Advancements in oral cancer detection

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Abstract

Identifying the lesions which are suspicious has attained great attention amongst the oral physicians. In this review, we provide an update on most recent insights into cancer diagnostics. To facilitate a better systematic approach, these newer methodologies help to ensure that patients receive a prompt diagnosis and adequate treatment.

Introduction

Early detection and prompt treatment offer the best hope to the patient with oral cancer, providing the best chance of cure. The common procedure for oral pre cancer and cancer detection include visual inspection, later confirmed by biopsy. An early detection of these cancers helps in better and faster treatment for improving the prognosis to some extent and the available advanced diagnostic adjuncts aid as a helpful tool for the early diagnosis of oral cancer to the medical practitioners in treating patients suffering from it. The conventional histopathology based on light microscopy, however, has recently been complemented with ultrastructure, immunohistochemistry (IHC) and molecular diagnostic methods.1 Hence, techniques which can distinguish between different lesions in a reliable and noninvasive way would be very beneficial and also avoid under diagnosis and the need of repeated biopsies. Non-subjective biological parameters such as tumour ploidy, cell proliferation and hormone receptor status can provide more precise diagnostic and prognostic information. Oncologic imaging is undergoing remarkable advances. Techniques like autofluorescence spectroscopy and imaging can assist in detecting and classifying lesions even before clinical manifestations of certain lesions. Several authors have demonstrated that imaging systems that record the spatial distribution of tissue fluorescence at specific excitation/emission wavelength combinations can be used to survey large areas of oral cavity mucosa to non-invasively detect early changes associated with oral cancer in real. Results
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from 50 biopsies taken from areas with loss of fluorescence in 44 patients showed a sensitivity of 98% and specificity of 100% for discriminating normal tissue from severe dysplasia, carcinoma in situ, or invasive carcinoma, using histology as the gold standard. An important finding was the ability of fluorescence visualization to aid clinicians in identifying early neoplastic lesions that were initially missed during traditional white light examination.

There has been a dramatic increase in the development of many potential diagnostic techniques for oral cancers in the last few years and yet many researchers are on the look out for better and faster aids for diagnosing these lesions. This review is an attempt to put more light into the available data in this field.

Diagnostic aids in cancer detection can be classified under the following headings:

A. Clinical Methods

2. Vizilite
3. Colonoscopy
4. VELscope

B. Photo Diagnosis

1. Fluorescence endoscopic imaging
2. Autofluorescent spectroscopy
3. Fluorescent photography

C. Pathology Methods

1. Exfoliative cytology
2. Oral CDX system
3. Imprint Cytology

D. Molecular Methods

1. Quantification of Nuclear DNA content
2. Tumor Markers
3. Microsatellite markers

A. Clinical Methods

Vital Staining

Niebel and Chomet were the pioneers who used dye material to detect oral cancer in 1964. In vivo studies using these stains reveal cytological details that might otherwise not be apparent. However, staining can also reveal where certain chemicals or specific chemical reactions are taking place within cells or tissues and thus aid in accelerating biopsies, diagnosis and treatment.

1. Methylene blue

The earliest technique developed by Paul Ehrlich in 1885 involved the immersion of freshly removed tissue in methylated blue. A set of MB dye system includes two bottles of solution, viz., dye rinse solution (Bottle A) containing an active ingredient 1% methylene blue, with the addition of 1% malachite, 0.5% eosin, glycerol, and dimethylsulfoxide. Pre- and post-rinse solution (Bottle B) contains 1% lactic acid, flavoring agents, and purified water.

Staining procedure: All subjects are supposed to rinse their mouths with Bottle B solution, after brushing their teeth, for twenty seconds to remove food debris,
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excess saliva and to provide a consistent oral environment. The mucosa of the target area is gently dried with gauze and power air sprayed to ensure that the lesion is not contaminated with saliva. Patients are asked to gargle and rinse the mouth with 1% methylene blue dye (Bottle A) for twenty seconds, and then expectorate. In order to remove the excess dye, Bottle B solution is given again for rinsing for twenty seconds. The pattern of dye retention is assessed by the intensity of stain retention on the lesion.

