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Pathology anatomy diagnostic for oral hairy leukoplakia: A review article

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Abstract
Introduction: Oral Hairy Leukoplakia (OHL) is a disorder of mucocutaneous epithelial cell hyperplasia caused by the Epstein-Barr virus (EBV), and is the first managed pathological manifestation of EBV infection. OHL occurs almost exclusively in persons with immunosuppressive conditions and is usually seen in patients with human immunodeficiency virus (HIV), although there have also been reports of the incidence of OHL in patients with other non-HIV immunosuppressive conditions. The diagnosis of OHL can be made based on the presumptive diagnosis, namely the discovery of bilateral lesions on the lateral part of the white / gray tongue that cannot be removed by rubbing and can show vertical verugation. However, exact diagnosis requires information further pathology anatomy examination. Aim of this study to review pathology anatomy examination for OHL.
Discussion: The histopathological features found in OHL are not pathognomonic. In OHL can be found epithelial hyperplasia, hyper and parakeratosis and ballooning, vacuoalization, koilocytic epithelial cells with no or minimal inflammation, 28 as well as nuclei with ground-glass image and nuclear beading. Candida sp. is also common on the histopathological features of OHL. Sampling for histopathological examination can be done with a punch biopsy or brush biopsy. Exfoliative cytology as a method of diagnosis of OHL is a relatively new, innovative study. The feature found on exfoliating cells is Cowdry type A inclusions which are characteristic of EBV virus infection. In situ hybridization (ISH) is a reliable technique, specific nucleic acids (eg DNA, RNA) at the single cell stage in distribution and quantitative. ISH is the most accurate and sensitive technique, and is the gold standard for the diagnosis of OHL. Immunohistochemical examination of OHL showed the presence of EBV particles. Immunohistochemical analysis using antibodies to EBV antigen from the viral lysis cycle such as viral capsid antigen (VCA), and early antigen (EA-D).
Conclusion: OHL immunohistochemical examination has low sensitivity and high specificity. Polymerase Chain Reaction (PCR) can regulate the viral load in the subclinical state but cannot determine the location of EBV infection. PCR has a high sensitivity but low specificity.

Keywords: oral hairy leukoplakia, pathology, anatomy, diagnostic, examination

Introduction

Oral Hairy Leukoplakia (OHL) is a disorder of mucocutaneous epithelial cell hyperplasia caused by the Epstein-Barr virus (EBV), and is the first pathological manifestation associated with EBV infection [1]. Clinical manifestations of OHL are white asymptomatic lesions, choppy and painless, plaque that cannot be removed by rubbing, it is often located bilaterally at the lateral border of the tongue [2]. OHL was first described by Greenspan *et al.* in 1984 in a group of homosexual patients in San Francisco [3]. OHL occurs almost exclusively in immunosuppressed individuals and is usually present in patients with human immunodeficiency virus (HIV), although there have also been reports of OHL in patients with other non-HIV immunosuppressive conditions, such as solid organ transplant recipients or hematopoietic stem cells, patients with hematologic malignancies, such as multiple myeloma or acute myelogenic leukemia, and in patients who need a pen systemic steroid medication [4]. In 1994, a reported prevalence of OHL was 20% in patients with asymptomatic HIV infection in the United States and 36% in patients with Acquired Immunodeficiency Syndrome (AIDS) [5] in Tanzania. The reported mean OHL prevalence varies according to the clinical criteria used and the character of the study population, for example the type of immunosuppression and the clinical stage of the patient. Epstein-Barr virus is a human herpesvirus that is associated with important human diseases, including infectious mononucleosis syndrome, malignant lymphoma, and nasopharyngeal carcinoma. In adults worldwide, the serologic prevalence of EBV is estimated to be 95%. Circulating latently infected B lymphocytes are believed to be areas of persistent lifelong EBV infection. The productive replication of EBV occurs on the surface of the oral mucosa and produces an infectious virus which is transmitted into the saliva [6]. The diagnosis of OHL can be made based on the presumptive diagnosis, which is when bilateral lesions are found on the lateral part of the tongue, white / gray which cannot be removed by rubbing and can show vertical verugation. However, definitive diagnosis requires demonstration of EBV in the lesion. If no

