ABSTRACT

Oral submucous fibrosis (OSMF) is a common premalignant condition occurring in Southeast Asia and Indian subcontinent. An early diagnosis and prompt treatment with patient counseling helps in the cure of the disease.

Aim: The aim of this pilot study is to study and assess the redox ratio in patients with OSMF and normal patients.

Materials and methods: This pilot study was carried out on in 10 patients visiting the Oral Medicine Department of our institution. After a thorough history, clinical examination and incisional biopsy the proven cases of OSMF were taken up for autofluorescence study. The representative site in the buccal mucosa was chosen based on clinical examination and the site was subjected to excitation with a light of wavelength 350 and 450 nm. This corresponds to the excitation wavelength of NADH and FAD respectively. The resulting emission intensities were obtained and the redox ratio was calculated. For control, about 10 cases of age-matched patients who had the habit of tobacco usage but without any lesions were chosen.

Results: The redox ratio of patients with OSMF was 0.58 ± 0.06 and in normal patients was 0.37 ± 0.04.

Inference: The redox ratio is an indication of metabolic activity of the tissue being examined. From this study it could be concluded that an increase in redox ratio with a decreased cellular activity is seen in patients with OSMF.

Keywords: Autofluorescence spectroscopy, Oral premalignant conditions, Oral submucous fibrosis, Optical imaging.


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Conflict of interest: None declared

INTRODUCTION

Oral submucous fibrosis (OSMF) is a chronic disorder characterized by inflammation and progressive fibrosis of the lamina propria and deeper connective tissues involving the oral cavity and upper aerodigestive tract. Oral submucous fibrosis was first described by Schwartz in 1952 among five Indian females living in Kenya and he coined the term atropica idiopathica (trophica) mucosae oris. Several other descriptive terms have been attributed; submucosal fibrosis of palate and pillars, diuse oral submucous fibrosis, idiopathic scleroderma of the mouth, idiopathic palatal fibrosis and sclerosing stomatitis. The etiology of the disease over the intervening years was thought to be multifactorial and several agents have been implicated, including the consumption of large amounts of chillies, nutritional deciency, genetic predisposition and autoimmune diseases. It was earlier designated as a precancerous condition but the consensus workshop of WHO in 2005, London, redesignated these lesions as potentially malignant condition. This disease is predominantly a disease of South and southeast Asian countries where the consumption of smokeless tobacco products is very high.

The disease is easily curable in the early stages by steroids, immune modulation, proteolytic enzymes, antioxidants and biogenic stimulation provided the patient quits the habit. Though the disease can be diagnosed by clinical methods and histopathological means, an exact assessment of the metabolic events is not possible through the above modalities. Moreover, the invasive scalpel biopsy is usually carried out only in a single spot as decided by the clinical features which may not be the site with the worst changes.

The metabolic mapping offers an early bird advantage as initial carcinogenic changes always starts with the alteration of cellular metabolism before the discernible histopathological and clinical changes become evident. Neoplastic cells require an increased amount of energy to sustain their rapid replicative potential. This increase in energy demand is associated with an increased concentration of nicotinamide adenine dinucleotide reduced (NADH) and flavin adenine dinucleotide (FAD), which are primary electron acceptors and donors. The electron transport chain is the most efficient way of producing energy in the cell. This process takes place in the mitochondria where NADH and FAD act as coenzymes and help in the funneling down of energy into ATP—‘The energy currency of the cell’. Hence, the monitoring of the relative concentrations of NADH and FAD provides a glimpse of the energy production of the cell which can shed light on the metabolic changes associated with carcinogenesis. These changes may become evident a little earlier to the morphological events associated with carcinogenesis.

The biomolecules of NADH and FAD are autofluorescent and hence their concentrations can be measured rapidly, repeatedly and noninvasively. Fluorescence is a...
phenomenon in which absorption of light of a given wavelength by a fluorophores (fluorescent molecule) is followed by the emission of light at longer wavelength. By measuring the intensity of the emitted light which is a combined product of the concentration of the fluorophores, extinction coefficient (absorbing power) and fluorescence quantum yield, the concentration of the fluorophore would be known.\textsuperscript{5,6}

The most common optical method for metabolic imaging is the redox ratio which is the ratio of the fluorescence intensity of FAD to the combined sum of FAD and NADH. This optical redox ratio provides the relative changes in the oxidation-reduction potential of the cell which in turn gives a clue about the metabolic state inside the cell. The redox ratio is sensitive to changes in the cellular metabolic rate and vascular oxygen supply. A decrease in the redox ratio indicates increased cellular metabolic activity, as is typically observed in cancer cells.\textsuperscript{6}

The aim of this pilot study is to estimate and assess the redox ratio of patients affected with OSMF of varying clinical grades in the buccal mucosa and compare them with age-matched controls of normal subjects.

**MATERIALS AND METHODS**

Autofluorescence spectra were collected from 10 patients of OSMF and from 10 normal subjects who were males in the age group of 18 to 37 years. A detailed history and clinical examination was carried out and incisional biopsy of the lesion was performed to confirm the diagnosis before collecting the autofluorescence spectra. A thorough oral prophylaxis was performed 1 week before the recordings were taken. On the day of recording, the patient was asked to refrain from eating for at least 1 hour before data collection and he/she was asked to rinse his mouth with water thoroughly before data collection. The autofluorescence data was collected only from the buccal mucosa using the specified wavelengths as mentioned below. The patients affected with OSMF were classified as group 1 and normal subjects as group 2.

