Head and Neck: Oral leukoplakia

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Identity

Note
Oral leukoplakia (OL) is the most common potentially malignant disorder of the oral mucosa (Neville and Day, 2002; Haya-Fernández et al., 2004; WHO, 2005). Besides oral leukoplakia, actinic cheilitis, lichen planus, and erythroplakia are also considered potentially malignant conditions affecting the oral cavity.

These lesions can precede oral squamous cell carcinoma, which is the most common malignancy of the oral cavity (Neville and Day, 2002; Haya-Fernández et al., 2004). It is believed that those conditions are reasonably classified as "potentially malignant" as it has been observed that some lesions evolved to malignant ones during follow-up. In addition, typical alterations of potentially malignant lesions are seen co-existing in the margins of squamous cell carcinoma. Moreover, a proportion of these lesions show cytological, morphological, chomosomal, genomic, and molecular alterations that are also observed in malignant lesions (Warnakulasuriya et al., 2007).

Classification

Note
In spite of diverse and even more recently published definitions for oral leukoplakia, the most widely known is still the one proposed by World Health Organization (WHO) in 1978, which states that leukoplakia is a predominantly white patch that cannot be characterized clinically or histopathologically as any other definable lesion (Kramer et al., 1978; WHO, 2005). Oral leukoplakias can be classified from the clinical or histopathological viewpoint. Such classifications are discussed in detail in the topics below.

Clinics and pathology

Etiology

Tobacco use is the main risk factor associated to OL development. OL is six times more frequent among smokers than non-smokers (van der Waal, 2009). The effects of alcohol, betel, and diet are associated as well, but their exact role is yet to be established (Neville and Day, 2002; Campisi et al., 2007; Napier and Speight, 2008; van der Waal, 2009). A recently published review showed that there is not enough evidence for a casual association between human papilloma virus and OL (Feller and Lemmer, 2012). Besides, there are the idiopathic OL, for which no obvious aetiological factor can be identified. It is believed that such lesions are significantly more prone to develop into cancer than those OL with known causative factors (Napier and Speight, 2008).

Epidemiology

It is accepted that the OL prevalence varies from 1% to 5% (Napier and Speight, 2008; van der Waal, 2009), with a global prevalence estimated to be 2.6% (Petti, 2003). However, isolated reports show rates from 0.5% to 26.92% (Petti, 2003; Napier and Speight, 2008). Middle-aged and elderly men are the most affected individuals, and growing indexes are observed towards age.

OL often arises in cheek and alveolar mucosa (Neville and Day, 2002). Conversely, lesions in the floor of the mouth and lateral border of tongue seem to present displastic or malignant alterations more frequently (Neville and Day, 2002; Napier and Speight, 2008).

Clinics

OL can present as homogeneous and non-homogeneous lesions. Homogeneous OL is a white patch slightly elevated, thin, white to gray, uniform, and can present...
well defined borders or gradually mix with normal adjacent mucosa (Figure 1). Non-homogeneous OL can be nodular, verrucous, or speckled (erythroplastic) (Figure 2) (Warnakulasuriya et al., 2007; van der Waal, 2009). The proliferative verrucous leukoplakia presents a multifocal evolvement, mainly in elderly female patients who do not present known risk factors (Figure 3). These lesions are usually resistant to treatment and show a high risk for malignant transformation (Warnakulasuriya et al., 2007; van der Waal, 2009). Some alterations of the oral mucosa can mimic OL, and these lesions must be considered as OL differential diagnosis. So, for the establishment of a correct diagnosis of OL, such lesions must be excluded (Warnakulasuriya et al., 2007; van der Waal, 2009):

1. Frictional lesion
2. Candidiasis
3. Linea alba
4. Leukoedema
5. Chemical injuries
6. Hairy leukoplakia
7. Nicotinic stomatitis

Once a provisional clinical diagnosis of OL was made, a biopsy must be performed in order to obtain the histopathological features. This is of paramount importance because it is believed that the presence and degree of epithelial dysplasia is a great indicator of evolution and prognosis (see below) (Warnakulasuriya et al., 2008).

