Chapter 5

Clinical trial for detection and classification of oral lesions using diffuse reflectance spectroscopy

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5.1. Introduction

Visual identification of pre-cancerous lesions in the oral cavity for pathological investigation is a challenging proposition in outpatient clinics. The present study explores the possibility of diffuse reflectance spectroscopy (DRS) with white light illumination to discriminate normal or healthy tissue from hyperplastic and dysplastic tissues using a portable and compact point monitoring system. DR spectra in the 400-700 nm region was collected from the buccal mucosa and alveolas (left and right buccal mucosa, retromolar area and angle of the mouth) in 96 patients and 34 healthy volunteers in a clinical trial. Principal component analysis (PCA) was performed on the normalized spectral data with linear discriminant analysis (LDA) as the classifying technique to discriminate healthy tissue from hyperplastic and dysplastic tissues. The DR spectral data were compared against the histopathology results of biopsy. The receiver operator characteristic (ROC) curve analysis was also performed for group comparisons and the results are presented.

5.2. Materials and methods

5.2.1. Study population and study settings

Study population consists of 34 healthy volunteers maintaining good oral hygiene (without any inflammations/visible lesions in their oral cavity) selected by a dental pathologist and 26- hyperplastic, 20- dysplastic and 50- squamous cell carcinoma (SCC) patients registered in the outpatient clinic of Government Dental College (GDC), Trivandrum. Only patients with

lesions in the left and right buccal mucosa, retromolar area and in the angle of the mouth were included in the study. The healthy volunteers were students within the age group of 20-35 years. Other inclusion criteria for participants were; (a) more than 20 years of age, (b) has no previous history of treatment for cancer with radiation or chemotherapy, (c) has no history of application of any medication orally for at least seven days, (d) no life threatening medical conditions which require emergency response and (e) willingness to provide written informed consent. Before enrollment, the study subjects were examined for suspicious lesions in their oral cavity, including white/red patches, non-healing ulcers and proliferative growth. Oral lesions, clinically suspected of erosive lichen planus were not included in the patient group.

A written informed consent was taken from all the participants after explaining the study procedures in detail. A study information sheet was provided to all participants and an independent (third) person explained the study procedures to the subjects if he/she is illiterate before obtaining the written informed consent. Prior to measurements, the study subjects were asked to rinse their mouth with 0.9% saline solution to reduce the effect of any recently consumed food. A detailed structured questionnaire was administered to collect demographic, behavioral and clinical data. The study subjects were asked to stop their habits of smoking, tobacco chewing and alcohol use at least 12 hours before spectral data measurements. Biopsy samples were taken from the respective measurement sites of all patients.

5.2.2. Spectral data processing

All spectral data recorded were normalized to the peak intensity for individual spectra and were averaged with respect to different lesion groups. Data were separated into standard data (training) and blind data (validation) sets, with all spectroscopic measurements from a single patient randomly assigned to either the training set or the validation set. The training set was used to define a set of spectral features; (a) to reduce the data to a diagnostically

relevant subset of the spectral features and (b) to develop a diagnostic classification algorithm to classify tissue sites based on the identified subset of spectral features.

5.2.3. Statistical procedure

DR spectra were pre-processed to minimize inter-patient variation by normalizing each spectrum to its peak spectral intensity. Although, the spectral measurements were taken from 13 anatomical sites of healthy volunteers, only buccal mucosal data were included in the analysis. Eight principal components (PCs) accounting 99.5% of the total information given by the original spectral data was extracted using PCA. The significant PCs (p-value <0.05) were the input variables of LDA. Furthermore, a diagnostic algorithm was developed using the discriminant functions with leave-one-out (LOO) method of cross validation. Diagnostic accuracies were determined from the discriminant function scatter plot. ROC curves were also constructed using the discriminant function scores to assess the potential of DR spectral data to differentiate pre-cancerous/cancerous lesions from benign/normal. The area under the curve (AUC) and its 95% CI were estimated. The significance of AUC value was tested using non-parametric assumption.