facilities are available to establish a definitive diagnosis, a lack of response to antifungal therapy or evidence of immuno deficiency status can confirm the presumptive diagnosis [7]. OHL is often misdiagnosed as candidiasis oris, especially in immuno deficient patients, so its management is often inappropriate. This referral is expected to help provide knowledge about diagnostic enforcement in OHL cases, both in the form of histo and Cyto pathological examinations as well as examinations to establish a definitive diagnosis, namely In Situ Hybridization (ISH) and immunohistochemistry. From this review, it is also hoped that clinicians will know the options for both invasive and non-invasive OHL examinations which are useful in making the diagnosis by considering patient comfort.

Discussion

OHL was first observed in 1981, but Greenspan *et al.* In 1984 have described in a group of homosexual patients in San Francisco it is common to find a benign, asymptomatic, white lesion that cannot be removed even with rubbing. These lesions are rare in a healthy population. Oral Hairy Leukoplakia (OHL) is a disorder of mucocutaneous epithelial cell hyperplasia caused by the Epstein-Barr virus (EBV), and is the first pathological manifestation associated with EBV infection [3].

In 1994, a reported prevalence of OHL was 20% in patients with asymptomatic HIV infection in the United States and 36% in patients with Acquired Immunodeficiency Syndrome (AIDS) 5 in Tanzania. In 2006, Moura *et al.* reported a prevalence of OHL in Brazil of 28.8% [8]. Kerdpon *et al.* reported a prevalence in northern and southern Thailand of 38.8% and 21.8%, respectively [9]. Reported mean OHL prevalence was different. -different according to the clinical criteria used and the character of the study population, for example the type of immunosuppression and the clinical stage of the patient. In general, the prevalence of OHL in developed and developing countries varies between 0.42-38%. An increase in the prevalence of OHL is closely related to high EBV exposure, a decrease in CD4 + counts and an increase in HIV levels in the blood [1].

In the early HIV pandemic, it can be seen that a quarter of HIV positive sufferers have OHL. Since the change of AIDS therapy from monotherapy to combination therapy using HAART there has been an improvement in both virological and immunological parameters so that it indirectly reduces the incidence of oral lesions including the incidence of OHL by 10% -25% [2]. OHL cases are usually found in immunodeficiency due to HIV infection, but can also be associated with other immunosuppressive conditions such as organ or bone marrow transplantation, chemotherapy, hematologic malignancies and long-term use of systemic steroids [10]. The occurrence of OHL in individuals without HIV infection is low in frequency, although the number of cases with chronic immunosuppression is common. (Non-hiv) Schimidt-westhausen *et al* (1993) and Ammatuna *et al* (2001) reported the incidence of OHL in individuals receiving organ transplants as much as 4-58% [11].

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Pathogenesis

The pathogenesis of OHL remains unclear, but it is believed that OHL is a benign lesion characterized by large volumes of productive EBV replication. EBV (also known as human herpesvirus 4) originates from Herpesviridae subfamily gamma [12]. This virus remains latent for life by residing in B lymphocyte memory cells circulating in peripheral blood, which serve as a cellular reservoir of persistent latent EBV infection [13, 14]. This virus is transmitted via mucosal excretion, for example, saliva, through EBV-infected oropharyngeal cells during viral reactivation [15]. It is unclear whether EBV originates from reactivation of latent strains of tongue epithelium or is acquired through contact with EBV-infected saliva or via circulating EBV-positive B lymphocytes [14].

