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Oral hairy leukoplakia: An exfoliative cytology study

Ajay Reginald, B. Sivapathasundharam[1]

Abstract

Oral hairy leukoplakia (OHL) is a white, hyperplastic, vertically corrugated lesion that occurs on the lateral border of the tongue, usually unilateral. Caused by the Epstein–Barr Virus (EBV), the lesion is said to be an early indicator of an immune deficiency status, thereby unmasking subclinical systemic conditions. OHL mimics many other white lesions of the oral cavity; therefore, it becomes imperative to identify the lesion. This study used exfoliative cytology, a noninvasive procedure, which helped in identifying the cellular changes brought about by the virus in the oral epithelium. The study revealed a subclinical phase of OHL, where the cellular changes were seen even before the appearance of the clinical lesion.

Keywords: Exfoliative cytology, immunosuppressed, oral hairy leukoplakia

Introduction

The oral cavity presents with a plethora of manifestations of systemic pathologies. These oral lesions could be early indicators of serious underlying systemic conditions. The ability to recognize such manifestations is key to providing optimal and appropriate care, ensuing early medical intervention and ultimately prolonging a patient's life and enhancing its quality.

Oral hairy leukoplakia (OHL) is one such oral lesion that might be the first sign of HIV infection in some patients. OHL was first seen in a group of isolated patients by Greenspan in 1984 and was described as asymptomatic, white, nonscrapable, vertically corrugated hyperkeratotic hair-like projections that appear on the lateral border of the tongue [Figure 1].

Its association with HIV prompted many people to believe that OHL could be used as a diagnostic and prognostic marker of HIV infection or AIDS, although today it represents an immunosuppressive state rather than being a marker for HIV infection.

This warrants that the clinician makes a careful diagnosis of OHL, since many lesion-like candidiasis, frictional keratosis, etc. might mimic OHL clinically.[1] OHL is said to be associated with the Epstein–Barr Virus (EBV), which infects 90% of the world population without causing symptoms. OHL is thus recognized as a focus of EBV replication, with viral DNA and a productive cycle antigen.

Department of Oral & Maxilofacial Pathology, Narayana Dental College and Hospital, Chinthareddypalem, Nellore, Andhra Pradesh, [1]Department of Oral & Maxillofacial Pathology, Meenakshiammal Dental College, Maduravoyal, Chennai, India

Correspondence: Dr. Ajay Reginald, Narayana Dental College and Hospital, Chinthareddypalem, Nellore – 524 002, Andhra Pradesh, India. E-mail: jayberna@gmail.com

When a clinical diagnosis becomes uncertain in such white lesions, the diagnosis is established by histologic examination of tissues from a biopsy specimen to identify the tissue pattern and for the identification of EBV. Nevertheless, immediate biopsies of such lesions require invasive procedures, which might not be possible on all patients owing to their immune-compromised status. In addition, sophisticated and expensive equipment are required to demonstrate EBV, which might not be at the immediate reach making them impractical. Thus a simple reliable and accepted technique like exfoliative cytology can be used as a diagnostic parameter, wherein the cellular changes brought about by the EBV can reliably be identified.

The study aimed to detect the presence of subclinical OHL infection using exfoliative cytology in HIV individuals.

Materials and Methods

The study material consisted of 150 individuals. All selected individuals were screened for HIV infection using ELISA and were divided into 3 groups for the study, with 50 in each group.

Criteria for selection

All selected individuals were subjected to thorough oral examination and patients clinically diagnosed with OHL were treated with antifungal therapy:

Group I – 50 ELISA-positive HIV individuals with clinical OHL

Group II – 50 ELISA-positive HIV individuals without clinical OHL

Group III – 50 ELISA-negative HIV-negative patients with no OHL comprised the control.

Lateral tongue scraping was done and directly smeared onto the glass slides and fixed in isopropyl alcohol, before staining with the standard Papanicolaou stain for cytological demonstration.

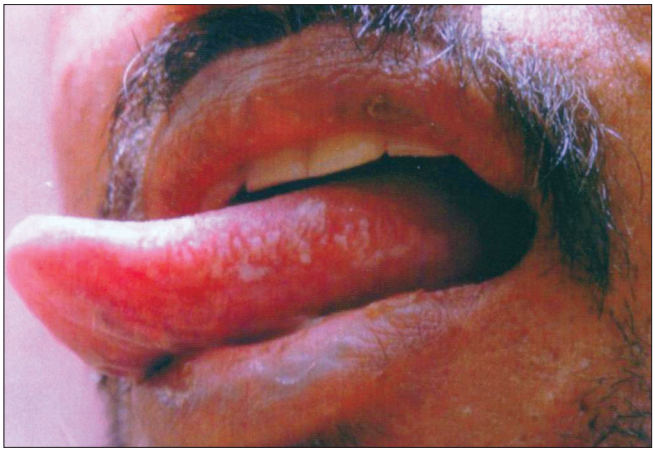

Figure 1: Clinical picture of oral hairy leukoplakia showing hyperplastic vertical corrugations on the lateral border of the tongue