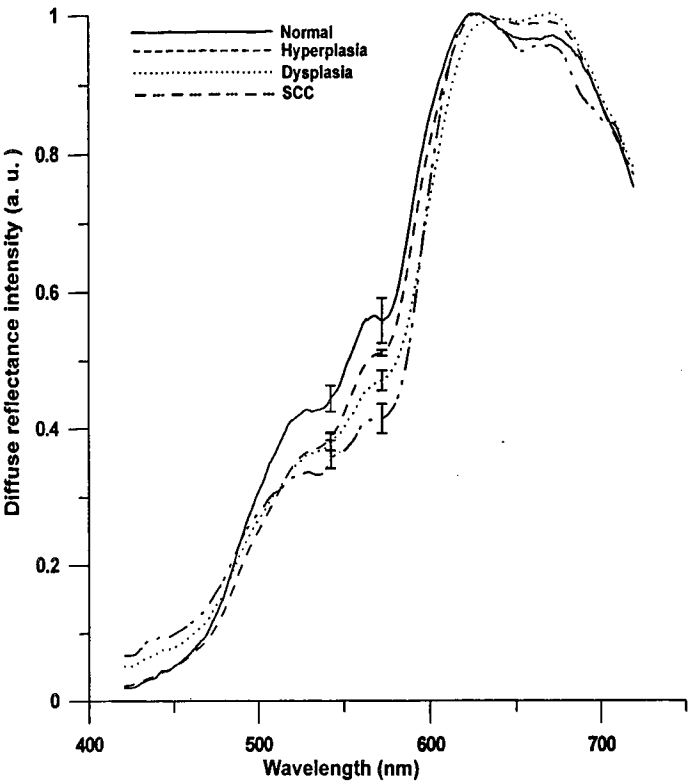

Fig. 5.1. Averaged DR spectral features from the oral cavity of 34 healthy volunteers and 96 patients (26- hyperplasia, 20- dysplasia, 50- SCC). Each individual spectrum is normalized to the peak intensity. The error bars relate to the standard deviation at 545 and 575nm.

5.3. Results

5.3.1. Demographic details

Healthy subjects comprised of 34 volunteers, whereas the diseased sample group consisted of patients with differing lesion grades viz., 26 hyperplastic, 20 dysplastic and 50 SCC. The raining data set and validation set were randomly selected from the whole set of patients studied and included 22 normal/ healthy samples, 16 hyperplastic (benign), 12 dysplastic (mild, moderate and severely -dysplastic) and 30 SCC cases (well, moderately and poorly - differentiated) for the training set, and 12 normal, 10 hyperplastic, 8 dysplastic (3-mild, 4-moderate and 1-severely dysplastic) and 20 SCC (9-well, 7-moderately