Figure 1: Homogeneous oral leukoplakia in the left lateral border and ventrum of the tongue. Figure 2: Non-homogeneous oral leukoplakia. White plaques intermixed with red patches.
Pathology

Microscopically, OL can vary from hyperkeratotic epithelium to lesions with different degrees of dysplasia (WHO, 2005; Brennan et al., 2007). The term "dysplasia" is generally employed in the sense of a disordered development (Izumo, 2011).

In a stratified squamous epithelium, architectural disturbances affecting normal maturation and stratification may occur. When such alterations are accompanied by cytological atypia, which can be detected as variations in the size and shape of the keratinocytes, the term "dysplasia" is applied (WHO, 2005; Warnakulasuriya et al., 2008).

The frequencies of dysplastic or malignant alterations in OL vary from 15.6% to 39.2%, and a rate of 19.9% was found in a retrospective study of 3300 white lesions of the oral cavity (Waldron and Shafer, 1975). Despite many efforts towards new evaluative methods, the histological analysis is still the most useful method for grading epithelial dysplasia in OL (Warnakulasuriya et al., 2008).

In this field, the WHO's system for grading epithelial dysplasia in OL is widely accepted among pathologists. However, it is not able to reflect the clinical behaviour of every single lesion and does not provide a clear therapeutic guideline to clinicians (Izumo, 2011).

Moreover, in spite of its wide acceptance, this system presents great variability and low reproducibility (Warnakulasuriya et al., 2008; van der Waal, 2009). According to it, lesions are classified considering the architectural features and cytological alterations listed below (WHO, 2005).

Architectural features:
- Irregular epithelial stratification
- Loss of polarity of basal cells
- Drop-shaped rete ridges
- Increased number of mitotic figures
- Abnormally superficial mitoses
- Premature keratinisation in single cells (dyskeratosis)

Cytological alterations:
- Nuclear pleomorphism: abnormal variation in nuclear shape
- Cellular pleomorphism: abnormal variation in cell shape
- Anisonucleosis: abnormal variation in nuclear size
- Anisocytosis: abnormal variation in cell size
- Increased nuclear size
- Increased nuclear-cytoplasm ratio
- Atypical mitotic figures
- Increased number and size of nucleoli

Considering the epithelium divided into "thirds", lesions are classified into five categories:
1. **Hyperplasia**: increase in cell number in the spinous layer and/or in the basal/parabasal cell layers. There is regular stratification and no cellular atypia (Figure 4).

2. **Mild dysplasia**: architectural disturbance only in the lower third of the epithelium with cytological atypia (Figure 5).

3. **Moderate dysplasia**: architectural disturbance extending into the middle third of the epithelium is the initial criteria, but the degree of cytological atypia may require upgrading it to "severe" (Figure 6).

4. **Severe dysplasia**: architectural disturbance affecting greater than two thirds of the epithelium, with cytological atypia (Figure 7).

5. **Carcinoma "in situ"**: theoretically, indicates that malignant transformation has occurred but invasion has not. Full or almost full thickness architectural disturbance is present in viable cellular layers with pronounced cellular atypia. Atypical mitotic figures and abnormal superficial mitoses are common.

Concerning the microscopic classification schemes for OL, a new binary system was proposed (Kujan et al., 2006). According to it, pathologists would observe the same morphological criteria used in the WHO classification, but lesions would be classified as low-risk OL (former "no/mild/questionable" dysplasia) or as high-risk OL (former "moderate/severe" dysplasia). Shorten the degrees of dysplasia from five, i.e. no, mild, moderate, and severe, to two - low-risk and high-risk - would provide a more feasible and reproducible classification system. Also, it could offer a more reliable criteria upon which to rely for the selection of patient treatment. Accordingly, OL classified as "high-risk" would be more prone to develop into cancer and, thus, should be removed. On the other hand, low-risk OL could be clinically followed up as they are less expected to evolve (Kujan et al., 2006; Warnakulasuriya et al., 2008; van der Waal, 2009).