Recent studies have shown that monocytes, macrophages, or infected Langerhans cells in the peripheral blood circulation migrate through the lamina propria to the oral epithelium and infect late differentiated cells at the top of the spinous layer, which triggers the viral and disseminated productive replication of EBV [16, 17]. Cells these cells can be a source of reactivation of productive replication of EBV [6, 16, 18]. Severe immuno suppression can lead to reactivation of EBV replication in the oropharynx of EBV positive patients. Replication of EBV has also been found in normal oral epithelium, suggesting that replication alone is not sufficient for pathogenesis of OHL and other cofactors are required. The lateral part of the tongue is the most common site for OHL. The development of OHL on the tongue may be associated with the accumulation of saliva on the floor of the mouth and the resting position of the tongue in the EBV-infected saliva pool [4]. Another explanation is the decreased number of Langerhans cells in OHL lesions compared to oral mucosa without lesions. A comparative study between normal mucosa, showed decreased Langerhans cell density in the lateral part of the tongue and the sublingual region. Hence the normal epithelium on the ventral and dorsal lidaj is more susceptible to EBV infection [19]. Manifestation of OHL is clinically present as a well-defined white or gray-white plaque with an asymptomatic wavy texture.[20] The "hairy" surface varies in size from a few millimeters to extensive involvement from the tongue to the oral cavity.[12] These lesions usually occur on the lateral side of the tongue, but it can also be on the ventral and dorsal surfaces of the tongue, and, rarely, on the cheek mucosa. The characteristic appearance of OHL is caused by hypertopy of the tongue papillae. In general, these lesions are painless and cannot be relieved by blunt manipulation [20]. When the lesions become symptomatic, they may result from elevation or are co-infected with candidiasis.

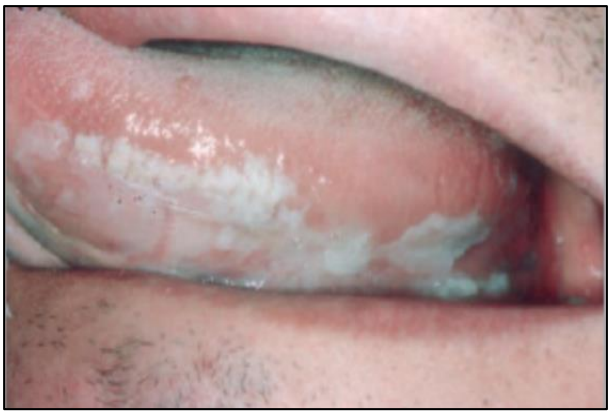


Fig 1: Clinical features of Oral Hairy Leukoplakia. It appears as a white, rough, non-easily removed patch on the lateral side of the tongue [21].

In HIV patients, the presence of OHL can provide a predictor of immune system condition and infection progression, because it is believed to be related to the number of CD4 + T cells. The rare incidence of OHL in healthy individuals and the association between OHL and HIV-positive patients of low CD4 + T cell counts as well as high viral loads suggest a role for EBV co-stimulation by HIV or an important role for CD4 + T cells in protection against this disease. OHL is not considered a prognostic factor except in AIDS patients. However, previous cases of OHL with hematologic malignancy have been reported in patients after chemotherapy, where the immunosuppression was more severe. Early recognition of OHL lesions is essential because of the high likelihood of an underlying immunodeficiency state and a potential indicator of poor prognosis [10].

Diagnosis and Pathology Examination

Clinical findings are generally sufficient to establish a presumptive diagnosis in patients with HIV infection. Further definitive criteria are needed to demonstrate the presence of EBV in the lesion, which is established by histopathology, exfoliative cytology, in situ hybdridization, immuno his to chemistry or polymerase chain reaction.

Histopathology Examination

Histopathological examination strongly supports diagnosis. Patients who are not included in the high-risk HIV sero-positive group but have lesions similar to OHL should undergo histopathological examination with hematocillin & eosin staining. The histopathological features found in OHL are not pathognomonic. In OHL can be found epithelial hyperplasia, hyper and parakeratosis and ballooning, vacuoalization, koilocytic epithelial cells with no or minimal inflammation, [25] as well as nuclei with ground-glass image and nuclear beading. The histopathological features of ballooning degeneration of keratinocytes of the stratum spinosum, hyperparakeratosis and absence or mild subepithelial inflammation are nonspecific. The presence of Candida sp. is also common on the histopathological features of OHL.