Autofluorescence spectra were collected using a spectrofluorometer (Fluoromax-2, ISA, Jobin Yvon-spx, New Jersey, USA). The excitation source (150 W ozone free xenon arc lamp) coupled with a monochromator which delivers light to the sample spot at a desired wavelength and the fluorescence emission from the sample is collected by emission monochromator connected to a photomultiplier tube.

The fluorescence intensity of NADH and FAD which are fluorophores by themselves were used to compute reduction and oxidation potential (Redox). The concentration of NADH was measured observing the emission intensity peak after excitation with a wavelength of 350 nm. The concentration of FAD was measured by observing the emission intensity peak after excitation with a wavelength of 450 nm. The final Redox ratio was calculated as follows:

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\text{Redox ratio} = \frac{\text{FAD intensity}}{\text{FAD intensity} + \text{NADH intensity}}
\]

where FAD intensity and NADH intensity are the emission intensity of FAD and NADH respectively.

**RESULTS**

The emission intensity peak after excitation with a wavelength of 350 nm occurred at 450 nm. This corresponds to the fluorophore NADH. The fluorescence intensity in counts per second for the molecule was entered into the proforma. The emission intensity after excitation with a wavelength of 450 nm occurred at 530 nm which corresponds to the fluorophore FAD. The two concentrations recorded separately were entered into the scoring proforma and the calculation as per the above equation was performed. These set of recording were taken for both OSMF patients (group 1) and normal patients (group 2).

In group 1 the mean redox ratio was 0.58 with a standard deviation of \pm 0.06 and in group 2 the mean redox ratio was 0.37 with a standard deviation of \pm 0.04. The values of all the patients is depicted in Table 1 and results in Table 2.

For statistical analysis, statistical package for the social sciences (SPSS) statistical software Version 19 (SPSS Inc, Chicago, IL, USA) was used and unpaired t-test was carried out which revealed that the results were statistically significant (p < 0.05).

**DISCUSSION**

OSMF is a commonly occurring potentially malignant disorder of the oral cavity in Southeast Asia. The most common reason being is the usage of smokeless tobacco products in this part of the world. The main ingredient of smokeless tobacco products is areca nut which has been designated as the fourth most common addictive substance in the world and is associated with a dependency syndrome. We have several options in the management of OSMF, such as topical applications, intralesional steroids, physiotherapy and surgical options.\textsuperscript{3} One of the important aspects in management is early and definitive diagnosis and regular monitoring of the disease activity during treatment. Though early diagnosis with OSMF is easy with a clinical examination, at present the monitoring of the disease activity under treatment is a little difficult. An ideal monitoring method should be noninvasive, rapid and easier to use. It is here the field of medical physics offers us an excellent tool called autofluorescence spectroscopy.
Autofluorescence spectroscopy is a rapid, noninvasive and easily applicable tool for the detection of alterations in the structural and chemical compositions of cells which is an ideal indicator of the disease activity. The concept of autofluorescence becomes a reality due to the presence of endogenous fluorophores like tryptophan, collagen, porphyrin, elastin, NADH and FAD which fluorescence naturally without the need for an external agent.

Fluorescence is a phenomenon in which absorption of light of a specific wavelength results in the emission of light at a higher wavelength by the fluorophores. The absorption of light by fluorophores is highly specific and there is no overlap in the absorption between the molecules. Hence, by monitoring the absorption and emission characteristics unique spectral signatures for each molecule can be drawn and their concentrations also assessed.6,8

The branch of autofluorescence spectroscopy has been extensively used in discriminating normal cells from malignant cells but studies pertaining to monitoring of disease progression are few to date.9

The most common means to monitor metabolic activity is to assess the ‘redox ratio’. The redox potential of the cell is determined by the relative concentrations of FAD and NADH which are the electron acceptors and electron donors in the electron transport chain within the mitochondria. The electron transport chain is the key component for energy production in oxidative phosphorylation by producing energy in the form of ATP by transferring electrons to molecular oxygen. This redox ratio is sensitive to the cellular metabolic rate and vascular oxygen supply. A decrease in the redox ratio indicates an increased cellular metabolic activity as is observed in cases of malignancy.5

In this study an attempt was made to compare the redox ratio of OSMF with that of normal subjects. The reason for choosing the buccal mucosa is that it is among the earliest sites to get involved in OSMF. In an earlier study carried out by Sivabalan et al in healthy subjects comparing buccal mucosa, palate tongue and vermilion border, had shown that the buccal mucosa offered the best potential to measure redox ratio with minimal variations between subjects.9

This study reveals significant elevation in the redox ratio of OSMF patients to that of the normal subjects. A higher than normal redox ratio is indicative of a diminished cellular activity and this is consistent with the fact that the collagen turnover in OSMF patients is lowered than normal.1

As previous studies have shown the fact the redox ratio tends to return to normal after the treatment, this modality may also act as an adjunct in the monitoring of the disease activity.9

CONCLUSION

The monitoring of redox ratio provides us with a window of opportunity to assess the presence of disease at several sites noninvasively. Further, it also provides us an option to continuously monitor the disease activity when under treatment and monitor the improvement of the disease in a noninvasive way. This pilot study has revealed statistically significant elevation of redox ratio in OSMF patients. Further research with a larger samples and continuous monitoring of redox ratio during therapy is mandated to improve the validity of redox ratio measurements using autofluorescent spectroscopy.

REFERENCES


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