Chapter 2

Binary System of Grading Epithelial Dysplasia in Oral Leukoplakias

Maria Auxiliadora Vieira do Carmo and Patrícia Carlos Caldeira

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/54466

1. Introduction

Cancers of the oral cavity and oropharynx account for approximatelly 3% of all malignancies among men and 2% among women in the United States, and oral squamous cell carcinoma represents 90% of these tumors. Despite great achievements concerning surgery, radiation and chemotherapy, survival rates in 5 years remain near 50 to 55%. As this survival time is directly related to the time of diagnosis of the lesion, prevention and early diagnosis remain important aspects to reduce incidence of the disease, as well as to enhance the survival rate of patients [1,2].

Oral squamous cell carcinoma can be preceded by potentially malignant alterations [1,3]. Such alterations are classified as potentially malignant due to the following evidence: 1) it was observed that these lesions evolved to malignant ones during follow-up; 2) typical alterations of potentially malignant lesions are seen co-existing in the margins of squamous cell carcinoma; 3) a proportion of these lesions show cytological and morphological alterations that are observed in malignant lesions; 4) some chromosomal, genomic, and molecular alterations are found in both, potentially malignant and malignant lesions [4].

1.1. Definition, epidemiology, and etiology of oral leukoplakias

In a recently published paper, leukoplakia has been defined as ''a white plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer" [5]. Nevertheless, the most used definition of leukoplakia is still the one proposed by the 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" [6,7].

Oral leukoplakia (OL) is the most common potentially malignant lesion of the oral mucosa [1,3]. In a published systematic review [8], the author estimated a global prevalence of OL of

2.6%, which is in accordance with the consensus that OL prevalence is between 1% and 5% [9,10]. However, isolated reports show variable rates from 0.5% to 26.92% [8].

OL is more frequent in middle-aged and elderly men, with higher indexes correlated with increased age. The most common sites are cheek, alveolar mucosa, and lower lip [1]. Nonetheless, lesions affecting the floor of the mouth, lateral border of tongue, and lower lip seem to present displastic or malignant alterations more frequently [1,9].

The main risk factor associated with OL is the use of tobacco. OL is six times more frquent among smokers than non-smokers [10]. The effects of alcohol, betel, human papiloma virus, and diet are associated as well, but their exact role is yet to be established [1,9-11]. In addition, there are some OL for which no obvious aetiological factor can be identified, and these lesions are named idiopathic leukoplakias. It is believed that such lesions are significantly more prone to develop into cancer than those OL with known causative factors [9].

1.2. Clinical and histological features

Clinically, OL can be classified as homogeneous and non-homogeneous lesions. Homogeneous OL arises as a white patch slightly elevated, thin, white to gray, uniform, and can present well defined borders or may gradually mix with normal adjacent mucosa (Figure 1 to 3). Non-homogeneous OL can be nodular, verrucous, or speckled (erythroplastic) (Figure 4) [4,10].

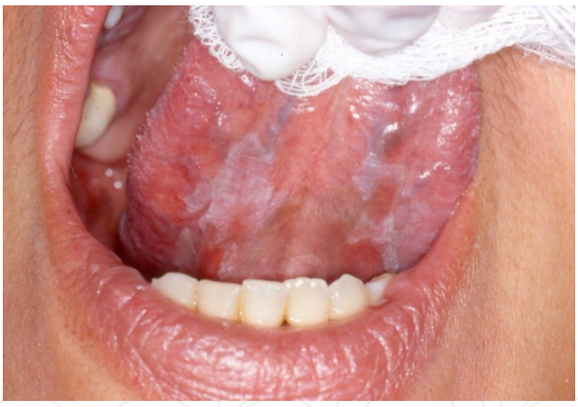

Figure 1. Homogeneous thin leukoplakia in the tongue.

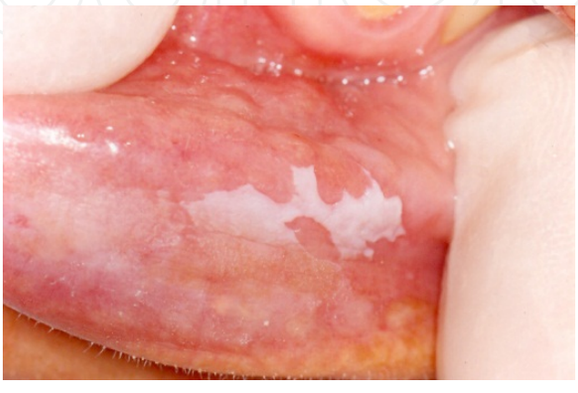

Figure 2. Homogeneous leukoplakia in the lower lip.

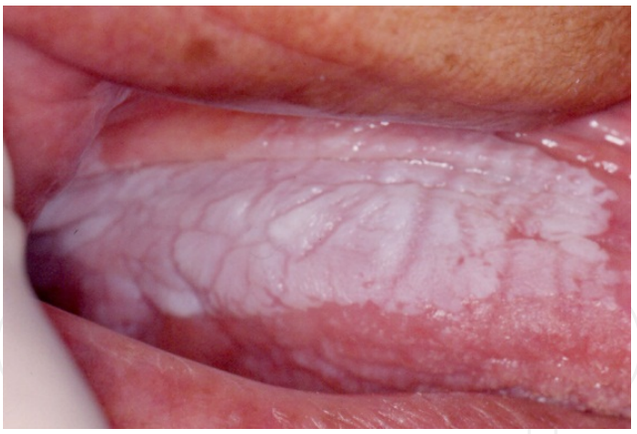

Figure 3. Homogeneous thick leukoplakia in the tongue.

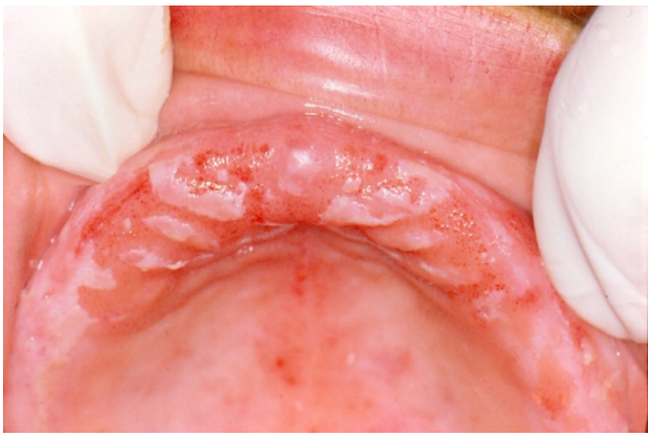

Figure 4. Non-homogeneous (speckled) leukoplakia in the upper alveolar ridge.

There is also the proliferative verrucous leukoplakia, characterized by multifocal evolvement, mainly in elderly female patients that do not present known risk factors (Figure 5 and 6). These lesions are usually resistant to treatment and show a high risk for malignant transformation [4,10].

