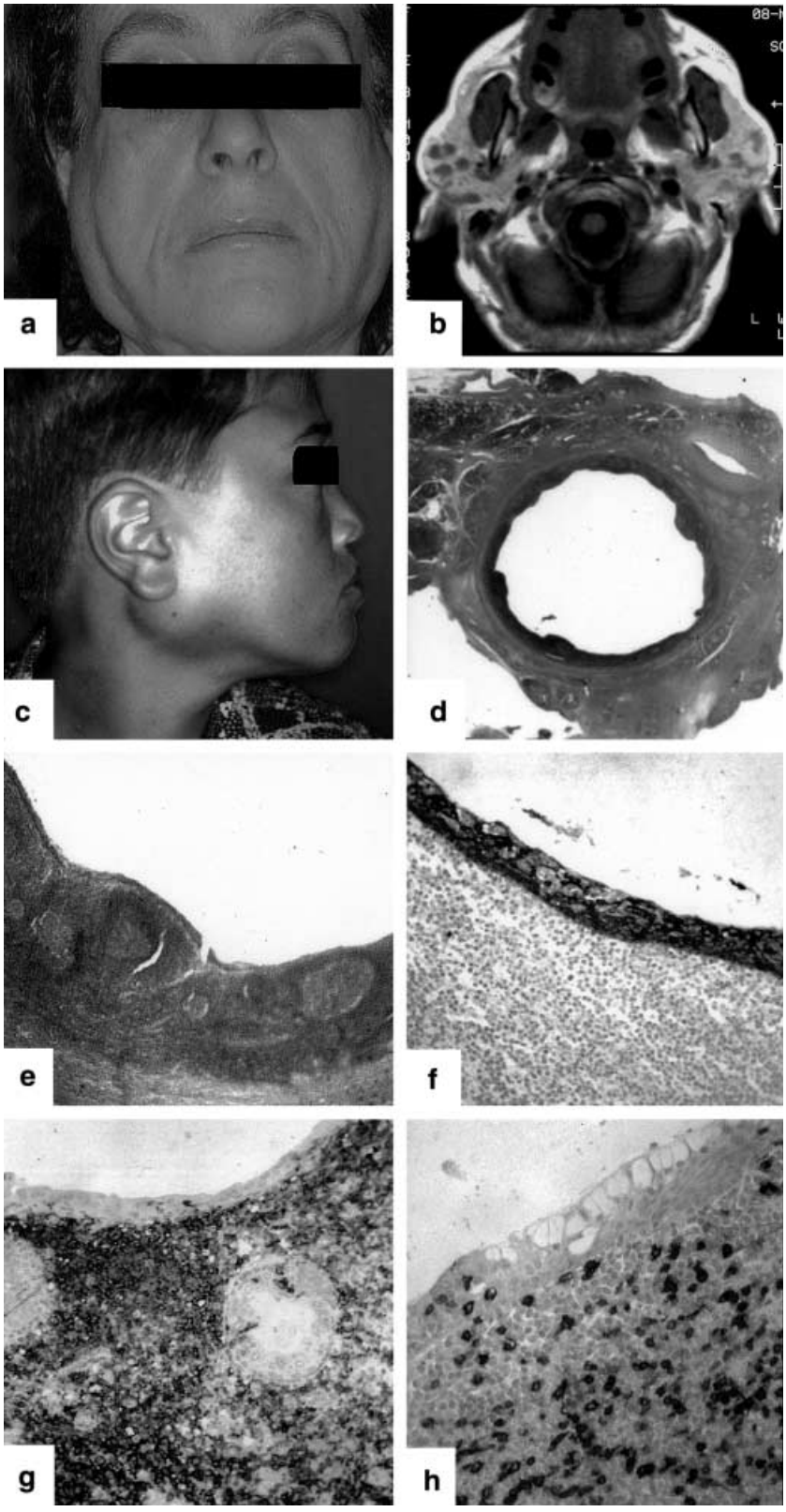

Figure 1 (**a**) Bilateral parotid swelling in a female HIV+ patient (case 1); (**b**) nuclear magnetic resonance: bilateral parotid swelling because of expansive multiloculated cysts both of the parotid glands and intrasalivary lymph nodes (case 1); (**c**) monolateral parotid swelling in a young HIV+ male due to large single parotid cyst (case 2); (**d**) low magnification of intraparenchymal lymphoepithelial cyst (LEC): the wall of the cyst protrudes into the lumen because of abundant lymphoid elements (H&E, original magnification 4×); (**e**) the wall of an intraparenchymal LEC: there are numerous lymphoid elements forming follicular structures, which create irregularities of the cyst wall. (H & E, original magnification 25×); (**f**) anti-cytokeratin immunostaining: the epithelial lining of the cyst is highlighted, while the abundant lymphoid intraepithelial component is unstained (original magnification 100×); (**g**) anti-CD20 immunostaining: intraepithelial and interfollicular lymphocytes and follicular center cells are CD 20+ (original magnification 100×); (**h**) anti-CD8 immunostaining: most of T-cells express CD 8 immunoreactivity (original magnification 100×)

The pathogenesis of this disease is still unknown. Some authors (Poletti *et al*, 1988) have hypothesized the important role of vascularization of the lymphoid component in cyst formation, while others have suggested an autoimmune origin (Itescu *et al*, 1993; Scully *et al*, 1993; Kazi *et al*, 1996), considering LECs as a manifestation of an HIV-induced autoimmune-like syndrome. Moreover it has been argued that cyst

formation derives from ductal obstruction and dilatation caused by follicular lymphoid hyperplasia of the intraparotideal lymphnodes, occurring during the initial phases of HIV infection (Poletti *et al*, 1988; Schattner and Bentwich, 1993). In fact it has been known that embryologically five to 10 lymphnodes are trapped within the parotid gland. These nodes contain salivary gland acini and ducts. With HIV-1 virus replication, salivary gland lymphoid hyperplasia develops and parotid swelling ensues. Other epidermotropic viruses have been claimed to be involved in the pathogenesis of LEC. EBV has been