Spectroscopy in the Diagnosis of Oral Cancer

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Abstract

Oral cancer has a remarkable incidence over the world and a fairly strenuous prognosis, encouraging further research on the prognostic factors and new techniques for diagnosis that might modify disease outcome. It is known to develop from pre-existing potentially malignant oral lesions or de novo. Identification of progressing lesion at this stage aids in early diagnosis of the severity of the disease & institution of preventive & treatment measures. Screening of patients for signs of oral cancer and precancerous lesions has relied upon the conventional oral examination & confirming it by biopsy & subsequent histopathological examination. However, they have various limitations. As the emphasis shifts from damage moderation to disease prevention or reversal of early disease in the oral cavity, the need for sensitive and accurate detection and diagnostic tools is absolutely essential. These systems rely on the fact that the optical spectrum derived from any tissue will contain information about the histological and biochemical makeup of that tissue. Such optical adjuncts may assist in identification of mucosal lesions including PML and OSCC, assist in biopsy site selection and enhance visibility of surface texture and margins of lesions and may also assist in identification of cellular and molecular abnormalities not visible to the naked eye on routine examination. The advantages of optical spectroscopy systems include diagnosis in real-time, non-invasive with high resolution surface and subsurface images.

Key words: Spectroscopy, Oral cancer, Diagnosis

Introduction

Oral cancer is the world’s sixth most common cancer, and global incidence and mortality rates are increasing. However, oral cancer is predominantly a disease of developing nations, particularly prevalent in India and other south-east Asian countries due to the habit of tobacco usage. Although patients with early disease have better chances for cure and functional outcome, most patients present with advanced tumors when treatment is more difficult, more expensive and less successful compared to earlier intervention. The best way to improve outcomes is to improve early detection and diagnosis. Conventionally it has relied upon visual examination and biopsy but inter-individual expertise in physical examination
as well as in histopathological confirmation, the
demand for human and financial resources, the
delay in time, amount of tissue gained via biopsy,
and the obligatory invasiveness of a biopsy are
factors affecting early diagnosis and thus adequate
and early treatment of cancer. Recent research has
demonstrated that optical methods can present a
viable approach for improving screening and
detection of oral malignancies. A number of bio-
optical methods are available which show the
trend towards an in situ identification of
pathological changes in clinical practice.

Methods

A web-based search for all types of articles
published was initiated using Medline/PubMed,
with the key words such as oral cancer, prognostic
factors of oral cancer, diagnostic method of oral
cancer, and imaging techniques for diagnosis of
oral cancer. The search was subsequently refined
to spectroscopy technique for head & neck
cancers. The sites of specialized scientific journals
in the areas of oral and maxillofacial pathology,
oral medicine, and oncology were also used.

Discussion

Light-based systems that are based upon the
assumption that abnormal metabolic or
structural changes have different absorbance and
reflectance properties for lights. Increased interest
in optical systems using tissue spectroscopy in now
been established to obtain diagnosis. Spectroscopy
refers to the use of visible light dispersed according
to its wavelength. It explores the optical
phenomenon resulting from the interaction of light
with biological tissues. Optical spectroscopy has
the potential to detect malignant lesions earlier,
before they become macroscopically visible, by
probing tissue biochemistry & morphology in vivo
in real time.

Different spectroscopic techniques are utilized
for the detection of premalignant oral lesions &
oral malignancies.