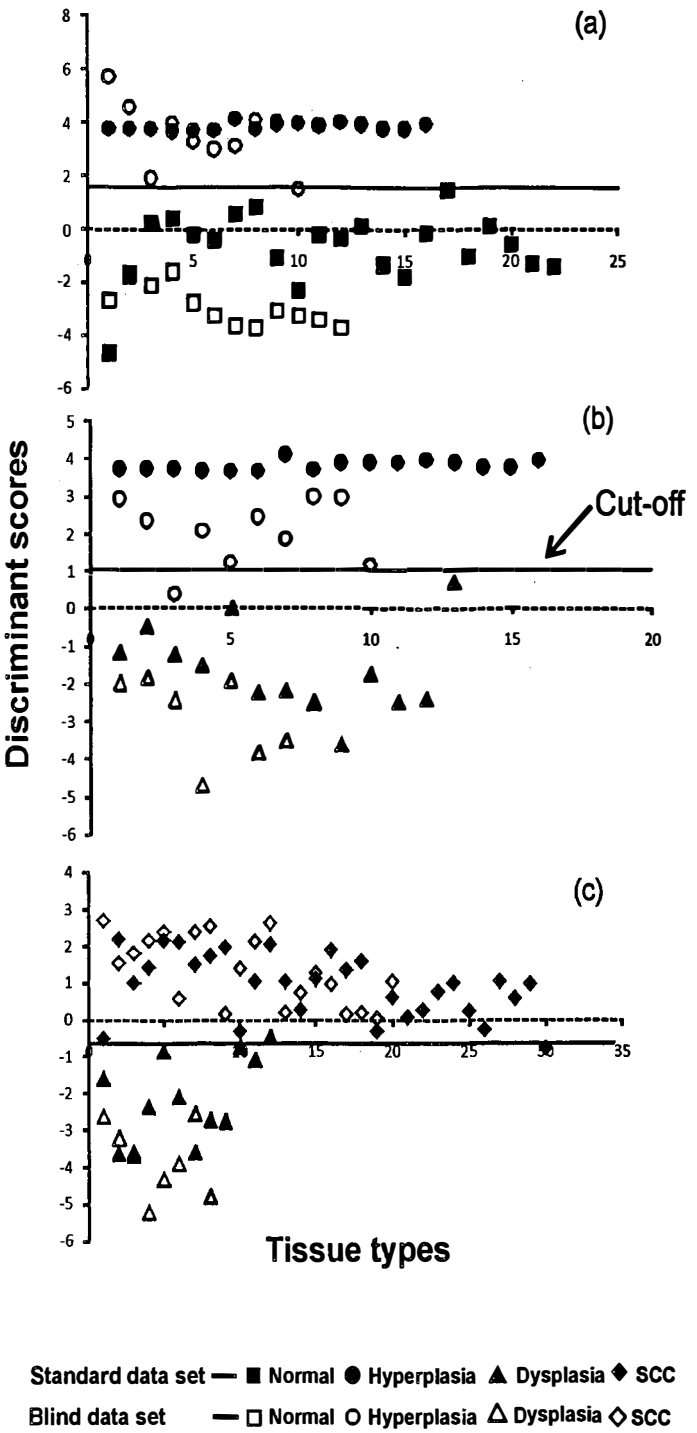

Fig. 5.2 Discriminant function scatter plot of different lesion pairs a) normal - hyperplasia b) hyperplasia - dysplasia and c) dysplasia - SCC. The solid symbols represents functions of standard data set and open symbols correspond to blind data set.

and 4-poorly -differentiated) cases for the validation set.

The mean age of healthy volunteers (mean age = 20 years, SD = 10 years) were significantly lower than the patient group (mean age = 50 years, SD = 15 years). While all the patients included in the study were current users of tobacco (pan-chewing), most of the (65 out of 70) male patients reported regular habits of smoking and alcohol consumption. In comparison, the healthy volunteers were free from tobacco and alcohol use.

5.3.2. Spectral features

However, the intensities of the oxygenated hemoglobin absorption dips were more prominent in healthy/ normal volunteers (0.44 ± 0.05 at 545 nm and 0.56±0.06 at 575nm) in comparison to the spectral data from patients (0.36±0.07 at 545 and 0.41±0.11at 575nm). Significantly lower spectral intensities were noticed in hyperplastic (0.41±0.01 at 545nm and 0.51±0.01 at 575nm), dysplastic (0.39±0.07 at 545nm and 0.47±0.07 at 575nm) and SCC (0.36±0.07 at 545 and 0.41±0.11at 575nm) lesions in comparison to DR spectra from normal/healthy volunteers (Table 5. 1). Furthermore, the spectral intensity ratio (R545/R575) increased significantly with tissue abnormality i.e., from healthy tissue to hyperplastic, dysplastic and SCC lesions ($p<0.005$).