For equivocal staining, Bottle B solution is applied with cotton rolls to wipe out the staining surface. If the patient has a highly suspicious lesion that is not all stained by the solution, the patient is instructed to revisit within fourteen days to repeat the test in order to reduce the false-negative rate. 6

Interpretation

Local, stippled, patchy and deep blue stains are marked as positive reaction.

Wide, shallow or faint blue stains are marked as negative reaction.

2. Lugols iodine

The stain was introduced by the Italian Camillo Golgi. Two grams of iodine and four grams of potassium iodide in 100 cc of distilled water form the LI solution. The selective character of staining intact mucosa with LI is dependent on the glycogen content present in the normal epithelium; this selective character of staining helps in delineating the inflammatory or carcinomatous epithelium from the normal epithelium, where the glycogen content is low. 7,8,9

Double staining technique helps in delineating the inflammatory lesions and is the main means in determining clinically, the degrees of differentiation of malignant lesions, as poorly differentiated malignant lesions without glycogen content fail to show LI retention. Toluidine blue (TB) with LI can be used for a pre therapeutic assessment of biologic aggressiveness of the disease. Depending on the retention of the dyes, the biopsy site can be determined. 10

The use of TB and LI serves as a useful adjunct in the diagnosis in patients, who are at risk and for selecting the site for biopsy with wide field cancers, prior to treatment. 11

Staining procedure: After recording the clinical features and photography of the clinically suspicious lesions, the lesion areas are applied prior with 1% acetic acid, using cotton bud for 20 s and further rinsed with water. LI is applied with a cotton bud for 10-20s and another photograph is taken.

Interpretation

- Brown stain is considered as positive for lesions.
- Lesions without any stain retention can be considered as negative.

3. Toluidine blue
Toluidine blue (TB) was first applied for in vivo staining by Reichart in 1963 for uterine cervical carcinoma in situ. Neibel, Chomet, Shedd and co-workers were the first to report vital application of TB for the detection of premalignant and malignant lesions of the oral cavity. They confirmed the property of TB to verify clinically suspicious lesions as neoplastic, to delineate margins of premalignant and malignant growth, and to detect unnoticed or satellite tumors.\(^5\)

TB is used based on the fact that dysplastic and neoplastic cells may contain quantitatively more nucleic acids than normal tissues. Also, malignant epithelium may contain intracellular canals that are wider than normal epithelium, which may facilitate penetration of the dye.\(^{13,14,15}\) The other views about the uptake of TB in dysplastic and carcinomas include the high density of nuclear material, loss of cell cohesion, and increased mitosis.\(^{12}\)

TB is generally prepared in 1% concentration for oral application. A 100 mL of 1% TB consists of 1 gm TB powder, 10 mL of 1% acetic acid, 4.19 mL absolute alcohol, and 86 mL distilled water to make up 100 mL. The pH is usually regulated to 4.5.\(^{17}\) The technique of application usually involves rinsing of the mouth twice with water for 20 s to remove debris. And 1% acetic acid is then applied for 20 s to remove ropey saliva. This is followed by 1% TB application for 20 s either with cotton swab when a mucosal lesion is seen or given as rinse when no obvious lesion is detected. Again, 2 rinses with 1% acetic acid are performed to reduce the extent of mechanically retained stain. Finally the mouth is rinsed with water.\(^{17}\)

Interpretation is based on the color; a dark blue (royal or navy) stain is considered positive, light blue staining is doubtful and when no color is observed, it is interpreted as negative stain. Under normal conditions, nucleated scales covering the papillae on the dorsum of the tongue as well as the pores of seromucinous glands in hard palate are frequently stained with TB.\(^{19}\)

Lingen et al\(^{20}\), in their review, mentioned the sensitivity and specificity of TB in the detection of oral cancer to be in the range of 78-100% and 31-100%, respectively. The evaluation of TB staining for detection of oral premalignant lesions and carcinoma in animal models showed high false-negative results in premalignancies (95.2%) raising doubt on whether TB is sensitive enough for the detection of premalignant lesions. In contrast, it was found that in vivo staining with TB is highly sensitive in detecting carcinoma.\(^5\)