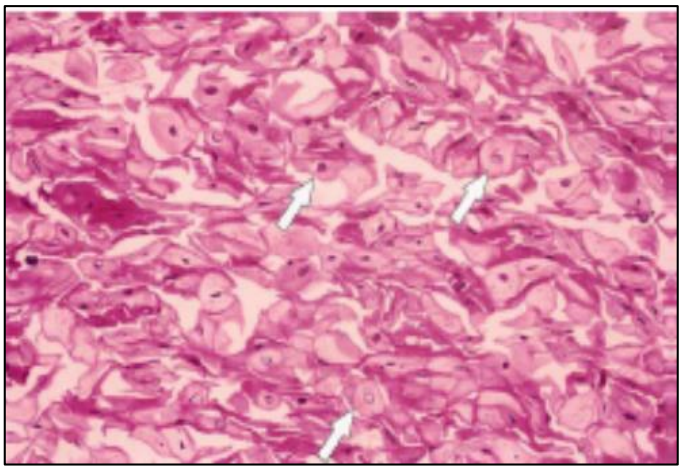


Fig 2: Epithelial cells taken from brush on OHL. A number of squamous epithelial cells were seen, including balloon cells with nuclear beading (arrow) (hematoxylin-eosin staining, 400x magnification) [26].

Sampling for histopathological examination is generally done through a biopsy punch, however, Walling *et al.* Suggested the use of a brush biopsy as an alternative non-invasive biopsy technique. Their study compared surgical biopsy and brush biopsy in diagnosing OHL, taking specimens using a brush biopsy instrument was done by rotating the small brush 10 times in the tongue epithelium area with an area of about 10x10 mm (100mm2). The cells on the toothbrush were then collected by agitation in a 1.5 ml polypropylene microsentive tube containing 1.0 ml of phosphate buffered saline. These cells are then centrifuged for 2 minutes with 7200 times the force of gravity. The cells were then fixed with 95% ethanol 1.5 ml. This procedure was well tolerated and did not provide any significant mucosal changes [26].

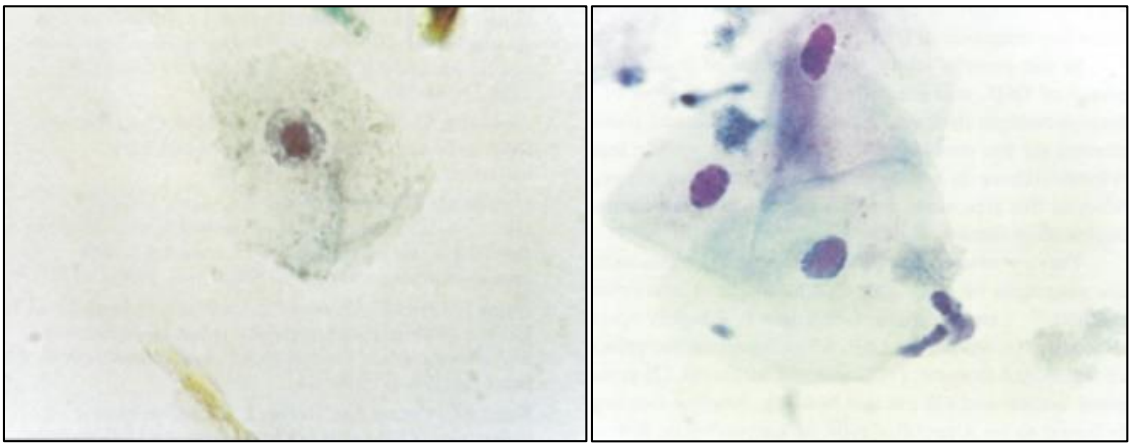
Fig 3: Example of punch biopsy of leukoplakia on the lateral side of the tongue. The punch biopsy tool is applied in a downward direction and a circular motion [27].

Cytopathology Examination

Cytopathology as a routine examination of smears of lesions on the lateral tongue has several advantages, namely (1) diagnosis of inflammatory changes by identification of the etiologic agent (EBV); (2) oncology analysis of epithelial cells; (3) the possibility to use the material by a variety of methods, including molecular studies and electron microscopy [11]. Exfoliative cytology as a method of diagnosis of OHL is an innovative study conducted by Lumerman *et al* in 1990. This finding was later confirmed by other researchers.