In a study with 218 patients with OL, the authors reported that high-risk OL was associated with a 4.57-fold increased risk for malignant transformation, compared with low-risk OL (Liu et al., 2010). Moreover, in another research with 138 patients with histologically confirmed oral dysplasia, 115 had OL and 23 had lichen planus. From these 138 lesions, 37 (26.8%) developed into cancer and the "high-risk" degree of dysplasia was an independent risk factor for transformation (Liu et al., 2011). The authors then suggest the utilization of high-risk dysplasia as a significant indicator for evaluating malignant transformation risk in patients with potentially malignant lesions.

In a recently published paper, our research group showed statistically significant differences for hMLH1 - a DNA repair protein - , p53 - a tumor suppressor protein - , and AgNOR - an indicative of cell proliferation - indexes between low- and high-risk OL. This suggests that the biological processes linked to the impairment of those proteins remain enhancing from low-risk OL to high-risk OL.

Thus, the use of the binary system would give support to a more reliable clinical approach involving the removal of high-risk OL (Caldeira et al., 2012).
Figure 6: OL with moderate dysplasia. Architectural disturbance affecting two thirds of the epithelium. Notice dyskeratosis in the granular layer of the epithelium (arrow). Figure 7: OL with severe dysplasia: architectural disturbance affecting greater than two thirds of the epithelium. Pleomorphic cells are seen in the upper third of the epithelium (arrows). Figure 8: p53 immunoexpression in OL, 200x magnification. (A): OL with mild dysplasia shows few immunopositive cells (arrows). (B): OL with moderate dysplasia shows an increased number of p53 labeled cells (arrows). Figure 9: hMLH1 immunexpression in OL, 200x magnification. (A): OL with mild dysplasia shows several keratinocytes labeled for hMLH1. (B): OL with severe dysplasia presents a decrease in the immunoexpression of hMLH1.
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Figure 10: AgNOR staining, 1000x magnification. (A): in normal oral epithelium, keratinocytes exhibit lower AgNOR counting than in (B) keratinocytes in OL with severe dysplasia.

**Treatment**

Surgical excision, cryosurgery, laser surgery, topical or systemic retinoids, therapy with mouth rinses with attenuated adenovirus, and photodynamic therapy are possible therapeutics (Brennan et al., 2007; Lodi and Porter, 2008; van der Waal, 2009).

**Evolution**

OL may persist unchanged, progress, regress, or disappear (Napier and Speight, 2008). The malignant transformation risk varies from 3.6% to 36.0%, and some features as presence and degree of dysplasia, female gender, time of duration, non-smoker patient, location at floor of the mouth or tongue, size higher than 200mm², and non-homogeneous type, seem to be associated with a worse prognosis (Cruz et al., 2002; Holmstrup et al., 2006; Hsue et al., 2007; Smith et al., 2009; van der Waal, 2009). Infection with human papilloma virus does not seem to be related to the progression of OL (Feller and Lemmer, 2012).

**Prognosis**

Recurrence rates are highly variable among studies, from 0 to 30.0% (van der Waal, 2009).

**Genetics**

**Note**

Many efforts have been done to identify molecular markers to predict cancer development in OL. According to the review by Smith et al. (2009), p53, Ki-67, and PCNA are the most frequently investigated markers, but loss of heterozygosity (LOH), survivin, matrix metalloproteinase (MMP9), and DNA content are pointed as potential markers for progression of OL. Nevertheless, the presence and degree of epithelial dysplasia in OL is yet regarded as the most relevant indicator of progression and prognosis, influencing the management of the patients (Napier and Speight, 2008; Warnakulasuriya et al., 2008; van der Waal, 2009).

Ki-67 is a cell cycle associated protein, of which expression is associated with cell proliferation. Ki-67 immunoexpression is used as a proliferation marker in pathology. Kodani et al. (2001) showed that OL presented lower indexes of Ki-67 than oral squamous cell carcinoma. Also, Zhao et al. (2005) demonstrated that OL with mild dysplasia shows low levels of Ki-67, while severe dysplasia shows a significantly higher expression than oral normal mucosa and mild dysplasia.

