ABSTRACT:

Purpose: With the advance of the modern technologies, the identification of dysplastic changes in the oral mucosa on a molecular level seems to have transferred into the dental office. With the new methods, however, new responsibilities and problems arise. The current review is an attempt to present the advantages and disadvantages of the clinical technologies used for early diagnostics of the oral precancerous lesions.

Materials and Methods: This study is based on an extensive literature review and personal clinical experience with these contemporary evaluating methods of oral precancerous lesions.

Results: The results from the pathohistological evaluation reveal stratified squamous epithelium with pronounced parabasal hyperplasia, disturbed maturation and loss of polarity of the basal cells. The latter demonstrates hyperchromatic polymorphic nuclei and single mitotic figures in half of the epithelial thickness. The morphological data points to a moderate epithelial dysplasia.

Conclusion: The loss of autofluorescence seems to be the most promising non-invasive method for identification of epithelial dysplasia in the oral cavity. The presence of some non-dysplastic lesions, which can also be non-fluorescent, i.e. produce “false negative” results, is one of the disadvantages of this method. Despite that most modern technologies are practical and convenient, they should be approached with a critical eye and should not be used as single and independent diagnostic means but only as an adjunct to the established classical methods of diagnostics.

Keywords: Oral precancerous, lesions, leukoplakia, erythroplakia,

INTRODUCTION

From a historical perspective the concept of oral precancerous lesions has undergone many changes and even today it continues to be confusing. In 1805 [1] a team of European physicians made the presumption that a group of benign diseases exist, which, if monitored over a long enough period, always develop into invasive carcinomas [2]. According to the current concept, a precancerous condition is defined as a condition at an increased risk of transformation into a neoplasm. Oral precancerous lesions have been described in great detail, starting back in the 1870s when Sir James Paget, one of the most renowned English surgeons, presumed that leukokeratosis, or nicotine stomatitis of the hard palate or the tongue, in avid smokers leads to an increased risk of transformation into a malignant disease [3]. He claimed that he had observed the first transformation into a cancer of this kind in 1851. Quite ironically, the current understanding of the aetiology of nicotine stomatitis as a premalignant condition does not involve the presence of carcinogenic substances but the reaction of the oral epithelium to the heat produced by the nicotine smoke [3, 4]. The hyperkeratinized (protective) mucosa of the hard palate is actually one of the areas least likely to develop an oral carcinoma [2]. Another white keratotic lesion is leukoplakia (Fig. 1). It poses a much greater risk of malignant transformation, which has been discussed even earlier than 1876 when the Hungarian dermatologist Schwimmer introduced this term. Nowadays, it has been proven that leukoplakia accounts for more than 80% of all oral precancerous lesions. It is present in approximately 3% of the population aged over 35 in the USA, and its prevalence increases with the increase of age and the increased use of tobacco [2, 3, 5, 6, 7].

Fig. 1. Leukoplakia – homogenous (white patch) form

Due to the persistent discussions on the correct meaning and use of the term premalignant conditions, the WHO has organized seminars in search of a new definition of the term precancerosis and the different oral precancerous lesions. The most recent seminar was held in London in 2005. The WHO recommended that the term precancerosis is discarded and that a more accurate term –
potentially malignant disorder, be used [8]. An attempt to fully discard the term leukoplakia was made, due to its progressively changing definition over time, but a more accurate term has not been agreed upon. Regarding all white mucosal patches in the oral cavity, the term leukoplakia is defined as “a whitish patch or plaque that cannot be identified clinically or pathologically as any other disease”, and is not associated with any physical or chemical causative agent, except the use of tobacco [2, 8]. This diagnosis excludes lichen planus, frictional hyperkeratosis, tobacco hyperkeratosis caused by tobacco chewing, nicotine stomatitis and alveolar ridge keratosis, i.e. all diseases which in the past have been described with the term leukoplakia. Nowadays the term is used in a strictly clinical sense, with no reference to a specific morphological change of the tissue, except for the proliferation of the surface epithelium containing keratin, which is responsible for the white colour of the lesion. It is known that if certain clinical changes occur within the leukoplakia, there is a higher risk of its transformation into cancer. Such is the case with the clinical lesion described as erythroleukoplakia (Fig. 2), which in most cases under a microscopic examination represents as epithelial dysplasia or even carcinoma in situ.