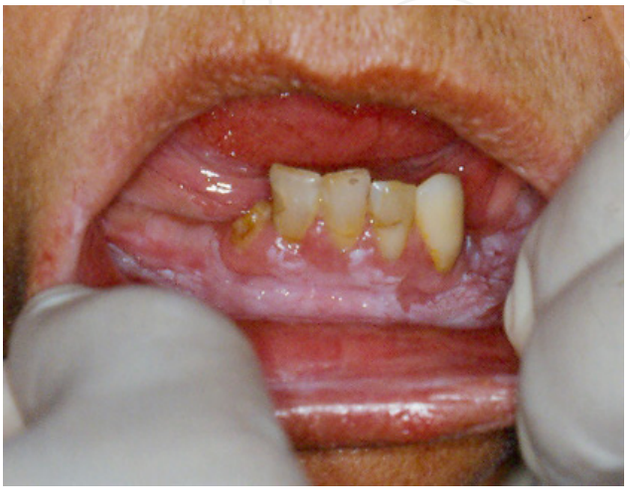

Figure 5. Proliferative verrucous leukoplakia. Notice the multifocal involvement in the lower gingiva.

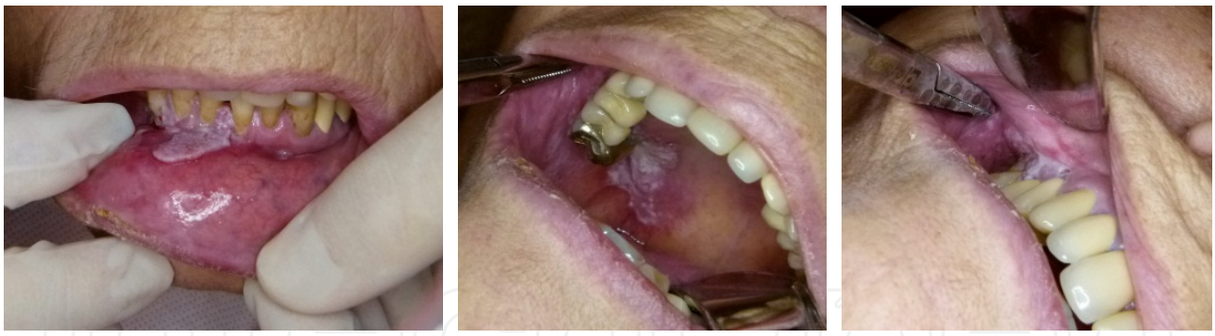

Figure 6. Proliferative verrucous leukoplakia. This elderly woman presented multiple lesions affecting different sites of the oral mucosa.

Many lesions must be excluded before formulating a diagnostic hypothesis of OL, such as chemical injuries, candidiasis, frictional lesion, hairy leukoplakia, leukoedema, linea alba, nicotinic stomatitis, among others [4,10]. Because of variable clinical presentation of the potentially malignant lesions, when a provisional clinical diagnosis of OL is made, a biopsy must be performed to obtain the histopathological diagnosis [12].

The microscopic presentation of OL can vary from slightly hyperkeratotic epithelium to lesions with severe dysplasia [13]. 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 3,300 white lesions of the oral cavity [14]. Epithelial dysplasia is characterized by the presence of architectural alteration and cytological atypia, and can be graded as mild, moderate, severe, and carcinoma *in situ* [10]. Nevertheless, there is a notable inter- and intra-observer variation in the interpretation and classification of dysplasia, which makes this method subjective with low reproducibility [12,15]. Thus, many different grading systems have been suggested to enhance the reproducibility and the predictive value for malignant transformation of OL.

It has been suggested a possible correlation between clinical and histopathological features of OL [16]. Following this proposal, thin and flat OL would show hyperkeratosis, acanthosis, and occasional lymphocytes. Thick fissured OL lesions would present, besides these microscopic alterations, mild to moderate dysplasia. The verrucous or granular OL would show irregular hyperkeratosis, drop-shaped rete ridges, a moderate amount of lymphocytes, and moderate to severe dysplasia. Finally, speckled OL and erythroplakia could show irregular hyperkeratosis, epithelial atrophy, numerous lymphocytes, and severe dysplasia or carcinoma *in situ*.

A research group published a proposal of a staging system for OL, in which a clinical feature of the lesion would be taken into account [17,18]. The lesion would be classified into one of the four stages (I, II, III, or IV), according to the association between two parameters. The first characteristic to be evaluated would be the size of the lesion, with four possible categories (L_1, L_2, L_3, and L_x). The second item concerned the histopathological presentation, focused on the presence of dysplasia, with three possible categories (P_0, P_1, and P_x). Therefore, a somehow similar strategy to that of TNM (extent of the tumor (T), spread to regional lymph nodes (N), and distant metastasis (M)) for oral cancer would be used to stage OL, and the authors intended to promote a uniform reporting of treatment or management of OL lesions.

1.3. Evolution and prognosis

OL may persist unchanged, progress, regress, or even disappear [9]. The malignant transformation risk varies from 3.6% to 36.0%, and some features such as the 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 [10,19-22]. Surgical excision, cryosurgery, laser surgery, topical or systemic retinoids, therapy with mouth rinses with attenuated adenovirus, and photodynamic therapy are possible therapeutics [10,13,23]. Recurrence rates are highly variable among studies, from 0 to 30.0% [10].

Many efforts have attemped to identify molecular markers to predict cancer development in OL. However, 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 [9,10,12].

2. Grading oral epithelial dysplasia

The term "dysplasia" is generally employed in the sense of a disordered development [24]. 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 [7,12].

Despite many efforts towards new evaluative methods, the histological analysis is still the most useful method for grading epithelial dysplasia in OL [12].

2.1. Relevance

The concept of a sequential developmental process from a normal epithelium through a dysplasia, ending in a carcinoma, was introduced from studying pathological changes in the uterine cervix [24]. It is believed that through this process there is an accumulation of genetic and epigenetic alterations and more and more layers of the epithelium are progressively involved, until it is replaced by atypical cells in full length. It is considered that the more severe the degree of dysplasia, the greater the likelihood of malignant transformation. Despite the imperfection of currently available systems, they remain essential, and the diagnosis is a prerequisite for the establishment of the treatment that provides the best prognosis [12,24].

2.2. Proposed systems

The elaboration of a classifying system is not a simple issue as the system may be, above all, an indicator of prognosis, guiding or at least helping in the establishment of the best treatment. Moreover, it should be reproducible, reliable, and as simple and objective as possible. Many classification schemes have been proposed over time, with variable

acceptance and employment. Herein, three of the most mentioned systems will first be discussed, followed by the recently suggested binary system.

2.2.1. Squamous intraepithelial neoplasia (SIN)

This classification is a modification of a previously suggested system for cervical pre-malignant lesions, named cervical intraepithelial neoplasia [25]. After that, this concept has been adopted and extended to other sites, including oral mucosa, named "oral intraepithelial neoplasia" [12,26]. The term squamous intraepithelial neoplasia is also used to encompass all sites of the upper aerodigestive tract [26]. However, there is no evidence that many of the potentially malignant lesions of the oral mucosa are committed on a path to malignancy. Moreover, the SIN terminology would not clarify the knowledge concerning this issue, which would not justify replacing the widely accepted concept of dysplasia. Additionally, the WHO consensus group did not favour this system [12].

According to this system, lesions are classified as:

- SIN 1, would be similar to mild dysplasia
- SIN 2, would be similar to moderate dysplasia
- SIN 3, would combine severe dysplasia and carcinoma *in situ*

2.2.2. Ljubljana classification of squamous intraepithelial lesions

This classification system was proposed by laryngeal pathologists in 1971 and additionally formulated in 1997 by a Working Group of the European Society of Pathology [27,28]. Very detailed criteria have been published and this system is more complex than the concept of dysplasia, so that even experienced pathologists would require time to adapt to it. Moreover, despite some publications, the usefulness of this grading system for oral lesions is doubtful [12,24].