1. Fluorescence Spectroscopy – It is a type of
electromagnetic spectroscopy which analyses
fluorescence from a sample. It involves using a
beam of light, that excites the electrons in
molecules of certain compounds & causes
them to emit light of lower energy typically but
not necessarily visible light. In case of
malignancy there are changes in the physical
and chemical characteristics of the tissues due
to the subcellular architectural changes in
cancer, such as nuclear grade and nuclear to
cytoplasm ratio, mitochondrial size and density,
amount of keratin, and elastin to collagen
ratio, and it is well known that all tissues
fluoresce and malignant tissues fluoresce less
than normal tissues, they have different
spectral characteristics. Commonly detected
fluorophores in living tissue include NADH,
collagen, elastin & cofactors such as flavins
(FAD, FMN). The fluorescence can either
occur as autofluorescence (if induced by UV
light) or as a laser induced phenomena. Dysplastic & malignant tissues have different
spectral characteristics as they tend to show
increased red fluorescence & decreased green
fluorescence. Significant increase in the
red/green fluorescence ratio is an accurate
predictor of dysplasia or malignancy. Optical
fibres may be introduced into the tissues
through a hollow needle; the tissue signals
are interpreted by spectrometers. The
reported sensitivity in fluorescence
spectroscopy technologies was up to 81% and
specificity was 100%. Autofluorescence
studies using peak intensity at 337 nm showed
sensitivity of 88% & specificity of 100%. Van
Staveren found a sensitivity of 86% &
specificity of 100% while distinguishing oral
dysplasia from normal mucosa using
autofluorescence. Light-induced fluorescence
spectroscopy can distinguish between benign
(normal and hyperkeratosis) and dysplasia
with a sensitivity of 92% and a specificity of
95%.

2. Enhanced Dye Fluorescence - Photodynamic drug can be applied which
enhanced porphyrins such as protoporphyrin
IX (protoporphyrin IX is an important
precursor to biologically essential prosthetic
groups such as heme, cytochrome C, and
chlorophylls). The fluorescence is slightly
enhanced by using exogenously applied
fluorescent drugs (e.g., 5-aminolevulinic acid
induced protoporphyrin IX). Recent advances
include the possibility to extract true spectra of
single fluorophores (chemical compound that can reemit light upon light excitation) by mathematically eliminating the undesired influences of scattering and absorption. As well, tumour-specific enzymes are about to be specifically targeted by fluorescent markers “smart probes” in order to improve both sensitivity and specificity. Ebenezar stated that the diagnostic algorithm based on discriminant function scores obtained by fluorescence excitation spectroscopy (FES) method was able to distinguish well differentiated squamous cell carcinoma from normal lesions with a sensitivity of 100% and specificity of 100%.  

3. **Elastic Scattering Spectroscopy** - The system uses a wide band of wavelengths from 400 nm up to 700 nm and recovers the scatter power, scatter amplitude, and absorption species from the reflectance from a 100 micron spot, allowing imaging of tissue at high frame rate. Thus the ESS is the optical signature of the tumor which greatly depends on the morphology of the tumor & the acquired data reflects both the scattering and absorptive properties of that tissue. The structures that induce the scattering are the nucleus, chromatin concentration & subcellular organelles. The probe of the system should be in contact with the tissues, and no light is collected from the surface reflections. Lovat et al stated that the sensitivity was 92% and specificity was 60% and it differentiated high risk sites from inflammation with a sensitivity and specificity of 79%. Elastic scattering spectroscopy recordings from normal and OSCC tissue may differ and studies on patients with leukoplakia have shown a sensitivity of 72.7% and specificity of 75% in differentiating cancer and dysplasia from benign lesions. Assessment of nodal metastases had a sensitivity of 98% and specificity of 68%, but false positives were found in 40%. When used to measure the extent of invasion in the mandible, sensitivity was 85% and specificity 80%. Muller et al used this to differentiate between various tissues & found the accuracy for normal to be 91.6%, abnormal 97%, dysplasia 64.3% & carcinoma 50 % when compared with histopathology. There are 2 main limitations: a lack of data on oral lesions and a lack of correlation between the sites investigated using optical and surgical methods. It’s other clinical applications include biopsy examinations, measurement of surgical margins, measurement of oxygen saturation in tissues, measurement of uptake of photosensitiser for photodynamic therapy & measurement of concentration of drugs or chemotherapy in tissues.

4. **Raman Spectroscopy** - It is a laser-based technique that enables chemical characterization and structure of molecules in the sample. Within biological tissues 4 principle components contribute to the spectra; water, lipids (cell membrane), nucleic acids (DNA, RNA) and proteins (hormones). The resultant spectra from these structures give a characteristic picture of that tissue. Operator can alter the wavelength & hence can probe into differing depths within the tissue due to different wavelength penetration. It thus helps to obtain a vibrational spectroscopic picture of the tissue content, thus provide immediate real time histology. The reported sensitivity of this technique was of 80.5% and specificity of 86.2%. The major disadvantage is that it’s a complex procedure & expensive too.