Table 5.1. Averaged normalized spectral intensity and DR intensity ratio at oxygenated hemoglobin dips at 545 and 575nm for different tissue types

Tissue types	Spectral intensity (p-value < 0.005)		Intensity ratio (p-value < 0.005)
	At 545 nm	At 575 nm	R545/R575
Normal/ healthy	0.44±0.05	0.56±0.06	0.78±0.06
Hyperplasia	0.41±0.01	0.51±0.01	0.80±0.01
Dysplasia	0.39±0.07	0.47±0.07	0.83±0.07
SCC	0.36±0.07	0.41±0.11	0.88±0.08

5.3.3. Lesion classification

In the training dataset, all the normal, hyperplastic and dysplastic lesions were classified correctly with cut-off values of discriminant score at -0.64 and 1.04 respectively, which

were the weighted averages of discriminant scores of normal - hyperplastic, and hyperplastic - dysplastic lesions (Fig. 5. 2). However, one case with SCC was misclassified as dysplastic while another case with dysplasia was misclassified as SCC, in this group of 12 dysplastic and 30 SCC cases, with a cut-off value of discriminant score at 1.58. In the validation dataset, out of the 10 cases of hyperplasia one was misclassified as normal in the normal - hyperplasia pair. In the hyperplasia - dysplasia pair one out of 10 cases of hyperplasia was misclassified as dysplasia. However, all cases of dysplasia and SCC were classified correctly with 100% accuracy.

Table 5.2. Overall Diagnostic accuracies obtained for different lesion pairs consisting of 80 sample in the standard data (prediction) set and 50 sample in the blind data (validation) set

Tissue types	Normal & Hyperplasia				Hyperplasia & Dysplasia				Dysplasia & SCC			
	Se (%)	Sp (%)	PPV (%)	NPV (%)	Se (%)	Sp (%)	PPV (%)	NPV (%)	Se (%)	Sp (%)	PPV (%)	NPV (%)
Standard data set	100	100	100	100	100	100	100	100	97	92	97	92
Blind data set	90	100	100	90	100	90	89	100	100	100	100	100
Over all	95	100	100	95	100	95	95	100	98.5	96	98.5	96

Se: Sensitivity; Sp: Specificity; PPV: Positive Predictive Value; NPV: Negative Predictive Value

Se = TP/(TP+FN), Sp = TN/(TN+FP), PPV = TP/(TP+FP), NPV = TN/(TN+FN)

TP: True Positive; FN: False Negative; FP: False Positive; TN: True Negative

The overall sensitivity and specificity for a discriminant score cut-off value of -0.64 to differentiate healthy Vs hyperplastic tissue were 95% and 100%, respectively. Similarly, a cut- off value of 1.04 yielded a sensitivity and specificity of 100% and 95%, respectively, to discriminate hyperplastic tissue from dysplastic tissue. Finally, a discriminant score cut-off value of 1.58 yielded a sensitivity and specificity of 98.5% and 95%, respectively, for differentiating dysplastic tissue from confirmed SCC tissue (Table 5. 2).

The ROC analysis with discriminant score yielded AUC of 0.983 (95% confidence interval (CI): 0.95-1.00) and 0.954 ((95% CI: 0.90-1.00) for discriminating dysplasia from SCC and

hyperplasia from dysplasia, respectively. While discriminating hyperplasia from healthy tissues the ROC analysis yielded an AUC of 0.987 (95% CI: 0.96-1.00) (Fig. 5.3). ROCs with normalized spectral intensity ratio data yielded AUC of 0.81 (95% confidence interval (CI): 0.70- 0.91) for discriminating dysplasia from SCC, 0.89 (95% CI: 0.82- 0.96) for discriminating hyperplasia from dysplasia and 0.84 (95% CI: 0.75- 0.93) for discriminating hyperplasia from normal tissue.