Briefly, lesions are classified into four groups according to this system:

- Simple hyperplasia, which is an increase in the stratum spinosum
- Basal/parabasal cell hyperplasia or abnormal hyperplasia, considered essentially a basal cell hyperplasia
- Atypical hyperplasia, also named risky hyperplasia, shows epithelial stratification, but with atypia
- Carcinoma *in situ*, characterized by loss of stratification throughout epithelium, but three to five layers of compressed cells may be present on the surface. Also, there is marked atypia and mitotic abnormalities

The first two degrees are considered mainly benign lesions, showing minimum risk for malignant transformation. The third degree would be a potentially malignant lesion, and the last one is actually considered a malignant lesion already. Additionally, the "atypical hyperplasia" and "carcinoma *in situ*" degrees are divided into basal cell type and spinous cell type [12,24].

2.2.3. World health organization

In 1997, the WHO published the "Histopathological Typing of Cancer and Precancer of the Oral Mucosa" and in the latest WHO's classification of Head and Neck Tumours, a grading system based on "thirds" was described [7,29]. This resembled the system described since the 1970's for lesions of the uterine cervix [24].

The WHO's classification system is truly 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 [24]. Moreover, in spite of its wide acceptance, this system presents great variability and low reproducibility [10,12]. According to it, lesions are allocated into categories considering firstly the architectural features, followed by cytological alterations [7].

The architectural features that should be addressed are:

- Irregular epithelial stratification (Figure 7)
- Loss of polarity of basal cells (Figure 8)
- Drop-shaped rete ridges
- Increased number of mitotic figures
- Abnormally superficial mitoses
- Premature keratinisation in single cells (dyskeratosis) (Figure 9)
- Keratin pearls within rete pegs

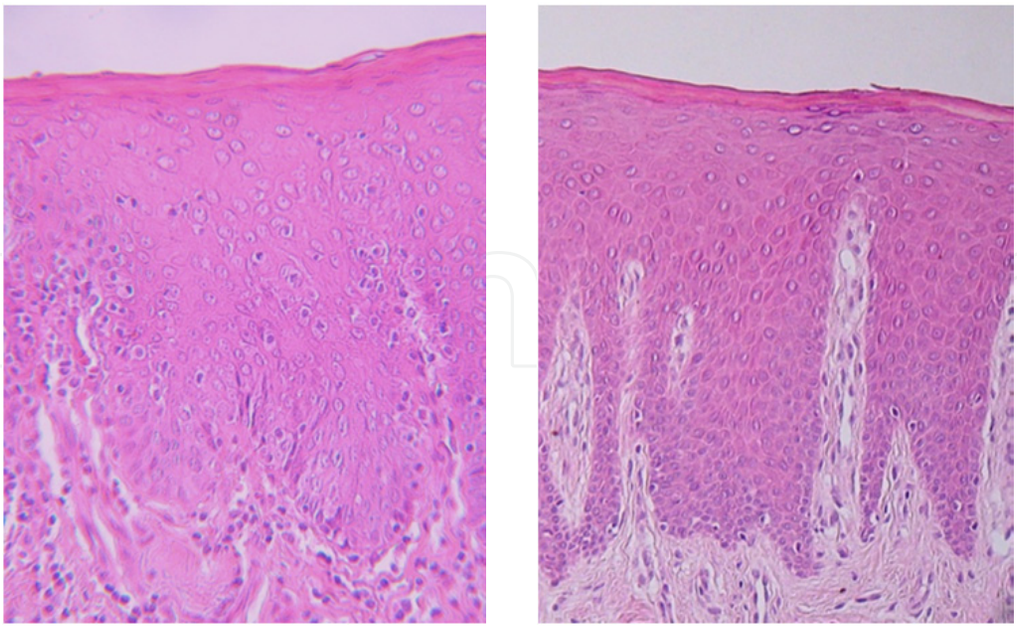

Figure 7. Left: specimen of an oral leukoplakia showing irregular epithelial stratification. Right: Normal oral mucosa. Hematoxylin and eosin, 200X magnification.

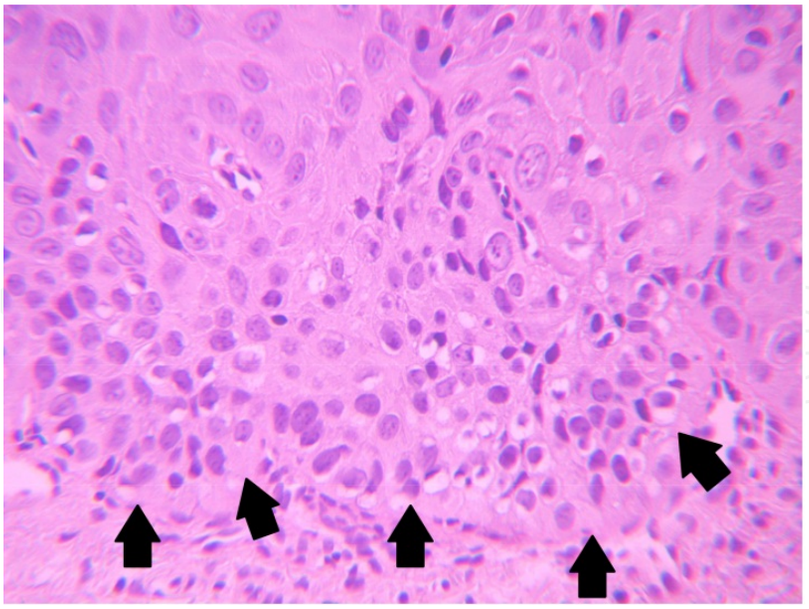

Figure 8. Loss of polarity of basal cells in a photomicrograph of an oral leukoplakia specimen (arrows). Hematoxylin and eosin, 400X magnification.

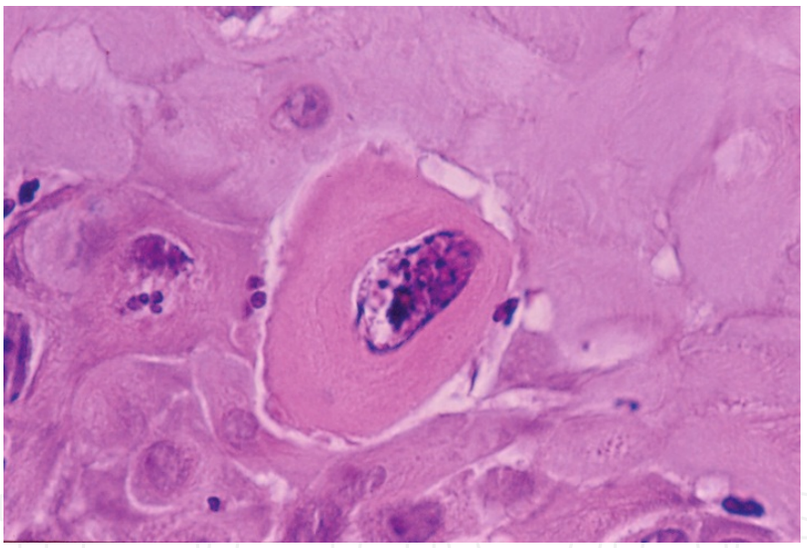

Figure 9. A keratinocyte showing dyskeratosis. Hematoxylin and eosin, 1000X magnification.

