Recent advancements in the diagnosis of oral premalignant and malignant lesions: A comprehensive review

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ABSTRACT

The diagnosis and treatment of lesions are currently based on histopathology, which is the gold standard for the diagnosis of cancer and dysplasia at the molecular level. Recently, there has been an increasing trend of optical spectroscopy methods for the detection of oral premalignant and malignant lesions and the same is highlighted in this paper. It depends on the optical spectrum derived from any tissue that contains information about the histological and biochemical make-up of that tissue. It provides tissue diagnosis in real-time, non-invasively, and *in situ*.

Key words: Advances, diagnosis, oral cancer, optical spectroscopy

INTRODUCTION

In day-to-day clinical experience, dental and medical practitioners often encounter a wide spectrum of oral mucosal lesions. Oral cancer is generally preceded by some benign lesions for a varying length of time. Individuals with precancer run a risk that is 69-times higher for them to develop oral cancer as compared to tobacco users who do not have precancer. The recognition and management of precancer, therefore, constitute a vital oral cancer control measure.[1] Currently, the most effective way of combating oral cancer is by early diagnosis, followed by adequate treatment. The clinician's dilemma is differentiating cancerous lesions from a multitude of other ill-defined, controversial, and poorly understood lesion that also occur in the oral cavity. Most oral lesions are benign, but they may have an appearance that may be easily confused with

a malignant lesion and some are considered premalignant because they have been statistically correlated with subsequent cancerous changes. Conversely, some malignant lesions seen at an early stage may be mistaken for a benign change.[2]

Periodic clinical examination of the oral cavity is the mainstay for early detection of oral cancer as it has been shown to reduce mortality of oral cancer by 32% in high-risk individuals. With the aim of improving the efficiency of this diagnosis, advanced techniques are being developed to complement clinical examination and to facilitate the identification of initial carcinomas.[3] Advancements in the field of oral cancer research have led to the development of diagnostic tools at both the clinical and molecular level for the early detection of oral cancer. Clinical diagnostic tools available for the early detection of oral cancer include toluidine blue dye, Lugol's iodine, Oral CDx 1 brush biopsy kits, contact microscope, and Vizilite.[4] Several clinical studies have evaluated the efficiency of *in vivo* staining with toluidine blue and methylene blue in the detection of dysplasia and malignant lesions.[3,5] The mechanism is based upon selective dye binding by dysplastic or malignant cells in the oral epithelium. Its value is based on simplicity, inexpensiveness, non-invasive technique, and accuracy.[2]

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HISTOPATHOLOGICAL TECHNIQUES

The use of oral exfoliative cytology in clinical practice declined due to the subjective nature of its interpretation, and only a small number of abnormal cells are identifiable in a smear. The more recent application of quantitative techniques, together with advances in immunocytochemistry have refined the potential role of cytology, stimulating a reappraisal of its value in the diagnosis of oral cancer.[6] Oral cytology and morphometric staining in combination with flow cytometry is used to analyze the malignant keratinocytes in oral premalignant or malignant lesions to measure exposure to tobacco carcinogens; this helps in establishing a link to premalignant and malignant transformation before a lesion is noted.[7] Liquid-based cytology is a reliable substitute for the conventional smear in predicting the early changes of malignancy in terms of both sensitivity and specificity and for minimizing the pitfalls associated with practical preparation of the obtained cells. Kujan *et al.*, concluded in their study that brush-collected epithelial cells with liquid-based cytology is a useful tool for cytomorphological evaluation, HPV detection, and immune-cytochemistry in oral mucosa, which is simple, rapid, non-aggressive, and relatively painless. It is useful in screening programs and for the surveillance of patients with confirmed cancerous and precancerous lesions.[8]

ADVANCEMENTS AT MOLECULAR LEVEL

Nevertheless, the era of the pathologist relying entirely on the examination of tissue sections stained by histochemical methods, which is gradually being replaced over time with advanced immunologic and molecular techniques. Polymerase chain reaction has emerged as one of the most powerful technique that is used almost universally for the amplification of genes and their RNA transcripts. It is used to detect mutations in cancer-associated oncogenes, tumor suppressor genes, monoclonality in B- and T-cell lymphomas, chromosomal translocations, chronic myelogenous leukemia, and minimal residual neoplastic disease.[9] It is used as a screening technique for detection of malignant cells in human secretions in urine, sputum, and saliva by studying the low numbers of unique DNA fragments.[10]