**Vizilite**

Vizilite is a nontoxic chemiluminescent light used for visualizing oral mucosal lesions after acetic acid mouth rinse. It is known by the name oral lumenoscopy.\(^{23}\) One of the studies revealed that Vizilite can be used as a general oral mucosal examination system and may in particular improve the visualization of leukoplakias.\(^{24}\) Vizilite Plus technology helps in identifying soft tissue abnormalities which, shows a
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Glowing that is different from that of normal tissue thus making it more visible. Oh and Laskin in 2007 recommended the use of dilute acetic acid rinse and observation under Vizilite for early detection of oral cancers. The Vizilite system has a sensitivity of 100% and specificity ranging from 0-14.2%. The Vizilite system has a sensitivity of 100% and specificity ranging from 0-14.2%.

**Colposcopy**

The main purpose of colposcopy is to detect intraepithelial and early neoplastic lesions of the cervix, vagina and vulva. Most of the times however, a colposcopic examination is indicated as an integral part of every gynecologic examination in concert with cytological examination to further investigate a cytological abnormality on pap smears.

Procedure: On application of saline on abnormal epithelium, it appears much darker than its normal counterpart. Using a filter of blue or green colour and high power magnification, abnormal vascular patterns can be evaluated. Then 5% acetic acid is applied to the lesion for sixty seconds. A grid is placed over the lesion and area estimated to have the most extensive cell changes based on colposcopic criteria such as vascular pattern, intercapillary distance, surface pattern, color tone and opacity, as well as the clarity of demarcation of the mucosal lesions can be selected for biopsy.

**Interpretation**

In relation to normal oral mucosa, two basic types of capillary networks can be seen with direct intraoral microscopy (colposcopy): hair pin capillaries and network capillaries.

In areas of dysplasia and carcinoma-in-situ, a specific vascular pattern, punctation (previously called ground), is seen commonly. Punctuation is characterized by dilated, often twisted, irregular and hairpin-type vessels.

Another pattern of the vessels in dysplasia is called mosaic. If the vessels do not reach the epithelial surface but extend only partially into the epithelium, they appear as red lines as surrounding blocks of epithelium. The colposcopic image resembles tiles of a floor. After application of acetic acid, this pattern is accentuated because of acetowhiteness of the atypical epithelium, forming a honeycomb pattern. True mosaic vessels are usually seen in sharply demarcated areas.

When it is difficult to describe the pattern of the vessels, the term atypical vessels is used. Capillary, punctation, mosaic, or atypical patterns are encountered in malignant lesions. Therefore, if one of them is present, it is an indication for biopsy and histopathological examination.

**VELscope**

The VELscope, recently introduced to the market as a diagnostic adjunct for oral cancer detection, is a hand-held device that was approved by Federation Dentaire Association for the direct visualization of auto fluorescence. The VELscope consists of a light source, which emits an intense light.
excitation blue light (400-460 nm) and a hand piece with a selective filter for direct visualization. A case reported by Poh et al. suggests the potential utility of VELscope in the operating room in assisting determination of the surgical margins of oral cancer.

The use of VEL scope is safe and simple; the entire examination can be done in about two minutes. However, it is a relatively new device and so far only a limited number of studies have been done on its effectiveness as a diagnostic adjunct for oral cancer.

Interpretation

Under the illumination of blue light, normal oral mucosa has pale green autofluorescence, while abnormal tissue has loss of autofluorescence and appears dark.

B. Photo Diagnosis

1. Fluorescence endoscopic imaging

Exogenous administration of 5-aminolaevulinic acid, a prodrug used in photodynamic therapy, leads to porphyrin accumulation in malignant and premalignant tissues. Preliminary data from the head and neck clinical trials indicate that red-to-blue intensity ratio of malignant tissue is larger than that of benign tissue due to protoporphyrin IX accumulation. It is found that the red fluorescence intensity distribution in the lesion area will be high.

An excess of exogenous ALA will initially overload the system and porphyrin intermediates will accumulate. The presence of the intermediates contributes to photosensitivity of normal cells, but these intermediates are rapidly metabolized into haem. In malignant tissue, a transcriptional down-regulation of ferrochelatase and ferrochelatase siRNA silencing causes endogenous protoporphyrin IX accumulation.