An ultrasktructural study of exfoliative cells was carried out by Frank and Greenspan and they found the presence of EBV DNA in the coilocyte cells. The picture found in the exfoliative cells of OHL lesions is Cowdry type A inclusion which is typical of EBV virus infection. This represents a central intranucleus and eosinophilic inclusion surrounded by a “clear” zone. The ground-glass nucleus exhibits eosinophilic / basophilic inclusions which homogenize the entire surface of the nucleus thereby providing an overview of the peripheral marginization of chromatin. Nuclear beading is a prominent feature of peripheral margination and can also be found in clumping of nuclear chromatin. This unique feature of nuclear beading stems from the multiplication of EBV within the nucleus which causes a shift of chromatin to the nuclear boundary [28]. Exfoliative cytologic examination together with detection of EBV is a non-invasive technique that

is useful in the diagnosis of OHL. This examination is carried out using fiber-tipped swabs or scraping the blunt tip at the location of the lesion which is then immediately removed on a glass object and fixed with isopropyl alcohol, then stained with Papaniculou stain to show nuclear changes. Reginald *et al* reported in their study that to some extent, OHL could be detected using exfoliative cytology. Of the 50 HIV patients with clinical OHL, [25] patients (50%) showed nuclear changes. While in 50 HIV patients without clinical OHL, it was found that 15 patients (30%) showed nuclear changes, this means that OHL can also be detected in the subclinical phase.



A **B**

Fig 4: Cytologic overview of OHL lesions. A. Keratinocyte cells with intranuclear inclusions (Cowdry A). B. Keratinocyte cells show intranuclear inclusions with a ground-glass image (Papaniculou stain, 1000x magnification) [28, 29].

The Hybridization in Situ Examination

In situ Hybridization (ISH) is a technique that can detect specific nucleic acid sequences (eg DNA, RNA) at the single cell stage in distribution and quantitative terms [30]. ISH is the most accurate and sensitive technique, and is the gold standard for the diagnosis of OHL. However, this technique requires a biopsy material which sometimes cannot be done if the patient refuses or the patient's condition is not possible (for example in a state of hemophilia, thrombocytopenia, and other weakness conditions). Mabruk *et al* stated that ISH is sensitive enough to detect early or subclinical infection of EBV. A similar report was reported by Leonard *et al.* ISH effectively combines histochemistry with molecular biology thus allowing rapid analysis of the distribution of RNA or DNA in tissue samples. The advantage of ISH is its ability to combine ISH with immunohistochemistry in the same tissue part [31].

The detection of viral nucleic acids by ISH in tissue sample cells provides a better understanding of the pathogenesis of viral diseases. EBV is known to contain about 80 gene proteins, not all of which have been identified. These genes are divided into three types, namely latent genes, early genes and late genes. Examples of latent genes are EBNA 1 to 6, LMP and EBER 1 and 2, while the early gene is BMRF1 and late gene, namely BMRF2. These genes can then be used as probes in the ISH process. (inet) Tissue sample fixation generally uses 10% formalin buffer fluid. Proteinase K is added to destroy protein-protein bonds and nucleic acid tissue in tissue samples that have been fixed with formalin and filled with paraffin [32].

The next process is hybridization with an EBV-DNA probe that has been labeled either with the non-radioactive biotin (1-s2), digoxigenin (jclin), or fluorescence (0344) label. Mabruk *et al* have used a pGEM vector containing EBV DNA to produce biotin (o3-24) labeled DNA probes. This EBV probe is used to detect EBV DNA in a sample of OHL tissue that has been fixed with formalin and filled with paraffin. Five micrometer sized sections were placed on a silane coated slide then processed and hybridized with an EBV-DNA probe labeled with biotin which had been dissolved to 2 ng per µl. The bound probe is then visualized after conversion to the substrate and chromogen (hydrogen peroxide and diaminobenzidine) becomes a brown local precipitate. The slides were then stained with a haematoxylin smear and examined under a light microscope. Positive and negative controls are also used [33].