The cytological alterations to be observed are as follows:

- Nuclear pleomorphism: abnormal variation in nuclear shape (Figure 10)
- Cellular pleomorphism: abnormal variation in cell shape (Figure 10)
- Anisonucleosis: abnormal variation in nuclear size (Figure 10)
- Anisocytosis: abnormal variation in cell size (Figure 10)
- Increased nuclear size (Figure 10)
- Increased nuclear-cytoplasm ratio (Figure 10)
- Atypical mitotic figures (Figure 11)
- Increased number and size of nucleoli (Figure 10 and 12)

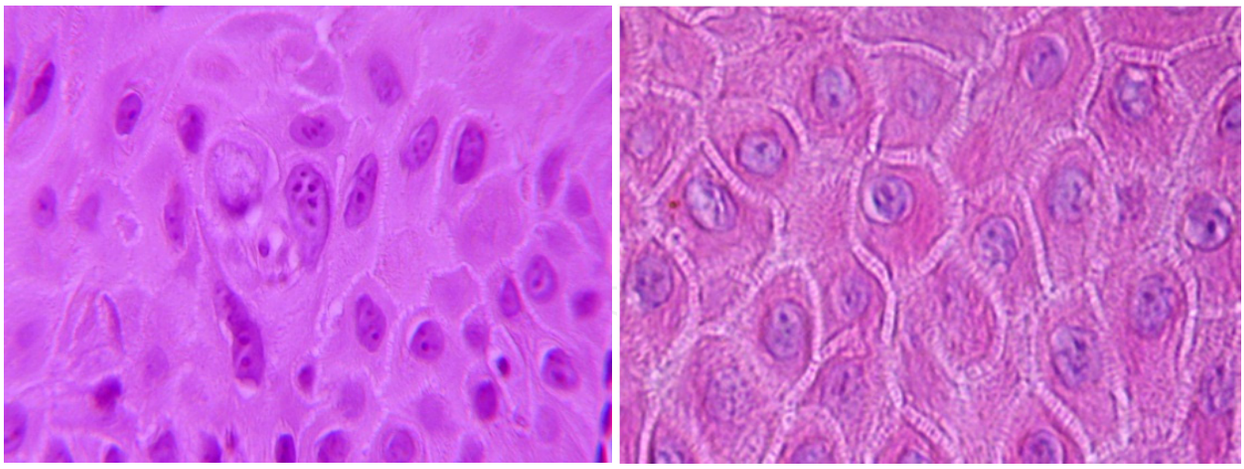

Figure 10. Left: in this specimen, it can be noticed anisonucleosis, anisocytosis, nuclear and cellular pleomorphism, increased nuclear size, increased nuclear-cytoplasm ratio, and increased number and size of nucleoli of keratinocytes in an oral leukoplakia. Right: normal keratinocytes. Hematoxylin and eosin, 1000X magnification.

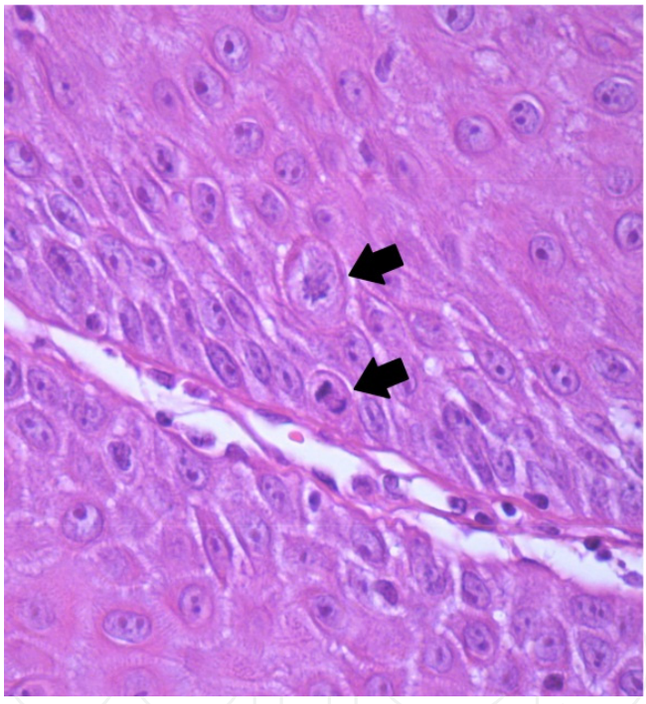

Figure 11. Keratinocytes exhibiting atypical mitotic figures (arrows). Hematoxylin and eosin, 400X magnification.

The observation of these alterations should be done considering the epithelium divided into "thirds". Accordingly, lesions should be classified into five categories, as described below:

1. Hyperplasia (Figure 13): describes a lesion showing an increase in cell number in the spinous layer and/or in the basal/parabasal cell layers. There is regular stratification and no cellular atypia.
2. Mild dysplasia (Figure 14): architectural disturbance only in the lower third of the epithelium with cytological atypia.

3. Moderate dysplasia (Figure 15): 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".
4. Severe dysplasia (Figure 16): architectural disturbance affecting greater than two thirds of the epithelium, with cytological atypia.
5. Carcinoma *in situ* (Figure 17): theorically, indicates that malignant transformation has occurred but invasion has not. Full or almost full thickness architectural disturbance in viable cellular layers with pronounced cellular atypia. Atypical mitotic figures and abnormal superficial mitoses are common.

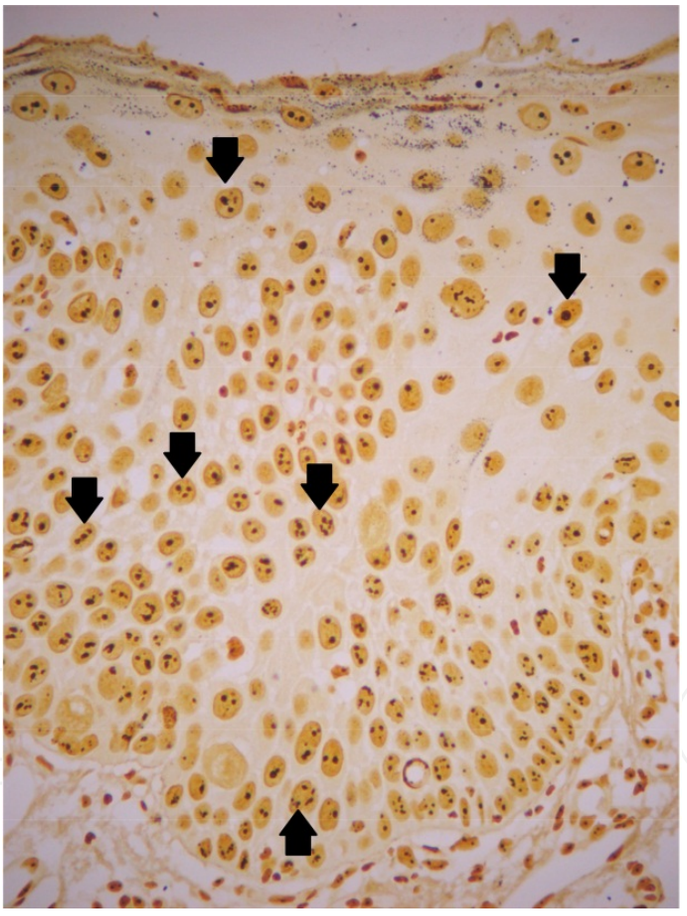

Figure 12. Oral leukoplakia specimen exhibiting increased number and size of nucleoli (arrows). AgNOR staining, 200X magnification.

