OPTICAL AID METHODS USEFUL TO SUPPORT AN ORAL CANCER DIAGNOSIS

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Oral cancer represents a significant challenge in the field of public health and medicine. In western countries, it constitutes from 6% to 10% of all malignant tumors. It is more frequent in males than in females. 90% of mouth cancers are squamous cell carcinomas (OSCC) and most cases occur in subjects over the age of 40, with an average age at diagnosis of about 60 years (Figure 1).

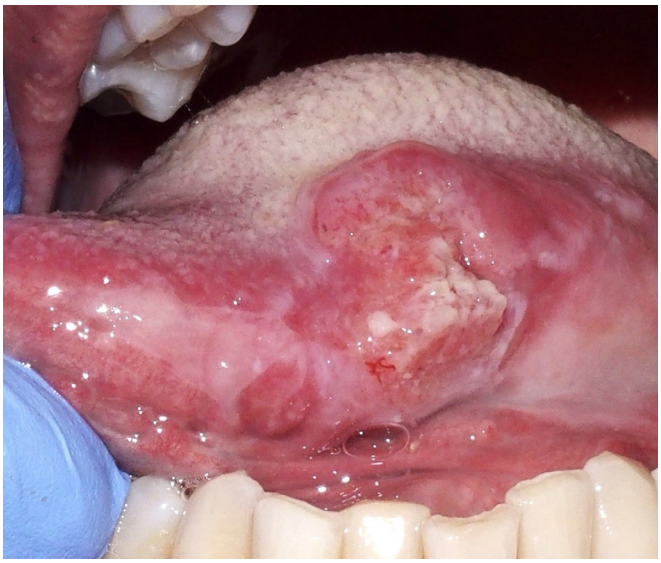

Figure 1.
Carcinoma squamocellulare della lingua in stadio avanzato

Significant risk factors for oral cancer include: tobacco use, alcohol consumption and the association between these two factors, which together constitute a higher risk than individual isolated factors. Other relevant risk factors are viral infections such as HPV, poor oral hygiene, poor dental conditions, chronic trauma to the mucous membranes and a poor diet.
Despite advances in combined therapeutic strategies (surgery, chemotherapy and radiotherapy), the overall survival of oral cancer patients has remained around 50% in recent years with no significant improvement in the stage of disease observed at the time of diagnosis.

The importance of early diagnosis

Early detection is crucial to improving patient survival and reducing morbidity resulting from disease and treatment. At the time of diagnosis, less than 40% of patients have localized disease. In the remaining cases, the disease is already invasive and in the regional or remote lymph nodes, with five-year survival rates significantly higher than cases of localized disease.
Rapid and accurate diagnosis, thanks to technological advancement, has become crucial to achieving a favorable prognosis and managing increasingly complex therapeutic challenges.
This aspect is particularly relevant in the case of oral cancer, where early diagnosis can mean less aggressive treatment and a greater chance of survival for the patient.
In fact, the literature agrees on the importance of the clinical and pathological stage at the time of diagnosis as a key prognostic indicator of oral squamous cell carcinoma. Recent studies show that the 5-year survival rate for stage I lesions reaches up to 90%, while for stage IV lesions, survival falls below 5%.
The complete clinical examination of the oral cavity remains the fundamental procedure for the detection of lesions of the oral mucosa that could represent a pretumoral disease or a malignant tumor of the mouth. To help the clinician in the examination of oral tissue, some techniques and devices have been developed and introduced on the market that can simplify the recognition of areas of mucosal injury. The evolution of optical aids in the diagnosis of pathologies of oral mucosa marks a fundamental chapter in modern

oral medicine.
In the presence of suspicious lesions that do not heal within 14 - 21 days, the surgical biopsy with histopathological examination of the removed tissue remains the gold standard for the diagnosis of malignant and premalignant pathologies of the oral mucosa.
Methods that help visual discrimination of the oral mucosa are also useful to guide the selection of the area of the lesion on which and to perform the biopsy.
The aim of this short review is to describe the technologies available to improve the early detection of oral diseases, highlighting their usefulness in the diagnosis of mouth cancer. By combining advanced optical methods with biomarker analysis, a more comprehensive and sensitive diagnostic approach is achieved, raising awareness and preparation in clinical practice for the early detection of oral cancer. This is crucial to increased survival, reduced morbidity and allows for less invasive treatments for patients that will have less impact on their quality of life.