In today's era, DNA sequencing is rapid and automatic with fluorescence-labeled nucleotides read by a laser during passage through an electrophoresis sequencing gel and parallel, tiny fiberoptic glass tubes, which contain a special polyacrylamide sieving medium for separation of fluorescence-labeled DNA fragments generated by the sequencing reaction.[11] Hybridization refers to the pairing of complementary RNA or DNA strands to produce a double-stranded nucleic acid by using radio-labeled or fluorescence-labeled DNA or RNA probe that binds to the target DNA or RNA of interest and helps in visualization. *In situ* hybridization is helpful in cancer detection and also for the study of clonality in lymphocyte populations of occult marginal zone lymphoma.[9] Flow cytometry is an important method used to analyze cell kinetics and protein expression in normal and tumor cells including growth factors, protein products of oncogenes, and markers of drug resistance such as P-glycoprotein.[9,12]

A major advancement in the quantitative study of mRNA is microarray technology known as DNA chips that helps determine the expression levels of hundreds and thousands of genes at the same time and providing a unique profile of increased or decreased gene expression in tissues. Laser capture micro dissection is a new and exciting technology for rapidly preparing relatively pure cell samples from tissue sections.[9] With the latest and rapidly expanding field of head and neck oncology, photodynamic diagnosis is relying on the optical spectroscopic properties of target tissues, at the time of measurement that is real-time, non-invasively, and *in situ*.[13] It helps to diagnose dysplasia and malignancy, performing guided biopsies, monitoring of hemoglobin tissue perfusion in free-flaps, therapeutic drug levels during chemo- and photodynamic therapy, assessment of surgical margins, and a role in sentinel node biopsy.[14] Vizilite is an easy, safe, and non-invasive technique capable of detecting early asymptomatic precancerous and cancerous lesions in the oral cavity based on the principle of chemiluminescence.[4]

OPTICAL INNOVATIONS

Autofluorescence spectroscopy is also known as laser-induced fluorescence spectroscopy/fluorescence polarization spectroscopy/time-resolved fluorescence spectroscopy/fluorescence lifetime imaging.[13] It is a non-invasive and easy tool for the detection of alterations in the structural and chemical compositions of cells in pre-malignant lesions and malignant tumors. Autofluorescence of tissues is due to fluorophores from tissue matrix molecules and intracellular molecules like collagen, elastin, and NADH.[15] Topical or systemic application of photosensitizers like 5-aminolevulinic acid-induced protoporphyrin IX renders pathological tissues to fluorescence when exposed to specific wavelengths of light.[16] Onizawa *et al.*, concluded in their study that autofluorescence in oral squamous cell carcinoma correlates with the progression of lesions and fluorescent protoporphyrin are produced in association with the cancerous tissue by using high-performance liquid chromatography. Ronchese *et al.*, reported that

vivid red fluorescence is suggestive of a poor prognosis and orange or no fluorescence is suggestive of a good prognosis.[17]

Ratio imaging compares a photochemical or its metabolic end-product, which is increased in disease states, to another intracellular compound that is known to be depleted in the same diseased state. Aminolaevulinic acid converted to protoporphyrin IX fluoresces red after excitation with blue light. The same excitation light results in green fluorescence of molecules such as NAD and FADH, which become depleted in high metabolic states. The red: Green ratio is important in diagnosing degrees of dysplasia and malignancy.[13] Elastic Scattering Spectroscopy is an emerging technique that generates a wavelength-dependant spectrum, which reflects structural and morphological change within tissues at scattering centers like the nucleus, chromatin concentration, sub-cellular organelles, structural proteins, lipids, and erythrocytes. It is fast, reliable, cheap, non-invasive diagnosis, *in situ*, and real-time. It is used for the diagnosis of malignancy, monitor chemotherapy levels, free-flap oxygenation levels, and to assess surgical margins and regional lymph nodes intra-operatively.[18] Jerjes *et al.*, concluded that elastic scattering spectroscopy technique is 98% sensitive and 68% specific to lymph nodes in comparison with histopathology on formalin-fixed neck dissection specimens.[19]

Optical coherence tomography is a new high-resolution optical technique that enables minimally invasive imaging of near-surface abnormalities in complex tissues, also known as confocal microscopy and Optical Doppler Tomography. It is based on low-coherence interferometry using broadband light to provide cross-sectional, high-resolution subsurface tissue images.[16] Raman spectroscopy is very sensitive and real-time imaging at molecular level, also known as vibrational spectroscopy/ultraviolet/visible/near-infra-red/infra-red based on vibrational energy levels of tissue molecules by discrete frequencies above and below the incident photon that emerge after interaction with tissue.[13] Electrical impedance spectroscopy helps monitor the effects of chemo- or photodynamic therapy during the treatment and follow-up by insertion of probes into the tissue under consideration.[20]