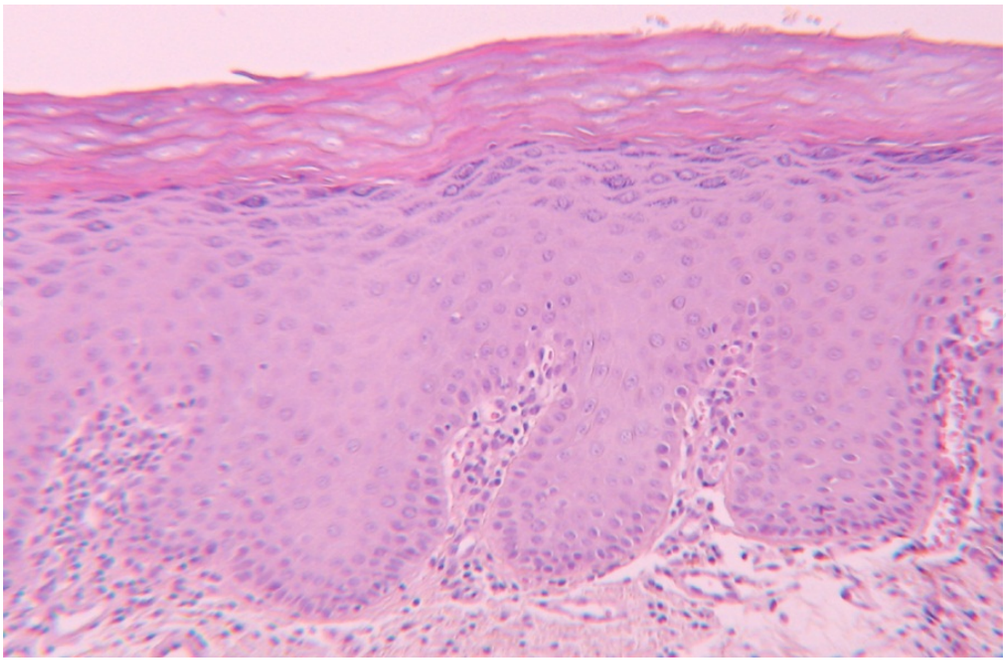

Figure 13. Sample of an oral leukoplakia showing hyperplasia. Note an increased number of basal / parabasal cells and a hyperkeratotic surface. Regular stratification is observed, as well as no cytological atypia. Hematoxylin and eosin, 200X magnification.

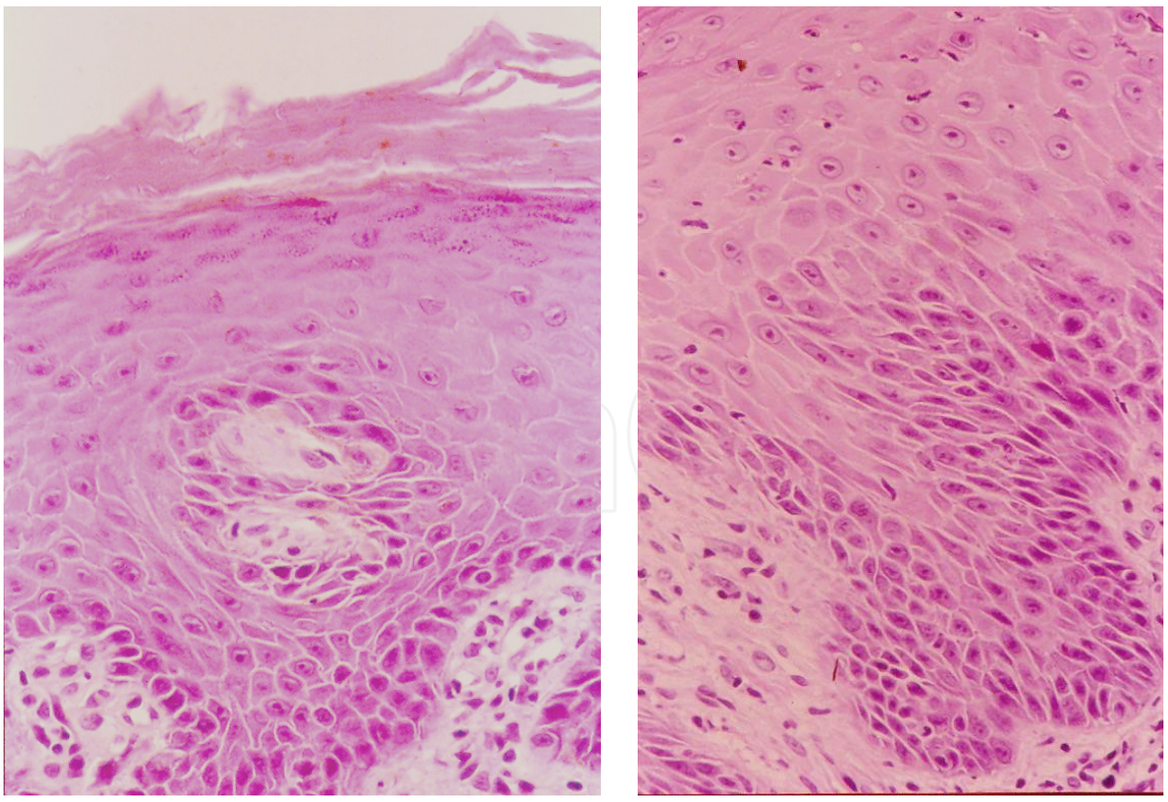

Figure 14. These specimens of oral leukoplakia exhibited mild dysplasia. Observe architectural disturbances affecting the lower third of the epithelium and cytological atypia. Hematoxylin and eosin, 200X magnification (left), 400X magnification (right).

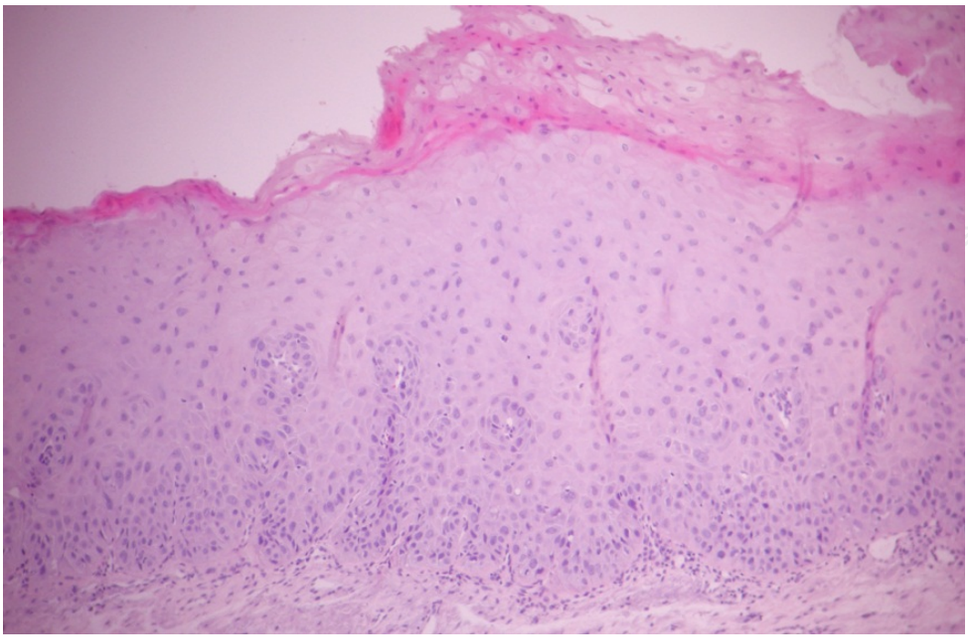

Figure 15. Microscopic presentation of an oral leukoplakia showing moderate dysplasia. Architectural disturbances extending into the middle third of epithelium, along with cytological atypia. Hematoxylin and eosin, 100X magnification.