Optical aids for the clinical examination of oral mucosa

Since the 1950s, optical ads have been developed and introduced to the market and studied for their effectiveness in clinical use. There are different devices and techniques to improve and facilitate visual discrimination of areas of abnormal oral mucosa or with suspected lesions during conventional clinical examination of the oral cavity.
Optical aids support conventional oral examination (COE) and are used to aid early detection of areas of the oral mucosa with premalignant lesions such as epithelial and malignant dysplasia such as carcinoma. The optical aids described here are toluidine blue staining, reflective lumenoscopy, narrowband imaging (NBI) and tissue autofluorescence (OFI), which exploit the differences in optical properties between normal and transformed epithelium, enabling the improvement of discrimination between normal and altered tissue quickly and non-invasively.

Toluidine blue

Techniques for in vivo superficial staining of the oral mucosa have been proposed with vital dyes such as toluidine blue, methylene blue, Lugol solution or fluorescin.
Toluidine Blue (Mashberg's stain, BT test,), also known as tolonium chloride, is an acidophilic metachromatic dye that selectively binds to the acid components of tissues, such as sulfates, carboxylates, and phosphate radicals. This dye has a particular affinity for nucleic acids and therefore binds to the nuclear material of tissues with a high content of DNA and RNA.
In the 1960s, the technique of using Toluidine Blue was introduced as a method for in vivo staining of the malignant epithelium of the mucous membranes. While normal tissues do not retain the dye, the differences between normal and malignant cells and tissues are visualized. The dye can be used as an oral rinse of 1%, or by direct application to suspect areas, in neutral aqueous solution or weak acid solution.
Procedure of coloring with the Toluidine Blue follows these steps:

1. Rinse with 1% Acetic Acid: This first step involves the use of the solution for the "cleaning" of the site from protein residues with a solution of 1% acetic acid.
2. Applying 1% Toluidine Blue: The mucous site is then treated with a 1% solution of Toluidine Blue for about 30 seconds. This allows the dye to bind to the DNA and RNA present in the tissue.
3. Rinse with Water: Next, the sample is rinsed with water to remove excess dye.
4. Buffering with 1% acetic acid: Finally, a further treatment with 1% acetic acid is applied to reduce the background staining level. This step helps to improve the contrast and sharpness of the image.

Despite its simplicity and low economic cost, unfortunately, the studies show values of forms of sensitivity and specificity for the detection of dysplasia and oral cancer, making limited the predictive value of this technique.
However, it is useful for clinicians to select sites for incisional biopsy within large suspect lesions.

Toluidine Blue's ability to bind selectively to areas with high concentration of nucleic acids makes it one of the tools available to highlight suspect areas of neoplastic or pretumor tissue (Figure 2)

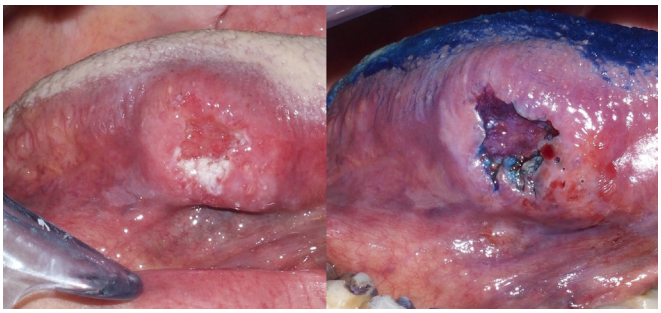

Figure 2.
Vital staining with toluidine blue of an ulcerated carcinoma of the edge of the tongue

Lumenoscopy

Another technique used to improve visual discrimination in the detection of areas of suspected lesions is reflective lumenoscopy (ViziLite®, Microlux/DL®).
This method consists of the surface treatment of the oral mucosa with 1% diluted acetic acid and examination with white-blue light (between 430 and 580 nm) to evaluate a particular chemiluminescence , the so-called "acetowhite" staining.
During the examination, the normal mucosa appears a bluish complexion, while the abnormal epithelium becomes visible as a whitish area, with lesions such as oral leukoplakia outlined with white color accentuation and edges (Figure 3).