identified, in HIV-negative patients, by *in situ* hybridization techniques only in malignant lymphoepithelial lesions, where the level of viral expression may be adequate for being detected with this technique. *In situ* hybridization have failed to detect viral expression in benign lymphoepithelial lesions and cysts where viral replication would be inhibited by a competent immune system of T lymphocytes. Otherwise polymerase chain reaction have succeeded in proving the presence of EBV infection in benign lymphoepithelial lesions and cysts of salivary glands, suggesting a role for this epidermotropic virus in benign parotideal lesions, even if the exact pathogenetic mechanism is still unclear. Although cytomegalovirosis is a common finding in the salivary glands of HIV patients, the association of CMV inclusions with LEC has been reported only in one case (Vargas *et al*, 2001).

The FNA is a relatively non-traumatic procedure, performed with the use of a 22-gauge needle with a 20 ml syringe attached to a syringe holder, and could represent both a diagnostic and a therapeutic tool in parotid LECs (Allen *et al*, 1999; Kavishwar *et al*, 1999; Li *et al*, 2000). The main target of a clinicopathologic approach in parotid LECs is to differentiate LECs not only from reactive lesions of parotid gland, such as benign hyperplastic intraparotid lymph node, salivary cyst because of salivary duct obstruction, lymphocytic sialoadenitis, but also from neoplastic lesions of epithelial or mesenchymal origin, and from extranodal malignant lymphoma (Poletti *et al*, 1988; Tao and Gullane, 1991; Kazi *et al*, 1996; Allen *et al*, 1999).

In conclusion, although LECs can occur both in HIV+ and HIV− patients, it is important to recognise the frequent association between early stage of HIV infection and multiple parotid LECs which could represent the only initial manifestation of the viral infection.

No surgical therapy is usually required for these lesions and aspiration of cystic fluid with FNA is quite resolutive, although evidence of further relapses does exist. Surgical excision may become necessary when pain, because of persistent and progressive swelling of the parotid gland, does occur.

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