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Bio-optical imaging: A Paradigm Shift in Early Detection of Oral Cancer

Esha Mukherjee[1], Aumiyo Das[2]
[1]*BDS, Nair Hospital Dental College, Mumbai, Maharashtra*
[2]*Final year BDS, Nair Hospital Dental College, Mumbai, Maharashtra*
Corresponding Author: Esha Mukherjee
C/2904, Azziano Building, Rutomjee Urbania, Off Eastern express highway, near majiwada junction, thane west, Mumbai 400601, Maharashtra, India Phone no +91 9821524742

ABSTRACT:- Oral cancer is a major cause of morbidity and mortality throughout the world. It accounts for only 2% of the overall incidence of cancer but is associated with a mortality rate of 50%. This can be attributed to the fact that oral malignancies are usually detected at their advanced stages. Hence, a significant turnover of statistics can be expected following the introduction of methods to detect this deadly disease at its inception. Bio-optical imaging is a promising imaging modality which can help us in early detection of pre-cancerous and cancerous lesions. The various imaging methods discussed in our review are Autofluorescence Spectroscopy, Fluorescence diagnosis, Molecular imaging probes, Surface enhanced Raman spectroscopy, Laser confocal endomicroscopy, Optical coherence tomography and confocal reflectance microscopy. Autofluorescence spectroscopy uses the intrinsic fluorescing properties of molecules like NAD, FAD, collagen, and elastin to detect molecular changes associated with neoplasia. Fluorescence diagnosis makes use of externally applied fluorophores like 5-Aminolevulinic acid unlike the former which uses the native fluorescence. Several molecular imaging probes have been introduced to detect and quantify biological processes at cellular and subcellular levels, the commonest of all being gold nanoparticles and nanoshells. SERS is used to differentiate between normal and dysplastic tissues by the differences in the vibrational bands that are recorded. It also helps in cancer detection using saliva as a diagnostic fluid. Laser Confocal endomicroscopy and Optical Coherence Tomography are non-invasive biopsy techniques which provide sufficient details for early detection of neoplastic changes. CRM provides high-resolution images similar to histopathological studies and can hence help in the definitive diagnosis of oral cancer. The aim of this article is to make the clinicians aware regarding the latest diagnostic modalities so that early dysplastic changes and/or suspicious lesions are not ignored and management of cancer begins at the earliest.

KEYWORDS:- Oral Cancer, Autofluorescence, Surface Enhanced Raman Spectroscopy, Laser confocal endomicroscopy, Optical coherence tomography, Confocal reflectance microscopy

I. INTRODUCTION

Cancer is a significant health problem affecting millions of people worldwide. [1] It is one of the chief causes of mortality in developing countries, accounting for 13 percent of deaths annually. [2] Oral cancer is the sixth most common cancer affecting humans. Its incidence is increasing at a dangerous rate with about 300,000 new cases per year across the globe.[3] This can be attributed to the ever-increasing consumption of Tobacco and its products which is one of the most important etiological factors. Oral cancer has a high mortality rate of 50% and a considerably low 5-year survival rate of less than 50%.[4] This alarming statistics clearly indicatesthe failure of detecting this deadly disease during its early stages. This is in spite of the fact that oral cavity is a readily accessible site for visual examination. What is probably lacking is the technology to detect neoplastic changes in an apparently normal oral mucosa and to study the malignant potential of pre-cancerous lesions.

The conventional techniques of screening and diagnosis, which involve visual inspection, endoscopic examinations, and tissue or brush biopsy procedures followed by histopathological analysis, have their own set of limitations. Firstly, they are subjective and sometimes, require the assistance of a professional (to accurately study the histopathological features). Secondly, there is difficulty in defining the exact margins of the lesion. And lastly, Tissue biopsies are invasive procedures and are painful to patients, especially in their morbid condition.[4] Hence, it is crucial to have new diagnostic methods that are quick and accurate and can assist us in reducing the burden of oral cancer by its early detection and appropriate management.