Fig. 2. Erythroleukoplakia with a moderate dysplasia (indicated by arrows)

It is considered that a clinical entity like leukoplakia has a potential for malignant transformation of approximately 4% (assumed risk for the entire life), although there are only a few clinical cases where patients were followed-up throughout their whole life [2, 7, 9]. The risk of transformation of the lesions of epithelial origin varies between approximately 4-11% for the moderate dysplasia and between approximately 20-30% for the severe dysplasia (Fig. 3), the malignant transformation occurring within 3 years after the dysplasia has been diagnosed [7-11]. The epithelium with a lower degree of dysplasia has a relatively more favourable biological behaviour. For this reason, the most significant studies on the monitoring of the oral dysplasia are focused on its severe forms or on the carcinoma in situ (often presenting as combined lesions), because the two conditions exhibit a similar biological behaviour [7, 9, 12].

How often can dysplastic cells be found in cases of erythroplakia and leukoplakia? The most recent studies show that in approximately 5-25% of the cases of leukoplakia the biopsy indeed reveals dysplastic epithelial cells, while in the cases with erythroplakia their numbers account for as much as the impressive 90% [2, 8, 12].

Fig. 3. Erythroplakia with severe dysplasia (indicated by an arrow)
In this regard, erythroplakia is considered as a high-risk process, unlike leukoplakia. Actually, the greatest part of the cases with leukoplakia does not demonstrate signs of cell atypia. How are they determined as high-risk lesions then?

Certain clinical signs like for instance: big size, nodular surface, erythema or fissures going through the white patch (speckled leukoplakia, erythroleukoplakia), the multifocalmiliary leukoplakia, the erosive and ulcerative forms are significant in this sense. (fig. 4) [7, 13, 14]

Fig. 4. Miliary homogenous form of leukoplakia with no dysplasia

These clinical signs are associated with a greater risk of dysplastic cells being present, therefore namely the affected areas should be the ones chosen for a biopsy test. This is true especially for the large-size leukoplakia lesions, as a negative result would lead to a false feeling of safety and poor monitoring of the lesion.

In the course of a few decades, the presence of the abovementioned clinical signs has been the major factor in the risk assessment of a dysplastic lesion and its transformation into cancer. While they might work remarkably well in the hands of an experienced clinician, most general dental practitioners do not possess enough experience so as to take an optimal decision when it comes to the diagnosis and monitoring of such lesions. The new technologies for identification of dysplastic cells inside the lesions offer help to the general dental practitioners and can be utilized in the dental office. These technologies make it easier for clinicians to distinguish the areas suitable for biopsy test, which allows for a more accurate risk assessment of the dysplastic lesion, and to identify any suspicious areas of the lesion. This consideration is of utmost importance, as a biopsy taken from an unsuitable spot is not indicative of the risk areas and does not allow for a timely diagnosis and treatment [15, 16].

All these factors indicate the growing need for a definitive solution of this problem at an early stage, starting from the general dental practice.

With the advance of the modern technologies, the identification of dysplastic changes in the oral mucosa on a molecular level seems to have transferred into the dental office. With the new methods, however, new responsibilities and problems arise [17]. The current review is an attempt to present the advantages and disadvantages of the clinical technologies used for early diagnostics of the oral precancerous lesions.