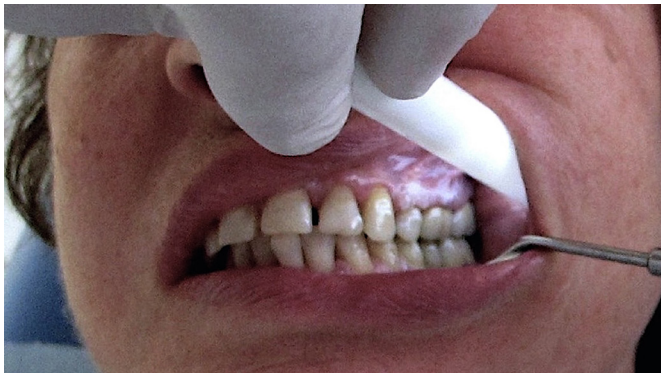

Figure 3.
Lumenoscopy reflective, with highlighted an upper gingiva leukoplakia

Lumenoscopy has a significant limitation as it fails to distinguish between different pathological conditions such as epithelial hyperplasia, dysplasia, carcinoma and inflammation. This represents an obvious disadvantage for the use of this technique as a useful diagnostic tool.

Lumenoscopy sensitivity has been estimated to be around 0.77, indicating that it has a good ability to correctly identify oral cancer patients. On the other hand, specificity is only 0.28, suggesting that the technique has a considerable limitation in excluding non-oral cancer patients, leading to a high number of false positives. Although lumenoscopy improves the visualization of white lesions, it's usefulness remains limited and scientific evidence does not support it's routine use in clinical practice, either general or specialist.
Several studies, however, have shown that short-wavelength white light is more useful than incandescent operating lights for the detection of oral squamous cell carcinoma (OSCC) and potentially malignant lesions of the oral mucosa (OPML).
In summary, while lumenoscopy represents an interesting technological development in the field of oral diagnostics, its effectiveness is limited by the low specificity and the difficulty of distinguishing between various types of epithelial abnormalities.

Narrow band imaging (NBI)

Imaging c.d. "narrow band" or NBI (Narrow Band Imaging) via illumination with multiple consistent wavelengths and their digital filtering allows discrimination of abnormal mucosal areas.
The NBI technique allows the visualization of the pattern of the superficial capillary net and the submucosal layer, exploiting the chromatophoric properties of hemoglobin present inside the vessels. The technique allows the accurate evaluation of the microvascular pattern of intrapapillary capillary loops (IPCL) and of its normal morphology or with characteristic alterations of the tumor or pretumor tissue areas and thus helps in diagnosis.
The instrument is a luminaire with a high-intensity

light source with narrow-band separation and filtration of the light beams, divided into a blue light (415nm) corresponding to the peak Soret absorption of hemoglobin, and a green light (540nm). The light beams are conveyed by means of an optical fiber and the image of the forming mucosa is detected by a camera, and subjected to computerized analysis, then displayed on a monitor.
Blue wavelength imaging allows for the visualization of superficial vascularity, while green wavelength light reveals deeper vessels and larger diameter.
In normal oral tissue there is a close interaction between the connective papillae and the epithelial ridges
(pegs net) forming uniform loops. In a neoplastic tissue this characteristic can be lost with a consequent alteration in the microvascular arrangement. In more advanced mucosal tumors, the high rate of capillary growth can be visualized by NBI as hyperchromatic areas (Figure 4).