Multiphoton Excited Fluorescence is a nonlinear, high-resolution optical method used in a variety of biological imaging applications by two-photon interactions that result in second-harmonic generation and two-photon excited fluorescence.[16] Nuclear magnetic resonance spectroscopy is sensitive by proteins linkup to DNA that allows the three-dimensional study of atoms in a molecule and helps in surrounding gene transcription and signal transduction.[13]

Table 1: Recent advancements in the diagnosis of oral malignant lesions and their summarization	
Diagnostic advancements	**Methodology in brief**
Oral cytology and morphometric staining with flow cytometry.[7]	Analyze the malignant keratinocytes in oral premalignant or malignant lesions to measure exposure to tobacco carcinogens
Liquid-based cytology[8]	Cytomorphological evaluation, HPV detection and immunocytochemistry in oral mucosa
Polymerase chain reaction[9]	Amplification of genes and their RNA transcripts to detect mutations in cancer-associated oncogenes, tumor suppressor genes, chromosomal translocations
Laser induced fluorescence[11]	Special polyacrylamide sieving medium used for separation of fluorescence labeled DNA fragments by the sequencing reaction
In situ hybridization[9]	Radio-labeled or fluorescence-labeled DNA or RNA probe binds to the target DNA or RNA that helps study clonality in lymphocyte populations of occult marginal zone lymphoma
Microarray technology[9]	Providing a unique profile of increased or decreased gene expression in tissues
Vizilite[4]	Detection of precancerous and cancerous lesions in the oral cavity based on the principle of chemiluminescence
Autofluorescence spectroscopy[15]	Alterations in the structural and chemical compositions of cells in pre-malignant lesions and malignant tumors based on autofluorescence of fluorophores from tissue matrix molecules and intracellular molecules like collagen, elastin, and NADH
Ratio imaging[13]	Compares a photochemical or its metabolic end-product, which is increased in disease states, to another intracellular compound known to be depleted in the same diseased state
Elastic scattering spectroscopy[18]	It generates a wavelength-dependant spectrum that reflects structural and morphological change within tissues at scattering centers like the nucleus, chromatin concentration, sub-cellular organelles, structural proteins, lipids, and erythrocytes
Optical coherence tomography[16]	It is based on low-coherence interferometry using broadband light to provide cross-sectional, high-resolution subsurface tissue images
Raman spectroscopy[13]	Based on vibrational energy levels of tissue molecules by discrete frequencies above and below the incident photon that emerge after interaction with tissue
Multiphoton excited fluorescence[16]	Biological imaging applications by two-photon interactions result in second-harmonic generation and two-photon excited fluorescence
Nuclear magnetic resonance spectroscopy[13]	Proteins linkup to DNA that allows the three-dimensional study of atoms in a molecule and helps in surrounding gene transcription and signal transduction
Immunohistochemistry[21]	Immunohistochemical detection of tumor-associated cancer cells produce telomerase enzyme that maintains telomere length and does not present in normal adult cells. Its detection in precancer may serve as a biomarker for high-risk lesions

IMMUNOLOGICAL MEASURES

Immunohistochemistry has provided insight into tumor histopathogenesis and contributed to more accurate determination of patient prognosis with various advantages, i.e., it can be performed on routinely prepared tissue sections linking morphology with immunologic phenotype, sensitive to detect antigens expressed at relatively low levels, antibody-antigen binding is very specific, equipment costs are low, and small laboratory space with technique is relatively simple and easily learned. Overexpression of the cell cycle-associated oncoproteins cyclin D as well as underexpression of tumor suppressor proteins p53, p16, and p27 are important tumor markers in oral cancer. Immunohistochemical detection of tumor-associated cancer cells produce telomerase enzyme that maintains telomere length and does not present in normal adult cells. Its detection in precancer may serve as a biomarker for high-risk lesions.[21] In addition, various recent methods for the diagnosis of oral premalignant and malignant lesions may be quickly summarized as given in Table 1.

CONCLUSION

We are in the era of information overload of 21st century. The fields of medicine and oral medicine are changing and we have come a long way. Advances in diagnosis and staging at the molecular level are expected to affect choice of treatment and patient outcomes. Oral healthcare providers should be aware of these advances in the evaluation and diagnosis of oral premalignant lesions and squamous cell carcinoma. Therefore, potential efforts still need to be taken as far as patient management and accuracy of diagnostic methods is concerned, which will enable the society as a whole to be more productive and healthier.

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