Interpretation

Normal oral mucosa exhibits blue color of the back-scattered excitation light in the fluorescence images, whereas the suspicious lesions display bright reddish fluorescence.

2. Autofluorescent Spectroscopy [AFS]

AFS is an easily applicable tool for detecting the alterations in the structural and chemical compositions of cells, indicating the presence of altered or diseased tissue. AF of the tissues is due to the presence of several endogenous fluorophores which include tissue matrix molecules and intracellular molecules such as tryptophan, collagen, and NADH. When excited by a particular wave length, these fluorophores emit fluorescence at a particular wavelength, for example, tryptophan gives maximum emission at 340 nm, collagen at 390 and NADH at 440 nm. This property of fluorescence emission at a particular wavelength is due to the biological nature of these molecules for AF spectroscopy. With respect to histopathology as the "gold standard", the diagnostic algorithm employed for delineating the normal oral tissues from all
Topical peroxide and vitamin D3 treatment for atrophic lichen planus:

A. Pathogenicity of Topical Peroxide and Vitamin D3

The use of topical peroxide and vitamin D3 in the treatment of atrophic lichen planus has been studied extensively. Topical peroxide has been shown to selectively inhibit the proliferation of keratinocytes in the outermost layers of the epidermis, leading to decreased cellularity and hyperkeratosis. Vitamin D3, on the other hand, has been shown to regulate the expression of cytokines and chemokines involved in inflammation and tissue remodeling. Together, these treatments work to improve the clinical features of atrophic lichen planus.

B. Clinical Efficacy

Numerous clinical trials have demonstrated the efficacy of topical peroxide and vitamin D3 in the management of atrophic lichen planus. These treatments are often used in combination with other therapies, such as oral retinoids or systemic immunosuppressants, to achieve optimal results.

C. Safety and Tolerability

Topical peroxide and vitamin D3 treatments are generally well-tolerated and have a low incidence of side effects. The most common adverse effects include transient irritation, erythema, and dryness, which can be mitigated by using a moisturizer or avoiding exposure to strong sunlight.

D. Conclusion

In conclusion, topical peroxide and vitamin D3 treatment is a promising strategy for the management of atrophic lichen planus. Further research is needed to evaluate long-term efficacy and to identify optimal treatment regimens.
tissue. This technique is considered accurate in evaluating surgical specimens from thyroid and parathyroid, breast cancer margins, sentinel lymph nodes and prostate but its role in the evaluation of resection margins of squamous cell carcinoma remains unclear.\textsuperscript{50}

D. Molecular Methods

1. Quantification of nuclear DNA content

Teeth are increasingly utilized as a source of nuclear DNA to aid identification of human remains especially when only skeletal remains are present. Teeth have been shown to survive better than any other tissue in the post mortem environment. Studies have revealed that teeth provide a preferable source of DNA over bone. Nuclear DNA is available in widely variable quantities in dentine and cementum. The quantity of DNA available in dentine is affected by age and dental disease, whereas that in cementum is not.\textsuperscript{48} Molecular techniques such as PCR can be used for its quantification.

2. Tumor Markers

Molecular techniques such as examination for abnormal protein expression, including tumor suppressor genes (TSGs) and other genetic changes can reveal neoplastic changes. Salivary testing is noninvasive, attractive and an effective alternative to serum testing. The possibility of developing home testing kits would further facilitate it as a diagnostic aid, enabling patients to monitor their own health at home and is important for those who live far from their treatment centers and especially for those at risk of developing oral squamous cell carcinoma.\textsuperscript{56}

3. Microsatellite Markers

Microsatellites are segments up to 200 bp DNA, containing variable number of tandem repeats of an identical 1-6 base pair motif, therefore known as short tandem repeats. Variations in the number of repeats give rise to multiple alleles for a given microsatellite, which can be differentiated by performing molecular investigations. Given the ease in allele haplotyping, the variety of alleles for microsatellites, and the frequency of microsatellites within the genome, microsatellites make useful markers for high-resolution ‘tagging’ of specific chromosomal regions for linkage analysis.\textsuperscript{18,21}

Conclusions

All diagnostic and adjuvant techniques for detection and diagnosis of oral malignant lesions have also the potential for early diagnosis of oral cancer and thus useful for improving treatment outcomes.

References

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