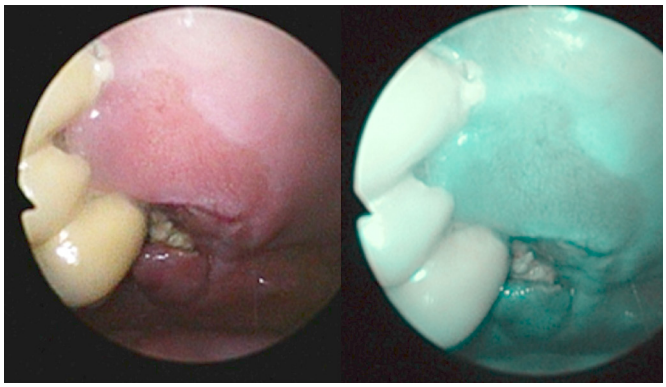

Figure 4.
Narrow band imaging; the image processed on the right improves the visualization and highlights the microvasculature of an area of dysplasia at the periphery of a gingiva carcinoma

Basically there are three characteristics visible by NBI light that distinguish a healthy tissue from a dysplasic:
1-change of vascularization given by tumor neoangiogenesis;
2- vascular destruction given by an uncontrolled proliferation;
3-displacement of the existing vascular system leading to an irregular pattern of vessels. An NBI image of high-grade dysplasia or oral cancer shows an increase in the number of vessels with altered morphology.
NBI is a safe and non-invasive endoscopic imaging method for the patient, providing the clinician with clear images for simplified therapeutic decision-making.
Apart from the high costs of the proprietary equipment Olympus I', for the usual professional dental settings, its application is more useful for the identification of areas of injury in the hollow viscera to wet surface, such as pharynx, larynx, esophagus or vaginal level; in addition, the existing projection and detection optics for the NBI technique are still poorly suited for use in the oral cavity, due to the characteristics of focus and depth of field; the technique while providing excellent results is currently more suitable for otolaryngology, gastroenterology and gynecology and urology.
Its application in these disciplines is very useful also for the definition of surgical margins during resection, in order to minimize the morbidity and the greatest possible preservation of healthy tissue.

Tissue autofluorescence

Methods to improve the optical imaging of mucous membranes and highlight oral lesions and malignant tissue include the detection of autofluorescence of the oral mucosa or fluorescence optical imaging (OFI).
This technique is used to exploit the capacity deriving from endogenous chromatophores, some molecules, so-called fluorophores, present inside cells and tissues, to emit fluorescent light radiation when light of a specific wavelength hits them.
When a tissue is illuminated with short-wavelength light, such as blue light, molecules in the epithelial cells and the connective tissue become excited and emit light at a longer wavelength, detectable by specific devices.
It is important to note that, while normal cells emit green light, abnormal cells, such as those that constitute a tumor or pretumor tissue, do not emit light, thus giving a loss of fluorescence.
The main epithelial fluorophores contributing

to normal autofluorescence are nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD), and collagen and elastin, fluorophores of own connective tonal, capable of fluorescence of green-hueyellowish when excited by a light radiation of wavelength in the range of 400-460nm. Haemoglobin, on the other hand, absorbs the exciting light, due to the presence in its composition of the heme group, thus causing a loss of autofluorescence of the mucosa in high-concentration areas. Other contributing factors include increased metabolism, increased nuclear area and pleomorphism, increased epithelial thickness, increased vascularity, breakdown of collagen cross-links, and fluorophore production by bacteria.

The clinical use of the technique is based on the concept that dysplasia and cancer cause obvious changes in the intrinsic autofluorescence of the oral mucosa. Oral carcinoma, even at an early stage, is often associated with a loss of fluorescence visualization (FVL) induced by a decrease in intrinsic tissue autofluorescence, which can be used to facilitate the detection of lesions and the selection of tissue portions for biopsy. Dysplasia and cancer typically cause a large loss of green autofluorescence, along with a small increase in red autofluorescence (Figure 5-6).