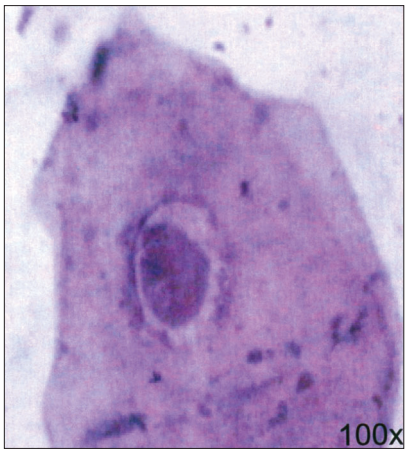

Figure 2: Photomicrograph showing PAP-stained, Cowdry Type A inclusion bodies along with a perinuclear halo 100× magnification

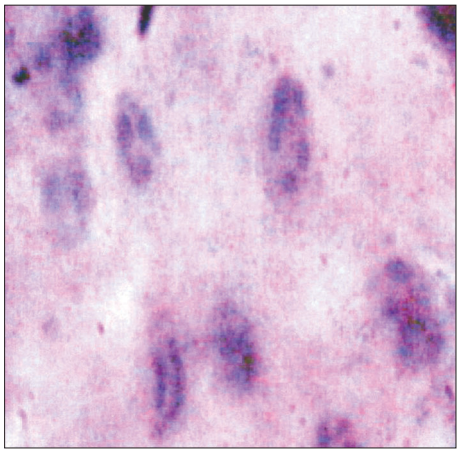

Figure 3: Photomicrograph showing PAP-stained ground glass appearance of the nucleus and a peripheral nuclear bearding 100× magnification

Prepared slides were studied under a light microscope and the features characteristic of OHL were identified and recorded accordingly.

These features include the following:

1. Cowdry A inclusion bodies: an eosinophilic and central intranuclear inclusion body surrounded by a clear space [Figure 2].
2. Ground glass nuclei: an eosinophilic or basophilic inclusion body homogenizing the whole surface and exhibiting peripheral margination of chromatin [Figure 3].
3. Nuclear beading: a prominent peripheral margination and clumping of the nuclear chromatin [Figure 3].

Results

It was found that 50% of cases in Group I showed nuclear changes irrespective of what they were [Table 1]. While in Group II, 30% of the cases showed nuclear changes, thus uncovering an underlying OHL lesion though not clinically visible.

Individual nuclear changes ranged from 32–42% in Group-I, while in Group II, a range of 2–22 % was seen [Table 2].

Discussion

OHL was first described and reported as an unique epithelial lesion of HIV seropositive homosexual men who later developed AIDS.[2] This association prompted many to believe that OHL was a form of opportunistic infection associated with HIV infection. But after reports of OHL being found in non-HIV-related immunosuppression, OHL has been accepted more as an indicator of depressed T-cell immunity in general rather than of HIV infection specifically.

Today OHL has been recognized as one of the oral manifestations of AIDS and has been included in the CDC classification system for HIV infection.[3] OHL has been described as an asymptomatic, white plaque or corrugated lesion that commonly occurs on the orders of the tongue. The patients reviewed in our study had similar features where the lesions were found on the lateral borders of the tongue, generally unilateral and had a corrugated appearance, though in one of the cases it was found to extend onto the dorsum of the tongue. The literature describes the lesion to be symptomatic at times with features of burning sensation and taste alteration. No such findings were evident in the cases studied by us. The patients were unaware of the presence of the lesion.

EBV is a human herpes virus that infects without causing symptoms in 90% of the population worldwide. In addition, it is said that EBV establishes a lifelong persistent infection in

Table 1: Percentage of nuclear changes in the study groups as a whole

Total no. of cases	No. of cases with nuclear changes	Percentage
50 – Group I, HIV positive with OHL lesions	25	50%
50 – Group II, HIV positive without OHL lesions	15	30%
50 – Group III, non-HIV individuals without oral lesions	0	0

Table 2: Individual percentage of Nuclear & Cytoplasmic Changes

Total no. of cases	Cowdry bodies no. of cases percentage	Ground glass nuclei – no. of cases/percentage	Nuclear beading/clumping – no. of cases/percentage	Perinuclear halo – no. of cases/percentage
50 – Group I, HIV positive with OHL lesions	NIL/0%	25/50 %	21/42%	16/32%
50 – Group II, HIV positive without OHL lesions	1/2%	11/22%	4/8%	4/8%
50 – Group III, non-HIV individuals without oral lesions	0	0	0	0

the saliva of immune-compromised patients and postulated that in these patients viruses can easily infect the epithelium resulting in hyperplasia.[4]

OHL has been associated with EBV infection and the demonstration of EBV DNA in lesions of OHL has found a strong cause-and-effect relationship. OHL lesions are said to be foci of replication for the virus.[5] This warrants the demonstration of EBV DNA within the keratinocytes before the diagnosis of OHL can be established. Several techniques are currently available to fulfill this requirement. These include the PCR and *in situ* hybridization which are no doubt highly sensitive in the detection of EBV, but such techniques are not performed at all laboratories due to economic restraints of obtaining such equipment. Also such techniques are time consuming and are a financial burden to the already financially overwhelmed patient with a myriad of medical problems associated with the disease.

