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Original Research

Fluorescence spectroscopic study on malignant and premalignant oral mucosa of patients undergoing treatment: An observational prospective study

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ABSTRACT

Background: To evaluate the changes of oral mucosa in malignancy and pre-malignant oral conditions using fluorescence spectroscopy during various phases of treatment.

Material and methods: The study involved patients of squamous cell carcinoma of the oral cavity and the pre-malignant lesions coming for the follow up/post-operative radiotherapy. The autofluorescence spectra were recorded in vivo using a Nitrogen laser based fluorimeter. Three sites of each patient were examined-right & the left buccal mucosa and the tongue. For a given pathology, spectra from all the individuals were grouped and mean spectra after different radiation cycles were compared. The quantitative analysis of the spectra involved extraction of diagnostically relevant spectral information through Maximum Representation and Discrimination Feature.

Results: As different patients had different response to the radiation, it was difficult to visualize any particular trend with increased number of radiation cycles. However, for a given pathology and an individual, when mean spectra after different radiation cycles and surgery were compared, the observation was: Intensity of the 460 nm fluorescence band for each pathology was increased with the number of radiation cycle. That had indicated tissue was being reverted back to its grossly normal features. As 460 nm fluorescence spike was a standard spectra for normal mucosa.

Conclusion: The results strengthened the hypothesis that fluorescence spectroscopy has considerable potential for use as a tool to evaluate the response to treatment in oral malignancy. These spectra of radiotherapy and surgically treated patients can be used as standards for treated patients in further studies.

1. Introduction

Oral cancer is the world's sixth most common cancer, and global incidence and mortality rates are increasing [1].

Oral squamous cell carcinomas are the most prevalent cancers in Indian and other South Asian countries [2]. High mortality rate is often attributed to the late detection of disease. Conventional oral examination, under normal light, followed by histopathological examination to determine morphological deformity in epithelium is the gold standard for cancer diagnosis. Epithelium undergoes various macroscopic and microscopic changes during the progress towards malignancy and during its treatment especially during radiotherapy.

Optical spectroscopy helps in studying the properties of physical objects based on measuring the ability of an object to emit and interact with the light. Diagnostic techniques based on fluorescence spectroscopy have the potential to link the biochemical and morphologic properties of tissues to individual patient care [3]. In particular, these techniques are fast, non-invasive and quantitative. Furthermore, they can be used to elucidate key tissue features, such as the cellular metabolic rate, vascularity, intra-vascular oxygenation and alterations in tissue morphology [3].

Here, we used a hand-held fiber optic probe instrument based on fluorescence/reflectance spectroscopy for precise tumor delineation, which has undergone some kind of treatment. The fluorescence of a biologic molecule is characterized by its quantum yield and its lifetime [4].Fluorescence spectroscopy is the measurement and analysis of

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various features that are related to the fluorescence quantum yield and/or lifetime of a biologic molecule (s). The quantum yield is simply the ratio of the number of photons emitted to the number absorbed [3]. The lifetime is defined as the average time the biologic molecule spends in the excited state before return to the ground state. The fluorescence quantum yield and lifetime are modified by a number of factors that can increase or decrease the energy losses. For example, a molecule may be non-fluorescent as a result of a large rate of non-radiative decay (thermal generation) or a slow rate of radiative decay (fluorescence) [3].

The fluorescence intensity of a biologic molecule is a function of its concentration, its extinction coefficient (absorbing power) at the excitation wavelength, and its quantum yield at the emission wavelength [5]. A fluorescence emission spectrum represents the fluorescence intensity measured over a range of emission wavelengths, at a fixed excitation wavelength. Conversely, a fluorescence excitation spectrum is a plot of the fluorescence intensity at a particular emission wavelength, for a range of excitation wavelengths [3].

Fluorescence spectroscopy in the ultraviolet and visible spectral regions has been developed and employed to differentiate diseased from non-diseased tissues, in vivo. The altered biochemical and morphologic state that occurs as tissue progresses from a non-diseased to diseased state, is reflected in the spectral characteristics of the measured fluorescence. This spectral information can be compared to tissue histology, the current gold standard, which indicates the absence or presence and grade of disease [3].