5.4. Discussion

This study carried out in the outpatient department (OPD) setting of a tertiary care hospital provides strong evidence on the utility of a low cost, non-invasive screening technique for early detection of a wide range of neoplastic lesions in the oral cavity. The DR spectral data at 545 and 575nm, generated from a portable and compact fiber-optic point monitoring system, efficiently discriminated the tissues with different grades of neoplasm in the oral cavity. The sensitivity and specificity yielded from this diagnostic evaluation study evidently support the inclusion of this technique for routine screening of oral cancer in clinical settings.

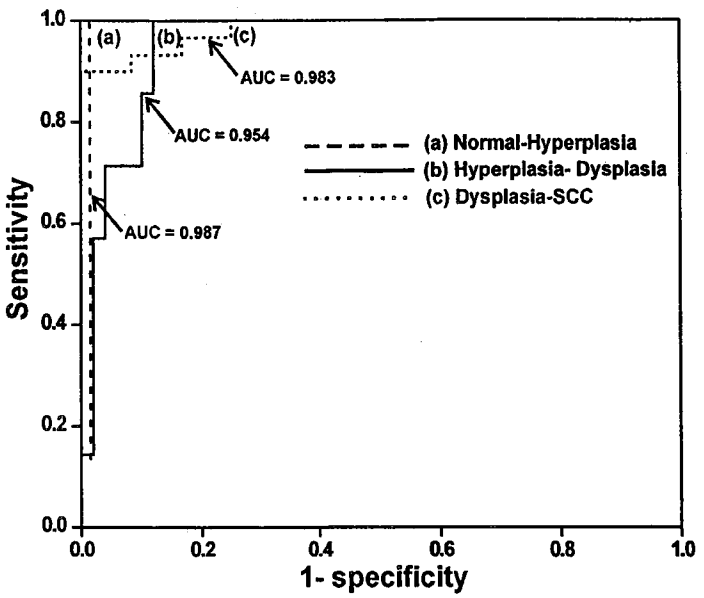

Fig. 5.3 ROC curves showing the diagnostic performance of discriminant scores of function for discriminating (a) normal- hyperplasia b) hyperplasia - dysplasia and (c) dysplasia - SCC.

Significant dips in the normalized mean DR spectra around 420, 545 and 575nm were observed from normal/healthy volunteers, hyperplastic, dysplastic and SCC lesions (Fig.5.1). Furthermore, the spectral intensities at these points were more prominent in healthy/normal volunteers in comparison to the spectral data from patients with early

neoplastic changes and SCC. DR spectral features mainly depend on the absorption and scattering properties of tissues and on oxygenation levels of hemoglobin (Schwarz et al, 2009; de Veld et al., 2005; Utzinger et al., 2001). Increased absorption at thickened epithelium, local architectural changes in the cellular and sub-cellular levels including changes in the nuclear to cytoplasmic ratio of the epithelial cells, stromal properties and neo-vascularisation contribute to the decrease in reflectance intensity with abnormality (Georgekoudi et al., 2002; Badizadegan et al., 2004). When tissues transform towards malignancy, the heme synthesis gets disturbed or broken down due to the reduced activity of ferrochelatase enzyme (Amelink et al., 2008, Lovat et al., 2006), thereby leading to lower production of hemoglobin and correspondingly lower oxygenated hemoglobin absorption around 545 and 575 nm.

The LDA of the significant PCs based on the spectral intensity data and further stratification of tissue types based on the discriminant score, yielded very high sensitivity and relatively good specificity, for discriminating normal tissue from abnormal tissues (suggestive of early neoplastic changes and SCC) in the oral cavity. The ROC curve analysis further confirmed the utility of this screening technique for early detection of neoplastic changes in the oral cavity. The AUC for the discriminant score analysis reached nearly 100% and conclusively proved the utility of this screening technique for early detection of oral cancer. Furthermore, the diagnostic algorithm developed based on PCA was comparatively better than the utility of simple spectral intensity ration (DR545/DR575) for discriminating different tissues types. To the best of our knowledge, this is the only study that examined a wide range of lesions in the oral buccal mucosa using different algorithms based on DRS and distinguished the changes suggestive of early neoplasia with relatively high accuracy.