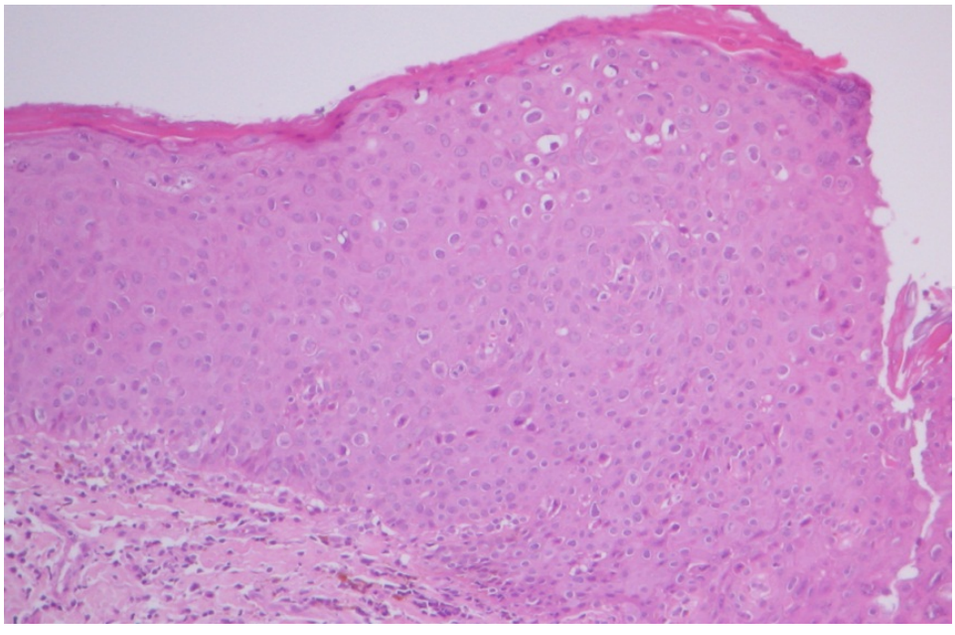

Figure 16. Histological section of oral leukoplakia exhibiting severe dysplasia. Architectural disturbances affecting greater than two thirds of the epithelium. Pronounced cytological atypia is evident. Hematoxylin and eosin, 100X magnification.

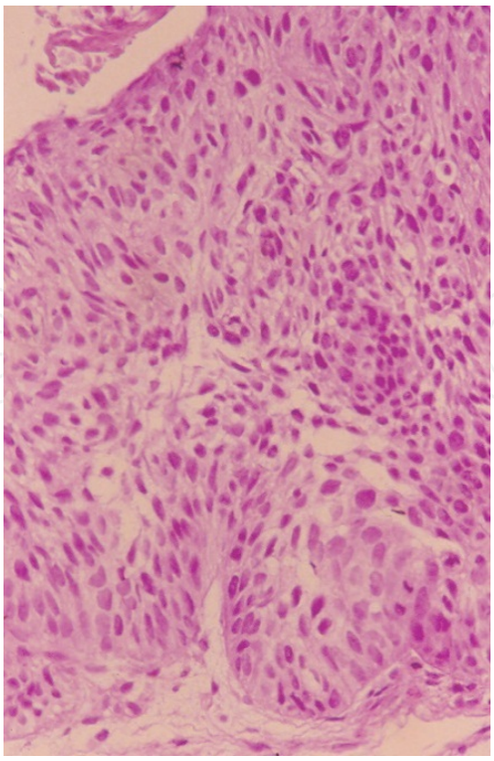

Figure 17. An oral leukoplakia that showed microscopic features of carcinoma *in situ*: architectural disturbances are observed in the full thickness of epithelium with pronounced cellular atypia. No superficial keratinisation can be observed. Hematoxylin and eosin, 200X magnification.

2.2.4. Binary system

As mentioned above, the proposed systems to grade epithelial dysplasia published so far, including the WHO's proposal, showed some shortcomings, such as great variability and low reproducibility. Thus, studies concerning classification criteria are being performed, looking for an enhancement for grading epithelial dysplasia in OL. In 2006, a new binary system was proposed, which could be a more feasible and reliable tool for grading epithelial dysplasia in OL [15]. According to this system, 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) [15,10,12]. This would provide more reliable criteria upon which to rely for the selection of patient treatment.

Interestingly, in 1988 the "Bethesda classification" for cervical cytopathology, already included only two grades [30]. According to this, lesions would be classified as low-grade squamous epithelial lesions, corresponding to former cervical intraepithelial neoplasia grade 1, and high-grade squamous epithelial lesions, corresponding to grades 2 and 3. This system has also been mentioned in some reports for oral lesions [26].

After the publication of those papers on the binary system for grading epithelial dysplasia in OL, a study was performed with 218 patients with OL, from which 39 (17.9%) developed into cancer [31]. The authors reported that high-risk OL was associated with a 4.57-fold

increased risk for malignant transformation, compared with low-risk OL. Those authors suggested that high-risk dysplasia would be a significant indicator for evaluating malignant transformation risk in OL.

Subsequently, the same research group published a study in which they identified significant risk factors for malignant transformation in a long-term follow-up cohort of patients with oral epithelial dysplasia [32]. Of the 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. Moreover, high-risk degree of dysplasia was associated with a 2.78-fold increased risk of transformation compared with low-risk degree. 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. According to them, this would also help guiding treatment in clinical practice. In spite of these great achievements, it must be mentioned that malignancy also developed in some patients previously presenting low-risk potentially malignant lesions [32].

In our first paper [33], we investigated the immunoexpression of hMLH1 (a protein of the mismatch repair system) (Figure 18) in OL with different degrees of dysplasia, according to the WHO grading system. We evaluated lesions showing no, mild, moderate, and severe dysplasia, and we found that the greater difference in the hMLH1 immunoexpression was detected comparing OL with mild and moderate dysplasia, with decreasing indexes. Therefore, we suggested that this result would be in accordance with the proposed binary system of grading dysplasia in OL, as the morphological dysplastic alterations observed in routinely stained slides may be related to molecular changes.