Optical imaging is the result of a constant hunt, and development of technologies which would help us in molecular characterization of cancer, leaving behind the use of conventional phenotypic markers.[1] There are various biochemical and structural transformations which take place in the epithelium and stroma, which

results in a shift in the optical and biological properties of neoplastic tissue.[4] Optical principles like endogenous and exogenous fluorescence can be used to detect these molecular alterations. There are some biomolecules which have an intrinsic fluorescing property like nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD) in the epithelium and collagen and elastin in the stroma. They exhibit excitation upon UV/visible radiation exposure. This property of the native fluorophores is used in autofluorescence spectroscopy. The quantitative and/or qualitative changes in the fluorophores due to carcinogenesis is detected by recording its emission signal.[4] Apart from the intrinsic fluorophores, even external fluorophores like 5-ALA can be applied on the surface of the suspected lesion for fluorescence diagnosis. Molecular imaging with gold nanoparticles and other biomarker targeted conjugates is a very effective optical imaging method. Cancer-specific biomarkers can also be studied with the help of Surface enhanced Raman spectroscopic technique (SERS) which uses antibody conjugated gold nanoparticles labeled with highly efficient reporter tags to analyze scattering signals. [4]Additionally, Laser confocal endomicroscopy and optical coherence tomography can be used in high-resolution imaging of oral tissues. Similarly, confocalreflectance microscopy helps in visualizing tissue morphology without the invasive biopsy technique. All the aforementioned modalities have been discussed further in this review.

II. AUTOFLUORESCENCE IMAGING

Direct visual surveillance of oral cavity is not only very subjective, it also demands good visual recognition skills of the examiner. To overcome the shortcomings of this method, digital fluorescence imaging can be used. It reduces the subjectivity and improves the accuracy of detection of pre-cancerous and cancerous changes. [5] Fluorescence imaging and spectroscopy have both been used with promising results. [6]

There are several biochemical, functional and structural transformations which take place in the neoplastic tissue and these alterations can be traced successfully with the help of endogenous fluorescence exhibited by aromatic amino acids(tyrosine, tryptophan, and phenylalanine), enzyme co-factors (NADP, FAD), porphyrins and structural proteins(collagen, elastin, collagen cross-links). [6] When the oral mucosa is subjected to UV/ visible light, these native fluorophores absorb some portion of the photons, get excited and emit lower energy photons, which isdetected as fluorescence of the mucosa. This fluorescence can then be recorded with a charged coupled device camera.[3] The Food and Drug Administration (FDA) has recently approved a new device called VELscope for direct visualization of fluorescence and detection of malignant and potentially malignant lesions. [6]

Oniwaza et al, detected fluorescence in the orange and read part of the spectrum when studying malignant lesions. This was attributed to the increase in the blood constituents, particularly protoporphyrin, which increases during tumor progression. Additionally, in many studies it was observed that along with increased red fluorescence, there was a loss of green fluorescence suggestive ofsubepithelial collagen degradation. Both of these together can be helpful in demarcation of tumor margins. [3] Though this technique helps in detecting neoplastic changes in its early stages, it has low specificity for detection of premalignant lesions and may give false positive results in many other inflammatory and benign lesions. [5] New and improved technologies to overcome these problems need to be explored to improve early cancer diagnosis. [4]

III. FLUORESCENCE DIAGNOSIS

Fluorescence diagnosis using fluorescence endoscopy is the process of topical or systemic application of tumor-selective photosensitizer to visualize the malignant lesions and demarcate the exact margins of the tumor. This helps in achieving excellent results in surgical excision and reconstruction. The most effective, out of all the photosensitizers evaluated, is 5-Aminolevulinic acid, which gets converted to Protoporphyrin IX, through the Heme biosynthetic pathway. PPIX is an intrinsic photosensitizer, which accumulates in the tumor cells and hence helps in detection of neoplastic changes. Apart from 5-ALA, another successfully administered photosensitizer is Hypericin, which also accumulates in abnormal tumor cells.[4] Both these photosensitizers exhibit a bright red fluorescence of malignant tissue against a dull blue background or normal mucosa. Though it is a very useful imaging technique especially while performing tumor resection surgeriesbut it has a few disadvantages. The molecular fluorophores may react with the available oxygen free radicals resulting in an irreversible loss of fluorescence properties. Therefore, they have low photostability. There is also a high background noise resulting from autofluorescence exhibited by the endogenous fluorophores. These drawbacks compelled researchers to look for better imaging modalities.