Studies have shown important histopathologic features by light microscopy that make diagnosis of OHL more practical.[6] Though not diagnostic they are characteristic and are found on the nuclei of vacuolated keratinocytes infected with EBV. These changes consist of aggregates of nuclear chromatin along the nuclear membrane similar to changes seen in cells affected with other herpes virus. These keratinocytes are present at various levels of the epithelium including the parakeratin layer, making them readily accessible for removal, for an exfoliative study.

A clinical diagnosis of a bilateral lesion on the lateral margin of the tongue with vertical corrugations was presumptive and for a definitive diagnosis, the demonstration of EBV in the lesion was necessary.[5] In the absence of facilities to demonstrate EBV, a lack of response to the antifungal therapy or a demonstration of an immunodeficient status was said to add weight to the presumptive diagnosis.

Cytopathology in dentistry is gaining widespread acceptance in oral cancer detection. However, exfoliative cytology in OHL diagnosis was an innovative study done by Lumerman *et al*. in 1990.[7] These findings were confirmed later by other investigators. An ultrastructural examination of exfoliative cells was studied by Frank and Greenspan and they were found to contain EBV DNA in koilocyte cells.[8]

The features found in studies on exfoliative cells from OHL lesions were[9] Cowdry Type A inclusions that were characteristic of the herpes virus infection. This denoted an eosinophillic and central intranuclear inclusion surrounded by a clear zone. Ground glass nuclei denoted an eosinophillic/basophilic inclusion that homogenized the whole nuclear surface exhibiting peripheral marginization of chromatin. Nuclear beading is a feature where a prominent peripheral margination and clumping of nuclear chromatin is seen. This unique feature of nuclear beading results from the multiplication of EBV in the nucleus causing displacement of the chromatin to the nuclear margin.

Considering such specific features as diagnostic, our study aimed to detect by cytopatholigc examination the subclinical features of OHL and to study the sensitivity of cytopathology using previously defined criteria on the Indian population. The few studies that tested cytopathology as a method for diagnosis of OHL demonstrated that it is indeed a reliable method and so also the results of our study showed the features to be a significant if not consistent.

The literature[10,11] shows a varied consistency of the cytopathology by various investigators. Out of the 50 smears

examined by us in HIV-positive patients with OHL, 25 smears (50%) showed nuclear changes. This could be attributed to a nonvirally induced hairy leukoplakia[12] (pseudo-OHL) or a lesion that mimics OHL clinically but has no attributes whatsoever to a viral lesion or may probably be a sampling error (nonrepresentative site). The literature also quotes that not all HIV-infected patients develop EBV-associated diseases, but persistent EBV replication and progressive immune dysfunction can eventually result in OHL.

A case reported by Greenspan[13] showed OHL in an HIV-negative individual on immunosuppressive therapy. It is substantiated that HIV alone is not responsible for immunosuppression of a type or degree sufficient to permit full replication of EBV in a site of latency of virus, followed by clinically apparent epithelial hyperplasia. Alternatively, it was said that EBV latent elsewhere is permitted in some immunosuppressed individuals to attach to and infect epithelial cells bearing the EBV receptors. The frequency of reports of OHL in non-HIV individuals seems to be surprisingly low in spite of the number of chronically immunosuppressed conditions seen. This raises the possibility that another, yet unidentified, factor is necessary for the occurrence of OHL.

Our third group showed no nuclear changes which could be explained by the fact that that a relation does indeed exist between decreased immunity and the presence of OHL irrespective of reported cases of incidental findings of OHL in immune-competent individuals.

While in the second study group of HIV-positive patients without OHL, a positivity of 30% (15 cases) was seen. This could mean that OHL caused by EBV could be detected at a subclinical phase also.

So it can be suggested with caution that OHL can be detected to a certain extent using exfoliative cytology. Further studies on the incidence of OHL under immune-compromised conditions and the comparison of the cytology of such cases with the more sensitive techniques like PCR and *in situ* hybridization to demonstrate EBV DNA could act as a calibrator to use exfoliative cytology as a diagnostic tool in early detection of immune deficiency, thus advocating early treatment.

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