Fluorescence spectroscopy can evaluate physical and biochemical properties of a specific oral site by analyzing the emitted fluorescence light, providing automated, noninvasive discrimination between benign and neoplastic epithelial lesions in many anatomic sites [6]. Several small clinical series [7–9] demonstrated that the fluorescence intensity from normal mucosa is generally greater than that from abnormal mucosa. Algorithms based on differences between fluorescence spectra could discriminate normal mucosa from dysplastic and carcinomatous tissue with high sensitivity and specificity [8–10]. In one study, using only a single emission wavelength of 472 nm, and 350 and 400 nm excitation, algorithm performance in the training set had 90% sensitivity and 88% specificity and in the validation set had 100% sensitivity and 98% specificity [10].

Overall, autofluorescence spectroscopy seems to be very accurate for distinguishing lesions from healthy oral mucosa, with high sensitivity and specificity, especially when malignant tumors are compared to healthy mucosa. However, the ability of the technique to distinguish and classify different types of lesion has been reported to be low [11–14].

The goal of this study is to evaluate the clinical applicability of fluorescence spectroscopy (a noninvasive technique for assessing the chemical and morphologic composition of tissue) for the in vivo detection of changes in oral cavity during treatment of neoplasia.

In the study, we had studied the changes obtained via optical spectroscope and compared the results obtained to the standard graphs set in the optical spectroscope.

The aim and objective of the study were:

To study the changes of oral mucosa in malignancy using optical spectroscope.

2. Materials and methods

An observational prospective study was conducted after institutional review board approval a total of 52 known patients of oral squamous cell carcinoma along with some form of premalignant lesions such as leukoplakia and submucosal fibrosis undergoing routine medical examination of the oral cavity at Out Patient Department were recruited in the study regardless of gender or age. Informed written consent was taken from each patient and their attendants explaining them that the procedure is painless, will take very less time and will help in assessment of their residual disease status.

Age, sex and details of tobacco chewing or details of smoking habit were recorded. The total duration of the study was 3 years. Patients were followed for a year.

The patients included in the study were known biopsy proven cases of either tongue or buccal mucosa squamous cell carcinoma. These patients were undergoing some treatment during the period of study. There were four categories of patients:

1) Some patients were diagnosed of malignancy and still not started treatment,
2) Patients underwent operative treatment only,
3) Patients underwent operative treatment and now undergoing radiotherapy and
4) Fourth group directly undergoing radiotherapy after diagnosis.

All patients first underwent a visual examination under torch light which was categorised a frank carcinoma, leukoplakia, submucous fibrosis, or normal. After visual inspection all three sites of a patient i.e. both sides buccal mucosa and tongue were evaluated by a fluorescence spectroscope. Three spectra were taken from all the three sites via a compact and portable spectroscopic system. In vivo autofluorescence spectra were measured using a compact and portable spectroscopic system. A sealed off, high pressure nitrogen laser, was used as the excitation source for inducing tissue fluorescence. Light delivery to and collection from tissue was achieved with a fibre optic probe consisting of seven 400 μm core diameter fibres arranged in a six around one configuration. The six surrounding fibres deliver laser light to the tissue surface while the central fibre collects fluorescence from the surface area directly illuminated by the excitation light. The fluorescence emission collected by the fibre optic probe was then dispersed and detected with a chip based spectrometer. For this study, the nitrogen laser was operated at 10 Hz repetition rate, 5ns pulse width, and average pulse energy of 50 ± 5 μJ at the tissue surface. An integration time of 500 ms was used for each spectral measurement. The overall spectral resolution of the system was ~5 nm. The spectral data acquisition was computer controlled.

The overhead room lights in the examining room were turned off temporarily during spectral acquisition to minimise the contribution of the ambient light in the acquired spectra. In between recordings of two patients, the fibre optic probe was cleaned and the probe just touched the mucosal surface of the patient's oral cavity, to make sure that the procedure is not painful for the patient. The patients once examined were encouraged to come for follow up so that their spectral analysis could be repeated at a different stage of treatment and further inferences could be drawn. So all in all a total of 52 patients that is a total of 156 sites and a total of 468 spectra were recorded to assess and evaluate the oral mucosal malignant changes using fluorescence spectroscopy. The work described had been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The work has been reported in line with the PROCESS criteria [22].

2.1. Observations

1) The age of the patients ranged from 33 to 65 yrs, with a mean of 40.52 yrs.
2) The overall ratio of male to female population was 3:1.
3) As far as tobacco habits were concerned, 81% of the patients were tobacco users, 8% were both tobacco and alcohol addicts, rest did not have any such habits.
4) In all the patients with oral malignancy included in the study, tongue was involved in 33% and buccal mucosa was involved in 67% of all cases.
5) Out of total 52 patients, 18 patients were also having lesions like leukoplakia and submucosal fibrosis (9 patients in each)