IV. MOLECULAR IMAGING

The goal of Molecular imaging is detection and quantification of biological processes and molecular alterations at cellular and subcellular levels. [7] It helps in studying the cardinal features associated with neoplastic changes and hence is a promising modality for early detection of malignant changes, staging of tumors and checking the efficacy of treatment modalities. [4] It requires a labeled probe which can be detected,

a ligand which has high affinity to the target, a method of amplification of signals received from the label and a high-resolution imaging system for detection of the label. [3]

Gold nanoparticles have been thoroughly studied in terms of their interaction with biomolecules and are used extensively in electron microscopic studies. [1] Colloidal gold nanoparticles illuminate neoplastic cells by providing an optical contrast between normal and dysplastic tissue. They can also be conjugated with antibodies for studying the expression of cancer-specific biomarkers. Gold nanoshells have been found to be very effective compared to the other types of nanostructures. They have a dielectric core of silica with a thin gold coating. They have extremely high scattering cross-section which is a critical property for optical imaging in living organisms. And this property is also the basis of its use as SERS nanosensors. These sensors help in studying the cellular chemistry and alterations at subendosomal resolution. [4]

Molecular imaging also helps in studying the vascular response. Vascular Endothelial Growth Factor(VEGF) is not only important in normal angiogenesis; it is also the prime target of several anti-angiogenic therapeutic modalities. VEGF expression in the endothelium increases in malignancies, and more so, in aggressive tumors. Single chain recombinant VEGF with cysteine tag is used to detect these changes. It is readily taken up by the vascular endothelium which has high specificity for the same. These single-chain VEGF probes can not only be used for cancer detection, it can also be used to design better treatment protocols. Bevacizumab is an antibody against VEGF which binds to all its isoforms and is approved for clinical application in a few systemic malignancy cases.[4]

V. SURFACE ENHANCED RAMAN SPECTROSCOPY

In 1930, Professor Raman from Calcutta University was awarded the Nobel Prize for discovering the Raman Effect, which is based on the interaction of light with matter. When photons strike the surface of matter, most of them pass through it unchanged, but certain molecules interact with these photons. This interaction results in their excitation to partial quantum state and emission of photons having the same frequency as that of incident photons. This phenomenon is called elastic scattering. Approximately 1 in 10^6 to 10^8 of photons get scattered at a different wavelength than that of incident photons, this is called Raman or inelastic scattering. And the difference in wavelength is recorded in Raman Spectroscopy. [6]

Raman Spectroscopy was found to be ineffective for surface studies as signals from the bulk overwhelms signals emitted from the surface. To overcome this problem, Surface Enhanced Raman Spectroscopy(SERS) was developed. It uses metallic nanoparticle substrates which are made to resonate with the external optical fields. Oscillating surface plasmons are created which produces local electric fields called hot-spots on the surface of the metal, with a resultant emission of strong Raman signals. It enhances the efficiency of Raman scattering by 10^4 to 10^6 fold. [4]

There are various structural and conformational transformations which take place at varying stages of dysplasia and Oliveria et al [8] demonstrated these changes by the difference in the vibrational bands of normal, dysplastic and cancerous tissue using Raman spectroscopy. SERS is also being used to study the specific biomarkers in the saliva of cancer patients. Olivio et al noticed three Raman peaks at 670. 1079 and 1627 cm^{-1} in the saliva specimens of oral cancer patients. Of particular importance, is the 1627 cm^{-1} which was also observed in other studies. [4]

Saliva as a diagnostic fluid can be advantageous in terms of accessibility and should be studied more extensively to be used in clinical scenarios.