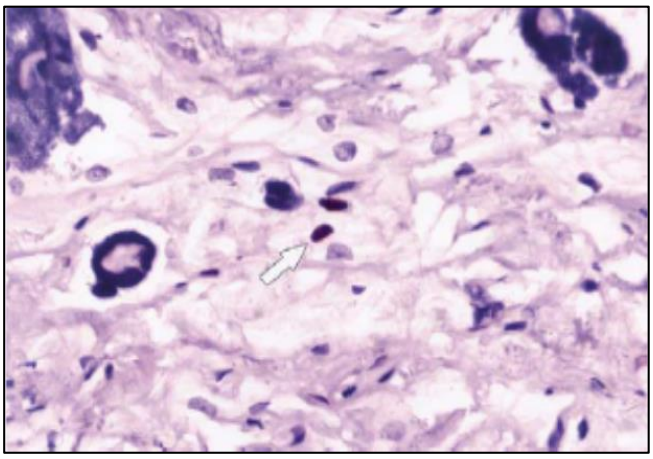


Fig 5: In situ hybridization smear of OHL cells taken from the brush for EBV DNA. The brown nuclei (arrows) represent the presence of a multicopy EBV genome, and this result is indicative of productive EBV replication in these cells. Hematoxylin stain, 600x magnification). (1s-2) [30].

Immunohistochemical Examination

Immunohistochemistry is a technique for identifying tissue or

cellular elements (antigens) through antigen-antibody interactions, in which antibody bonds are identified either through antibody labeling or through secondary labeling methods. The immunohistochemical method was first introduced by Coons and Jones as an immunofluorescence technique for detecting the presence of bacteria. (Basics) The development of monoclonal antibodies against various viruses allows the rapid diagnosis of many viral infections with a high degree of specificity. Immunohistochemistry cannot replace routine histology, but can help rule out a differential diagnosis. Some of the antibodies to EBV currently available are mouse monoclonal, CSI-4 and DAKO (BioTek Solutions TechMate 500). (Molina) Gratama *et al* found that immuno his to chemical examination of OHL showed the presence of herpes-type virus particles which were later confirmed to be EBV [34, 35].

This immunohistochemical analysis uses antibodies to EBV antigen from the viral lysis cycle such as viral capsid antigen (VCA), and early antigen (EA-D). (Jvirol) OHL immunohistochemical examination has low sensitivity and high specificity. (Molina) The immunohistochemical technique was started by placing a five micrometer sized section on a glass slide coated with silane, deparaffinizing it and then washing it with saline with Tris-buffer. The slides were then buffered with citrate and heated with steam for 20 minutes. Each section was then stained with monoclonal mouse antibodies with EBV proteins such as Early Antigen-Diffuse (EA-D) encoded by the BMRF1 gene and diluted 1: 200. The slides were then counter-stained with hematoxylin for examination under a light microscope. Positive and negative controls were also used as comparisons [35].

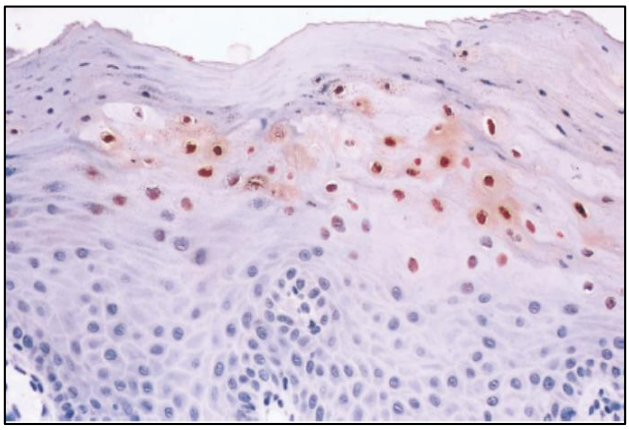


Fig 6: Immunohistochemical staining of surgical biopsy specimens with EBV early antigen-diffuse (EA-D). The red nuclei of balloon cells represent the expression of EA-D in these cells, and this result is an indication of the productive EBV replication from these cells. Haematoxylin stain, 200x magnification. (valacyclovir) [34]

Polymerase Chain Reaction Examination

Polymerase Chain Reaction (PCR) is a very sensitive examination technique requiring only a small amount of tissue sample, minimally invasive, easy to detect EBV on tongue tissue swabs. However, EBV is positive on PCR not only in patients with OHL but also in patients with other diseases that may be accompanied by EBV, it can also be in healthy patients. This is because EBV can affect children and adolescents without clinical symptoms and become latent. PCR can detect the quantity of virus in the epithelium in a subclinical setting but cannot determine the location of EBV infection. PCR has a high sensitivity but low specificity [13].

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