Diagnostic non-invasive methods for the examination of the oral mucosa. Autofluorescence – scientific rationale. Each cell in the human body contains molecules capable of auto-fluorescence, especially when they are activated (excited) by a specific wavelength [26]. The excitation and emission of fluorescence depending on how light is scattered and absorbed by the tissue. The scattering of light is caused by the differences in the refraction index of the various tissue components, while the absorption depends on the molecular composition of these components [26]. There are different fluorescent components in humans: tryptophan, porphyrins, collagen fibers, elastin, NADH, flavins (FAD), etc. [25]. This fluorescent signalling is used in the evaluation of the metabolic state of the tissues and for the identification of dysplastic cells. When violet or blue light is used in a dark room, auto-fluorescence can be easily observed through an eyepiece or glasses filtering the reflected light and transmitting only the light with wavelength characteristic of the fluorescent tissues. The wavelengths which excite the greatest degree of fluorescence of the biological tissues vary between 400 and 460nm, i.e. this is the violet and blue light. The device VELScope(R) (LED DentalInc, Canada) uses blue light (436nm) with a peak intensity, its wavelength inducing green fluorescence of the soft tissues. The device emits light from its handpiece, which is connected to a light source and the operator observes through a filtering eyepiece, which does not allow the transmission of reflected light.
Fig. 5. Scheme illustrating the diagnostic principle of VELScope, based on the natural fluorescence of the oral mucosa. http://www.velscope.com/velscope-technology/tissue-fluorescence/

An immature or dysplastic epithelial cell produces lower amounts of NADH and FAD as compared to a normal cell. Because of that mucosal area containing such cells do not fluoresce, appearing black, black-greenish or black-blueish when viewed through the eyepiece [18, 19-26]. Experimental data indicate that due to the intersection of the sub-epithelial fibers with the dysplastic cells, the latter lose their fluorescent activity, which leads to the appearance of a dark spot, visible through the filter [26]. According to the present studies, the autofluorescence of dysplastic epithel-
Hemoglobin (for instance in a carcinoma) can be 12 times greater as compared to that of the normal oral mucosa. Biopsies of the borderline areas between the “green” and the “dark” mucosa indicate that the chances of the green/blue mucosa to contain dysplastic cells are very low, while the chances that the “dark” mucosa might contain such cells are high [25, 27, 28].

The advantage of autofluorescent tests is that the light used for excitation of the oral epithelium cells penetrates to the deepest layers of the epithelium. Thus it reaches easily the dysplastic cells of the deeper epithelium layers, as well as the subepithelial collagen fibers. This deep penetration, however, might be a disadvantage in certain cases, as some non-dysplastic tissue changes also demonstrate lack of fluorescence during this test. These dark lesions are not dysplastic, but due to a change in the blood circulation, inflammation or infection, they might produce a false positive result. The presence of this phenomenon requires a thorough knowledge of the commonly observed oral lesions and assessment under visible light generated from a close distance. For instance, the excellent property of hemoglobin to absorb light, as well as the melanin deposition cause loss of fluorescence. If there is a multitude of dilated superficial blood vessels right beneath the epithelium, like for instance after light trauma or during inflammation, they can also imitate loss of fluorescence (a black spot).

Fig. 7 Example of autofluorescence.

Clinical examples
A case with Lichen planus (erosive form)
The areas with reduced fluorescence (dark zones) are considered to be suspicious for epidermal dysplasia, while the normal intact mucosa appears bright green [24]. The histological examination refuted dysplasia but demonstrated hyperemia, thinning of the epithelium and inflammation, which produced a “false” positive result.

Fig. 8. A case with Lichen planus

Multifocal lesions
The area from where the biopsy is obtained is important for determining the right diagnosis in some disseminated oral lesions. The area with most pronounced changes is chosen (strongly positive test, i.e. loss of fluorescence). With the red-white lesions, the dark zones can contain scattered areas of hyperkeratosis, which have a light green to white colour.
Clinical case 3. Occult lesions
Even the areas with no strongly pronounced macroscopic mucosal changes can be detected with the VELScope due to the loss of autofluorescence. The areas with loss of fluorescence are suspicious and suitable for biopsy.