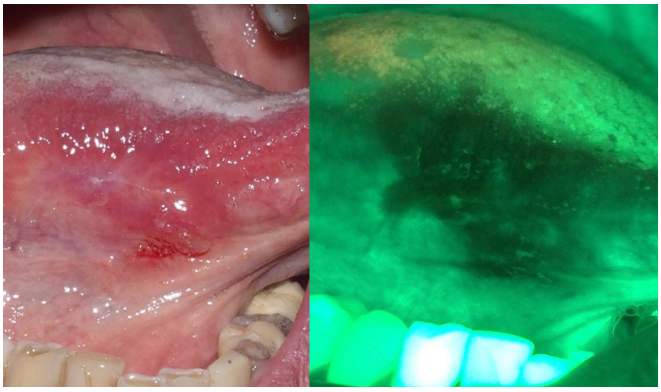

Figure 5.
Detection of tissue autofluorescence. In the photo on the right (Goccles' Pierrel) the loss of autofluorescence shows a carcinoma of the right edge of the tongue and its peripheral zone of dysplasia

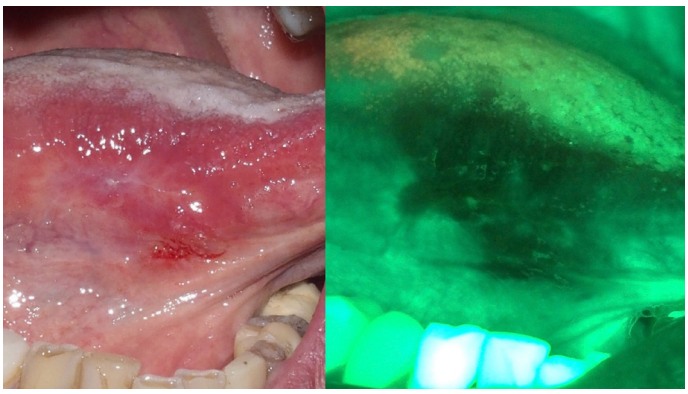

Figure 6.
Detection of tissue autofluorescence. In the photo on the right (Goccles' Pierrel) the loss of autofluorescence highlights a leucoeritroplachia with carcinoma in situ of the soft palate of the left.

Several scientific studies have been conducted and are in progress for the application of the evaluation of tissue autofluorescence of the oral mucosa with digital spectroscopic methods that employ special algorithms to evaluate the relationships between green and red fluorescence, with the possibility of arriving at correct diagnoses in over 95% of cases.

Studies evaluating the effectiveness of the detection of autofluorescence in clinical practice have shown medium-low sensitivity values due to the presence of false positives due to inflammatory lesions of the oral mucosa.

In the presence of inflammatory lesions of the oral mucosa with the use of tissue autofluorescence, false positives can be found, mainly due to high blood flow and hemoglobin concentration, with a loss of fluorescence.

The devices marketed are of various technological sophistication and ease of use, and some allow directly or indirectly the photographic detection of the result of their use in the oral cavity (VelScope®, Identafi®, Bio/Screen®, SapphireR Plus LD®, DentLight DOE®, OralID®, Lasotronix Diagnostic®, Goccles® Pierrel).

Conclusion

The techniques and methods of optical aids may assist the clinician in the detection of lesions of the oral cavity to be highlighted or occult, for the purpose of periodic screening of oral mucosa or as an aid in the evaluation of symptomatic clinical cases.

With some techniques, the detection of malignant and potentially malignant lesions can be simplified, even if no technique has shown definitive scientific evidence of sensitivity and specificity higher than the conventional oral examination.
Tissue autofluorescence detection and staining with toluidine blue are simple, inexpensive, fast and non-invasive procedures; however, interpretation of the result is closely related to operator experience and knowledge.
The methods can identify suspected lesions but are unable to distinguish between benign and malignant lesions; for this reason they must always be compared with histopathological examination.
These procedures can help the dentist and dental hygienist to improve the detection of areas of oral mucosa injury and encourage healthcare professionals to perform a more accurate examination of the oral cavity, so that we can anticipate the detection and correct diagnosis of oral cancer and premalignant lesions.

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