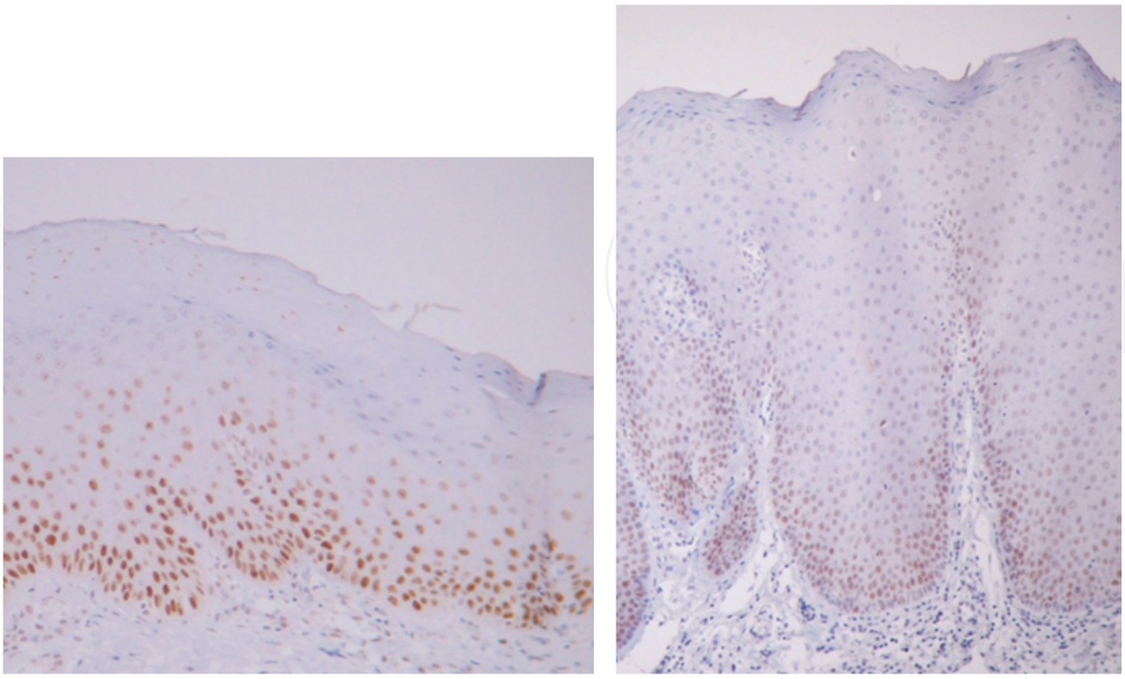

Figure 18. Immunoexpression of hMLH1 in oral leukoplakia showing no dysplasia (left) and severe dysplasia (right). Advance HRP, 200X magnification.

After that, we conducted a comparative immunohistochemical and histochemical study encompassing those same samples of OL [34]. At that time, the hMLH1 immunoexpression was compared to p53 immunoexpression (Figure 19) and AgNOR counting. Thus, we could assess the possible association between a protein of DNA repair, a tumor suppressor protein, and the cellular proliferation in OL with different degrees of dysplasia, *i.e.* no, mild, moderate, and severe. We concluded that it seemed reasonable that other molecular alterations may take place in early phases of carcinogenesis, related to tumor suppressor genes, like p53, as well as modifications in proliferation rates.

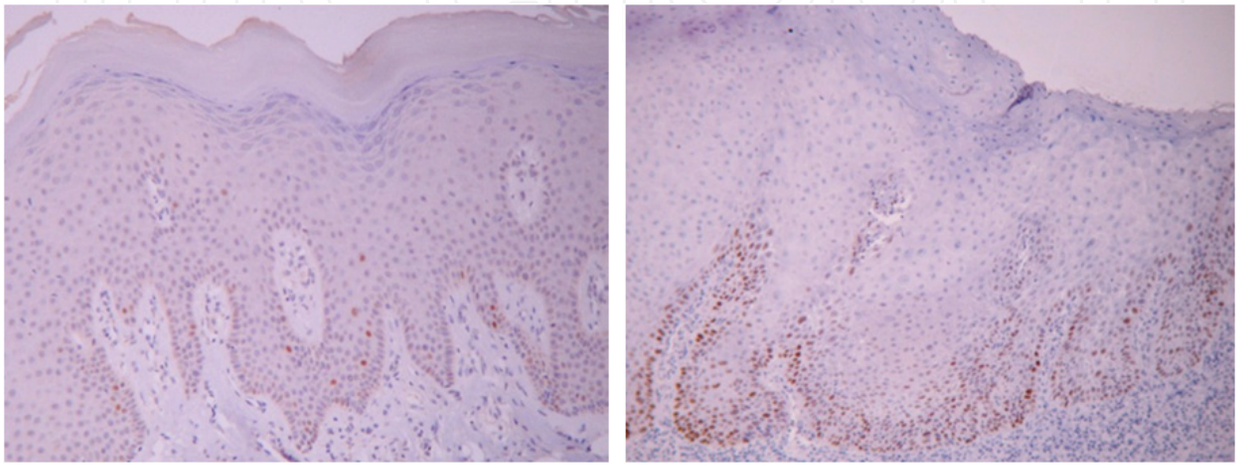

Figure 19. Immunoexpression of p53 in oral leukoplakia with mild dysplasia (left) and severe dysplasia (right). Streptoavidin-biotin, 100X magnification.

Recently, we decided to reevaluate those previous results in the light of the binary system to grade epithelial dysplasia in OL [35]. Therefore, we grouped OL formerly classified as showing no and mild dysplasia into low-risk lesions. Accordingly, OL previously classified as having moderate and severe dysplasia were defined as high-risk lesions. After that, we performed the statistical analyses again. Our findings showed statistically significant differences for hMLH1, p53, and AgNOR 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. Moreover, we could speculate that OL classified as low-risk may be reasonably named this way, since comparisons between hMLH1 and AgNOR indexes of this group and normal oral mucosa did not reach statistical significance, despite their different median values.

2.2.5. Other proposals

Apart from those investigations pointing towards an adaptation of the WHO classification to a binary system, there are also other recently published papers on different proposals to evaluate epithelial dysplasia.

As reviewed before, the Japanese Society for Oral Pathology reported a definition of carcinoma *in situ* and proposes the term “oral intraepithelial neoplasia”, which in turn could

be classified as differentiated and basaloid type [24]. The main difference between them would be the presence of keratinisation in the epithelium surface in the differentiated type. Additionally, some authors analyzed individual features of dysplasia in oral lesions and determined the reproducibility of scoring each one [36]. They suggested that those data might be used to improve or to develop simpler routine diagnostic methods.

3. Conclusion

To date, no system is free of presenting failures in identifying those OL prone to evolve to oral squamous cell carcinoma. Furthermore, the reproducibility and subjectivity are still key points to be addressed. Therefore, robust research on the predictive value, relevance, applicability, and feasibility of the binary system for grading epithelial dysplasia are clearly warranted. Such research should aim to establish of a reliable and reproducible method that, above all, could provide a better and less empiric clinical management of the patient.

Author details

Maria Auxiliadora Vieira do Carmo and Patrícia Carlos Caldeira
School of Dentistry, Universidade Federal de Minas Gerais, Brazil

Acknowledgement

The authors acknowledge National Council of Technological and Scientific Development (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for the received grants and research funding.