Fig. 9. A. Macroscopic view of lichen planus. B. Macroscopic view of lichen planus with VELScope. C. Macroscopic view of miliar leukoplakia. D. Macroscopic view of miliar leukoplakia with VELScope.

Lesions with bacterial or fungal origin:
Clinical case 4. Infectious lesions
Bacteria that use different cytosole molecules exhibit a red, pink, yellow or orange fluorescence. Fungi, like for instance Candida, fluoresce in yellow or yellow-orange. This phenomenon can be used both for diagnostics and for monitoring of the results of the treatment.

Fig. 10. A and B. Tongue with chromogenic scattering. During fluorescence orange tinge is observed.
A clinical case:
A 56-year-old patient H.Y. sought help due to a painful area in the zone of the lip commissure and was examined at the Department of Oral Pathology. The medical history and the objective examination revealed a lesion of about 2 sq.cm in size, with a nodular appearance in the centre and fissures in the periphery. The examination with VELScope showed a strong loss of fluorescence inside the lesion and in the surrounding tissues. After determining the outer borders of the area with lost fluorescence, an excision biopsy was performed with a CO2 laser and sent for histological evaluation. The working diagnosis (on observation) was determined as Erythroleukoplakia.

Fig. 11. A. Macroscopic view of erythroleukoplakia. B. Macroscopic view of erythroleukoplakia with VELScope. C. Postoperative macroscopic view of erythroleukoplakia. D. Postoperative macroscopic view of erythroleukoplakia with VELScope.

RESULTS:
The results from the pathohistological evaluation reveal stratified squamous epithelium with pronounced parabasal hyperplasia, disturbed maturation and loss of polarity of the basal cells. The latter demonstrates hyperchromatic polymorphic nuclei and single mitotic figures in half of the epithelial thickness. The morphological data points to a moderate epithelial dysplasia.

Fig. 12. Microscopic biopsy results. Presence of dysplasia, hyperkeratosis and acanthosis.
CONCLUSION:

The distribution of the oral pathology, especially in the elderly polymorbid patients, has a predominantly random nature with the prevalence of one or another lesion depending on the general health condition of the patient and/or the presence of local etiological factors, which confirms the need for periodic prophylactic examinations of the risk groups by a specialist in oral pathology. The methods for early non-invasive diagnostics of the premalignant lesions can broaden the knowledge about the number, distribution and nature of the mucosal lesions in risk patients. The lack of such studies in Bulgaria and the contradictory results reported by international authors support the need for detailed research and analysis of the existing non-invasive techniques. The evaluation of the efficiency, specificity and sensitivity of these methods can give an idea of the possibility for their integration in the dental practice and encourage the use of new non-invasive and easy-to-use methods for oral screening of the risk patient groups.

While most modern technologies are used they should be approached with a critical eye and should not be used as single and independent diagnostic means but only as an adjunct to the established classical methods of diagnostics. The loss of autofluorescence seems to be the most promising non-invasive method for identification of epithelial dysplasia in the oral cavity. The presence of some non-dysplastic lesions, which can also be non-fluorescent, i.e. produce “false negative” results, is one of the disadvantages of this method. Thus the gold standard in diagnostics remains the biopsy which is supported by the complementary non-invasive diagnostic methods. The validation of the method on a large number of clinical cases will be the subject of future work, which shall assess its specificity and sensitivity in the screening and early non-invasive diagnostics of the premalignant oral lesions.

REFERENCES:

12. Bouquot JE, Ephros H. Erythroplakia: the dangerous red mu-

Received: 09/11/2017; Published online: 02/04/2018

**Address for correspondence:**
Nikolay Veselinov Nikolov,
Department of Periodontology, Oral Pathology Department, Faculty of Dental Medicine, Medical University, Plovdiv
3, Hristo Botev Blvd., 4000 Plovdiv, Bulgaria
E-mail: dr_n.nikolov@abv.bg