Our research group investigated the immunoexpression of hMLH1 (a protein of the mismatch repair system) in OL with different degrees of dysplasia. We found that hMLH1 indexes decreased from a lower degree of dysplasia to a higher one, despite statistical significance. So, hMLH1 immunoexpression was inversely related to the OL degree of dysplasia. Our findings also suggest a role of such alterations in this pathway of DNA repair as an early event in oral carcinogenesis (Caldeira et al., 2011a).

Briefly, some information about the most frequent genetic alterations of OL are shown below. A complete and detailed revision on this topic was published by Mithani et al. (2007).

**p53 alterations**: The p53 protein can induce DNA repair, cell cycle arrest, cell death or senescence, showing a pivotal role in tumor avoidance (Joerger and Fersht, 2008). Its alteration is a common finding in human cancers, including those of oral mucosa (Kurokawa et al., 2003). Investigations demonstrated that the normal oral mucosa presents negative or low indexes of p53 immunoexpression (Kurokawa et al., 2003; Fan et al., 2006; Caldeira et al., 2011b). Likewise, p53 immunopositive cells were identified in OL with mild dysplasia, with increasing indexes from hyperplasia, to dysplastic lesions (Figure 8) and to oral
squamous cell carcinoma, with immunopositivity found in superficial layers of moderate and severe dysplasias (Kerdpon et al., 1997; Cruz et al., 2002; Kurokawa et al., 2003; Caldeira et al., 2011b). The detection of p53 in oral dysplastic lesions prompted many investigators to suggest that its abnormalities may constitute an early event in carcinogenesis.

**Loss of heterozygosity (LOH):** describes the elimination of a genetic loci containing tumor suppressor genes. In OL, LOH of the chromosome arms 3p and 9p seem to be related to a higher risk of malignant transformation. Fifty percent of OL contains allelic loss of either the 3p or 9p chromosome arms (Mithani et al., 2007).

**Microsatellite instability (MSI) and the mammalian mismatch repair system (MMR):** Microsatellites are DNA regions in which multiple repeated sequencies of nucleotides are found. These regions are prone to the occurrence of mismatched DNA, developing the MSI phenotype (Jascur and Boland, 2006; Jiricny, 2006). MSI was detected in many OL and there is a trend toward an increased prevalence of MSI in more aggressive histologic OL lesions (Ha et al., 2002). The MMR is responsible for maintaining genomic stability during repeated duplication, and microsatellites regions are hypersensitive to MMR dysfunction. The immunexpression of hMLH1 - one of the main MMR protein - was shown to decrease in OL with more severe grades of dysplasia (Figure 9) (Caldeira et al., 2011a). Taken together, these results may suggest that the altered function of MMR and the occurrence of MSI could be early events in the carcinogenic process, but these findings still need more investigation.

**Methylation / hypermethylation:** is an epigenetic alteration which can inactivate genes. In OL, it was described to occur in RAR-b2, p16, hMLH1, hMSH2, and MGMT (Ha et al., 2002; López et al., 2003; Youssef et al., 2004; Sengupta et al., 2007).

**AgNOR number:** AgNOR staining technique is used to assess cellular proliferation, and normal oral epithelium showed lower AgNOR number than dysplastic OL (Figure 10), which in turn presents lower indexes than oral squamous cell carcinoma (Chattopadhyay et al., 1994; Caldeira et al., 2011b). It was suggested that mean AgNOR number would be useful in distinguishing OL with mild and moderate dysplasia (Chattopadhyay and Ray, 2008).

**Telomerase activity:** these are the enzymes that degrade telomeres, which are a sequence of nucleotides that prevents DNA to undergo degradation and fusion. The telomerase activity was detected in OL.

**To be noted**

Note
In conclusion, OL is the most common potentially malignant oral disorder preceding oral squamous cell carcinoma. OL is often related to tobacco use and lesions may persist unchanged, enlarge, reduce or even disappear. Nevertheless, the presence and degree of epithelial dysplasia is still considered the most important predictor factor for malignant transformation. Also, it seems that the lesion duration, patient’s age, gender, the affected site, clinical appearance, and smoking habit are related to the risk of malignant transformation.

To date, as pointed out above, no single molecular marker is validated as a predictor, despite several investigations.

**References**


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