4. References

[1] Neville BW, Day TA. Oral cancer and precancerous lesions. CA: A Cancer Journal for Clinicians 2002;52(4):195-215.
[2] Scully C, Bagan J. Oral squamous cell carcinoma: overview of current understanding of aetiopathogenesis and clinical implications. Oral Diseases 2009;15(6):388-99.
[3] Haya-Fernández MC, Bagán JV, Murillo-Cortés J, Poveda-Roda R, Calabuig C. The prevalence of oral leukoplakia in 138 patients with oral squamous cell carcinoma. Oral Diseases 2004;10(6):346-8.
[4] Warnakulasuriya S, Johnson NW, van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. Journal of Oral Pathology & Medicine 2007;36(10):575-80.
[5] van der Waal I. Potentially malignant disorders of the oral and oropharyngeal mucosa; present concepts of management. Oral Oncolology 2010;46(6):423-5.

[6] Kramer IR, Lucas RB, Pindborg JJ, Sobin LH. Definition of leukoplakia and related lesions: an aid to studies on oral precancer. Oral Surgery, Oral Medicine, Oral Pathology 1978;46(4):518-39.
[7] World Health Organization. Tumours of the Oral Cavity and Oropharynx. In: Barnes L, Eveson JW, Reichart P, Sidransky D, editors. Pathology & genetics. Head neck tumors. Lyon: IARC Press; 2005. p. 177-9.
[8] Petti S. Pooled estimate of world leukoplakia prevalence: a systematic review. Oral Oncology 2003;39(8):770-80.
[9] Napier SS, Speight PM. Natural history of potentially malignant oral lesions and conditions: an overview of the literature. Journal of Oral Pathology & Medicine 2008;37(1):1-10.
[10] van der Waal I. Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management. Oral Oncology 2009;45(4-5):317-23.
[11] Campisi G, Panzarella V, Giuliani M, Lajolo C, Di Fede O, Falaschini S, et al. Human papillomavirus: its identity and controversial role in oral oncogenesis, premalignant and malignant lesions (review). International Journal of Oncology 2007;30(4):813-23.
[12] Warnakulasuriya S, Reibel J, Bouquot J, Dabelsteen E. Oral epithelial dysplasia classification systems: predictive value, utility, weaknesses and scope for improvement. Journal of Oral Pathology & Medicine 2008;37(3):127-33.
[13] Brennan M, Migliorati CA, Lockhart PB, Wray D, Al-Hashimi I, Axéll T, et al. Management of oral epithelial dysplasia: a review. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Edodontics 2007;103(Supp l):S19e1-12.
[14] Waldron CA, Shafer WG. Leukoplakia revisited. A clinicopathologic study 3256 oral leukoplakias. Cancer 1975;36(4):1386-92.
[15] Kujan O, Oliver RJ, Khattab A, Roberts SA, Thakker N, Sloan P. Evaluation of a new binary system of grading oral epithelial dysplasia for prediction of malignant transformation. Oral Oncology 2006;42(10):987-93.
[16] Bouquot JE, Gnepp DR. Laryngeal precancer: a review of the literature, commentary, and comparison with oral leukoplakia. Head & Neck 1991;13(6):488-97.
[17] van der Waal I, Schepman KP, van der Meij EH. A modified classification and staging system for oral leukoplakia. Oral Oncolology 2000;36(3):264-6.
[18] van der Waal I, Axéll T. Oral leukoplakia: a proposal for uniform reporting. Oral Oncolology 2002;38(6):521-6.
[19] Cruz I, Napier SS, van der Waal I, Snijders PJ, Walboomers JM, Lamey PJ, et al. Suprabasal p53 immunoexpression is strongly associated with high grade dysplasia and risk for malignant transformation in potentially malignant oral lesions from Northern Ireland. Journal of Clinical Pathology 2002;55(2):98-104.
[20] Holmstrup P, Vedtofte P, Reibel J, Stoltze K. Long-term treatment outcome of oral premalignant lesions. Oral Oncology 2006;42(5):461-74.
[21] Hsue SS, Wang WC, Chen CH, Lin CC, Chen YK, Lin LM. Malignant transformation in 1458 patients with potentially malignant oral mucosal disorders: a follow-up study based in a Taiwanese hospital. Journal of Oral Pathology & Medicine 2007;36(1):25-9.

[22] Smith J, Rattay T, McConkey C, Helliwell T, Mehanna H. Biomarkers in dysplasia of the oral cavity: a systematic review. Oral Oncology 2009;45(8):647-53.
[23] Lodi G, Porter S. Management of potentially malignant disorders: evidence and critique. Journal of Oral Pathology & Medicine 2008;37(2):63-9.
[24] Izumo, T. Oral premalignant lesions: from the pathological viewpoint. International Journal of Clinical Oncology 2011;16(1):15-26.
[25] Richart RM. Natural history of cervical intraepithelial neoplasia. Clinical Obstetrics & Gynecology 1967;10(4):748-784.
[26] Küffer R, Lombardi T. Premalignant lesions of the oral mucosa. A discussion about the place of oral intraepithelial neoplasia (OIN). Oral Oncology 2002;38(2):125-30.
[27] Kambic V, Lenart I. Notre classification des hyperplasies de l'epithelium du larynx au point de vue prognostic. Journal Français d'Oto-Rhino-Laryngologie, Audio-Phonologie et Chirurgie Maxillo-Faciale 1971;20(10):1145-50.
[28] Hellquist H, Cardesa A, Gale N, Kambic V, Michaels L. Criteria for grading in Ljubljana classification of epithelial hyperplastic laryngeal lesions. A study by members of the working group on epithelial hyperplastic laryngeal lesions of the European Society of Pathology. Histopathology 1999;34(3):226-233.
[29] World Health Organization. Pindborg JJ, Reichart PA, Smith CJ, van der Waal I, editors. Histological typing of cancer and precancer of the oral mucosa. Berlin: Springer; 1997.
[30] National Cancer Institute Workshop. The 1988 Bethesda System for reporting cervical/vaginal cytological diagnoses. JAMA 1989;262(7):931-4.
[31] Liu W, Wang YF, Zhou HW, Shi P, Zhou ZT, Tang GY. Malignant transformation of oral leukoplakia: a retrospective cohort study of 218 Chinese patients. BMC Cancer, 2010;16(10):685.
[32] Liu W, Bao ZX, Shi LJ, Tang GY, Zhou ZT. Malignant transformation of oral epithelial dysplasia: clinicopathological risk factors and outcome analysis in a retrospective cohort of 138 cases. Histopathology 2011;59(4):733-40.
[33] Caldeira PC, Abreu MH, Batista AC, Do Carmo MA. hMLH1 immunoexpression is related to the degree of epithelial dysplasia in oral leukoplakia. Journal of Oral Pathology & Medicine 2011;40(2):153-9.
[34] Caldeira PC, Aguiar MC, Mesquita RA, Do Carmo MA. Oral leukoplakias with different degrees of dysplasia: comparative study of hMLH1, p53, and AgNOR. Journal of Oral Pathology & Medicine 2011;40(4):305-11.
[35] Caldeira PC, Abreu MH, Carmo MA. Binary system of grading oral epithelial dysplasia: evidence of a bearing to the scores of an immunohistochemical study. Journal of Oral Pathology & Medicine 2012;41(6):452-3.
[36] Tilakaratne WM, Sherriff M, Morgan PR, Odell EW. Grading oral epithelial dysplasia: analysis of individual features. Journal of Oral Pathology & Medicine 2011;40(7):533-40.