Our study findings are supported by similar DRS analysis for distinguishing different tissue types. For example, Koenig et al (1998) used DRS to detect bladder carcinoma and distinguished malignant and dysplastic lesions from normal or benign lesions with 91%

sensitivity and 60% specificity. Mirabel et al (2002) reported a sensitivity of 72% and a specificity of 81% for distinguishing squamous intraepithelial lesion from normal squamous mucosa and normal columnar mucosa using DR spectroscopy with variable source-detector distance for the detection of cervical neoplasia. In a recent study, Mallia et al. (2010) examined the potential of DRS as an alternative to autofluorescence spectroscopy in tongue cancer detection. In that study the DR spectral intensity ratio (R545/R575) of oxygenated hemoglobin absorption peaks at 545 and 575 nm was used in detecting malignancy on the dorsal side of the tongue (DST) with a sensitivity of 86% and a specificity of 80% to discriminate benign hyperplasia from normal lesions, which was indistinguishable with autofluorescence spectroscopy. Similarly, while distinguishing hyperplastic from premalignant dysplastic lesions, the study yielded a sensitivity of 90% and a specificity of 86%.

Currently cancer screening is advocated in India only for cancers of cervix and breast as they are the most common cancers and these screening tests are considered reliable with relatively good accuracies. However, a World Health Organization (WHO) estimate suggests 63,441 incident cases of cancer of oral cavity in India in the year 2004. Furthermore, it is estimated that by 2015 this will go up to 78,419 cases [Nair et al., 2005]. A vast majority of these cases remain undiagnosed in the early stages and a conservative estimate suggests the incidence of at least 4 pre-malignant cases per one oral cancer case in this population. Therefore, a high risk screening approach for neoplastic changes in the oral cavity using the DRS algorithms among individuals over 35 years of age, with any lesions or patches in the oral cavity will aid in early detection of both pre-malignant and malignant conditions. The high sensitivity of the non-invasive DRS method would lead to a relatively smaller number of genuine cases being referred for invasive tissue biopsy and time consuming histo-pathology analysis, and the relatively good specificity would ensure low rate of missed diagnosis. Furthermore, there is hardly any recurring cost for DR screening technique. Since, the screening method is non-invasive and easy to carry out by non-specialist staff,

the technique can be employed in different clinical settings with minimum additional resources. The near real-time data aid in quick decision making with no waiting period for the patient. The above mentioned characteristics of the DRS technique make it a suitable mass screening tool for early detection of oral neoplastic changes. However, the usefulness of this technique for mass population screening needs to be examined in an independent study involving larger number of patients covering all other sites of the oral cavity.

Nonetheless, we have noticed that in malignant proliferative lesion, the present DR system failed to obtain true results due to inadequate penetration of light, which could be rectified by increasing the intensity of the white light or by modifications in the probe design. Another problem encountered was on patients with erosive lichen planus, wherein false positive results were obtained as a result of increased reflection of excitation light.

5.5. Conclusions

The DRS data analyses presented in this paper clearly establish the potential of this technique for early detection of malignant changes in the oral cavity. The relatively high accuracy obtained in this study with very low miss-classification rate recommends it as an ideal tool for screening of oral cancer in clinical settings. Furthermore, the following characteristics; (a) the non-invasive nature of the screening technique, (b) availability of real-time data for quick decision making, (c) non-requirement of massive additional resources and/or specialized staff for screening, and (d), absence of any recurring expenditure for the screening procedure, satisfy all the necessary requirements of a standard screening tool. The public health implications of this technique are considerable as it is adaptable to a variety of clinical settings and easy to utilize in community centers for screening of random population and detection of early neoplastic changes in the oral cavity.