VI. LASER CONFOCAL ENDOMICROSCOPY

Laser confocal endomicroscopy is a recent optical imaging modality that allows high-resolution imaging of tissue structures. A low-level laser is used to illuminate the tissues and the fluorescence light reflected back from the tissues is detected through a pinhole. Confocal refers to the alignment of the illumination and collection systems in the same focal plane, i.e. the laser is directed at the tissues at a particular depth and the reflected light from the tissues is detected by the same lens. The reflected and scattered light not in alignment with the pinhole is not detected. This results in high spatial resolution.This imaging modality can be based on reflectance or fluorescence. Fluorescence-based imaging requires local and/or intravenous contrast agents and produces high-quality images. [9] The various dyes which are safe for human use are fluorescein, Hypericin and 5- Aminolevulinic acid. They provide structural images of the oral cavity and help to differentiate the morphological features of normal and cancerous tissue. [4]

Haxel et al(10) used this imaging modality to study particularly five areas in the oral cavity- buccal area, tongue, the base of the tongue, floor of the mouth and tonsils. The results showed that LCM is suitable for studying the anterior parts of the oral cavity but the procedure triggered pharyngeal reflexes while studying the posterior parts hence was not satisfactory. Apart from this, there are several other shortcomings; for example, Fluorescin sodium fails to stain the nuclei, which is very important for distinguishing normal and dysplastic

tissue. Though Acriflavin can stain the nuclei, it doesn't penetrate the deeper layers of the tissue. The fluorescence signals lack specificity due to overlapping fluorescence from endogenous molecules and externally introduced fluorophores. [4] Hence further technological improvements and amendments are required to make this a more efficient imaging technique for early detection of cancer.

VII. OPTICAL COHERENCE TOMOGRAPHY

It is an in vivo 'optical biopsy' procedure which is analogous to conventional ultrasound procedure, the only difference being the use of light instead of sound. [6] It scans the topology of the tissue subsurface using a laser light in near-infrared region to a high spatial resolution of 10 to 20 micrometers. [4] It has a limited penetration depth of 1 to 2 mm but most epithelial cancers are restricted to 600 micrometers which is an ideal imaging depth for this imaging modality. Low coherence light and ultra-short laser pulses are used to measure the internal structures. The optical signal transmitted and reflected from the tissue provides information about the spatial orientation of microstructures. [11] Its application to study the orals structures dates back to 1998. It helps in studying the difference in epithelial thickness, the integrity of basement membrane, cell migration and mitotic cycle which are critical in differentiating between normal and dysplastic tissue. [12] Apart from studying the structural changes, optical Doppler tomography helps in quantifying the velocity of blood flow and other vascular changes associated with carcinogenesis. [4] 3D OCT is capable of visualizing histological features up to the depth of 2-3 mm. Studies also show a strong agreement between histopathological diagnosis and OCT diagnosis.

VIII. CONFOCAL REFLECTANCE MICROSCOPY

It provides high-resolution images of the tissue architecture by filtering out the scattered light which is not in focus. The image contrast results from the difference in the refractive index of the tissue structures. For example, the refractive index of the cell nucleus is different compared to the surrounding structures, which enables its visualization. Nuclear changes play a very important role in differentiating between normal, precancerous and cancerous tissue and these alterations can be very well appreciated with the help of Confocal Reflectance Microscopy. Apart from the nuclear changes, there are various other cytological features specific to dysplasia, for instance, large nucleoli, abnormal mitosis, increased rate of mitosis, cellular pleomorphism et cetera. And these features can be observed with this imaging modality. [13] However, when compared to paraffin-embedded histological analysis; lesser number of microscopic details are discernible in CRM.
Thus far, CRM has proved to be a promising imaging technique for screening and diagnosis of cancer, determining margins for surgical excision and monitoring the efficacy of treatment modalities. [4]

IX. CONCLUSION

There is an increasing burden of Oral Cancer across the globe. Millions of people are fighting this disease with every moment being a struggle for survival. The high mortality rates associated with this disease is due to its detection at advanced stages. The only way of combatting this deadly disease is by early detection of the premalignant lesions and malignant changes. The various imaging modalities mentioned in this review are the newer, emerging technologies which are noninvasive and effective techniques for surveillance of the oral cavity and cancer detection. Clinicians need to become more aware of these upcoming imaging and diagnostic tools and should put them in use for screening suspicious lesions and thereby improving the survival rates of those who are suffering from oral cancer and also the ones who are susceptible to it.

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***Corresponding Author:** *Esha Mukherjee*
C/2904, Azziano Building, Rutomjee Urbania, Off Eastern express highway,
near majiwada junction, thane west, Mumbai 400601, Maharashtra, India
Phone no +91 9821524742