5. **Trimodal spectroscopy** - It uses three independent optical diagnostic techniques (fluorescent spectroscopy, Raman spectroscopy and elastic scattering spectroscopy) to achieve better results, reaching sensitivity and specificity of 96% in differentiating between normal oral mucosa and dysplasia and OSCC and a sensitivity of 64% and specificity of 90% in distinguishing between dysplasia and OSCC. Tri-modal spectroscopy, although having the advantage of being accurate is however, expensive and time-consuming.

6. **Ratio Imaging** - This technique compares a photochemical or end metabolic product which is known to be increased in disease status to another product which is known to be depleted. The 5-aminolevulinic acid enhances
protoporphyrin IX which fluoresces red after excitation with blue light. The same excitation results in green fluorescence of molecules such as NAD and FADH, which are depleted in high metabolic rate of malignant tissues. Shin stated that the sensitivity of the fluorescence imaging techniques ranged from 60 to 97% and specificity from 75 to 99%.18

7. **Differential Path-Length Spectroscopy (DPS)** – Differential path-length spectroscopy is a recently developed fibreoptic point measurement technique that measures scattered photons that have travelled, and their predetermined path lengths are measured. The spectrum is analyzed mathematically and is translated into a set of parameters that are related to the microvasculature and to the intracellular morphology. The reported sensitivity was 69% and specificity was 85%.19

8. **Nuclear Magnetic Resonance Spectroscopy**- This technology allows three-dimensional study of atoms in the molecule; the larger the magnet, the more sensitive the device. It is possible to view the way of protein link up to DNA.

9. **Infrared Spectroscopy**- It distinguishes different biomolecules by probing chemical bond vibrations and using these molecular and submolecular patterns to define and differentiate pathological from normal tissues.

10. **Magnetic Resonance (MR) Spectroscopy**- It is a non-invasive diagnostic test for measuring biochemical changes in the tumors. While magnetic resonance imaging (MRI) identifies the anatomical location of a tumor, MR spectroscopy compares the chemical composition of normal tissue with abnormal tumor tissue. MR spectroscopy is conducted on the same machine as conventional MRI. The MRI scan uses a powerful magnet, radio waves, and a computer to create detailed images. Spectroscopy is a series of tests that are added to the MRI scan to measure the chemical metabolism of a suspected tumor. MR spectroscopy analyzes molecules such as hydrogen ions or protons. Proton spectroscopy is more commonly used. There are several different metabolites, or products of metabolism, that can be measured to differentiate between tumor types. MRS provides biochemical information of compounds present in human tissue and cells. Human brain contains hundreds of metabolites, but the proton MRS can only detect a few of them as the least millimolar concentrations are necessary for the metabolites to be detected. Various metabolites detected are choline (Cho), creatinine (Cr), N-acetyl aspartate (NAA), lactate, myoinositol, glutamine and glutamate, lipids, and the amino acids leucine and alanine. The frequency of these metabolites is measured in units called parts per million (ppm) and plotted on a graph as peaks of varying heights. By measuring each metabolite’s ppm and comparing it to normal tissue, the type of tissue present can be determined. MRS demonstrates chemicals or metabolites within cancers that can be used as biomarkers to identify cancer and explore changes associated with hypoxia and cancer treatment. Of these, lactate is been found in metastatic nodes from HNSCC and has the potential to be used in the assessment of cancer hypoxia for assessing head & neck cancers.4

**Conclusion**

Early detection of oral cancer is one of the most efficient ways to reduce the morbidity of the disease and its treatment. The information & the diagnostic potential provided by spectroscopic techniques allow us to understand the changes taking place during the onset of the disease & its subsequent progression. It not only helps in differentiating between normal & dysplastic mucosa but can also help in monitoring treatment & potential complications. They provide diagnosis in real time & are non-invasive. All imaging techniques presently require large multicenter controlled trials to determine the sensitivity & specificity of various spectroscopic techniques available to assess & improve their ability in detecting and management of potentially malignant & malignant oral lesions.
References


