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Detection of Oral Lesions by the Diffuse Reflectance Spectroscopy on Human Oral Tissue and Saliva Samples

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Abstract

A comparative study has been carried out on 172 human oral tissue and saliva samples for the detection of oral lesions on three groups: oral squamous cell carcinoma (OSCC), dysplastic and control (normal) by using diffuse reflectance (DR) spectroscopy (DRS). DR spectra obtained from oral tissues consist of blood valise near 410, 537 & 577nm and water absorption dip near 505 nm. However, human saliva consists only of water absorption dip near 510 nm. Variation in fluorescence intensity values among the three groups are observed in the spectra of human saliva and tissue. Classification among the groups has been done by employing principal component analysis (PCA) on the original DR data. Then Mahala Nobis distance model and receiver operating characteristic (ROC) analysis are applied successively. PCA is used for dimension reduction and further Mahala Nobis distance and ROC are utilized to classify the groups (OSCC, dysplastic and normal) as well as to find the diagnostic parameters such as sensitivity, specificity, and accuracy. DR spectroscopy for oral tissue was able to differentiate OSCC to normal, dysplasia to normal and OSCC to dysplasia with the sensitivities of 97%, 78%, 74% and specificities of 92%, 88% and 81%. For saliva, it differentiated respective groups with the sensitivity and specificity values of 88%, 74%, 68% and 88%, 81% and 77%. Results indicate that human saliva may be utilized for the differentiation of OSCC and dysplastic patients from normal volunteers.

Keywords: Oral Cancer; Tissue And Saliva; Diffuse Reflectance Spectroscopy; Absorbers; Principle Component Analysis; Mahalanobis Distance

Abbreviations: OSCC: Oral Squamous Cell Carcinoma; DR: Diffuse Reflectance; DRS: Diffuse Reflectance Spectroscopy; PCA: Principal Component Analysis; ROC: Receiver Operating Characteristic; OCT: Optical Coherence Tomography; SERS: Surface Enhanced Raman Spectroscopy; PCs: Principal Components

Introduction

There is progressive increase in oral cancer incidence due to lack of early symptoms as well as late diagnosis. It is thus very much essential to detect and diagnose oral lesions at an early stage. Early detection can result in a significantly higher chance of cure. Tobacco chewing, smoking in any form and consumption of alcohol are the key risk factors of oral cancer. Oral cancer has become eighth most common malignancy worldwide and in India it is one of topmost among males and females [1,2]. For early diagnosis, there is need to probe different possibilities in terms of diagnostic media and better and novel diagnostic techniques.

Among the conventional techniques (visual inspection, brush biopsy, toluidine blue, etc.), tissue biopsy with histopathological examination is the gold standard for the detection of oral mucosal lesions [3,4]. However, tissue biopsy is an invasive process. It is therefore need of non-invasive methods in terms of diagnostic techniques and diagnostic media. Non-invasive diagnostic techniques have been extensively researched for differentiating various cancerous lesions. Fluorescence, Raman, diffuse reflectance (DR), optical coherence tomography (OCT) and lifetime spectroscopy are some of the major spectroscopic diagnostic techniques used for cancer detection [5-12]. Research on early detection of oral carcinoma *(ex-vivo and in-vivo)* has been performed by many research groups using optical spectroscopy with promising results [13-19]. Diagnostic media such as saliva, blood and urine are studied for cancer detection. Human saliva has been investigated by many research groups for oral cancer detection using various techniques [20-23]. But using spectroscopic devices, study on human saliva has been done by only few research groups [24-28].

With the evolution of cancer, biochemical and morphological changes occur in the human oral tissue. Biochemical alternations are also known to occur in human saliva with the progress of cancer. Some of the major fluorophores in human oral tissue are collagen, tryptophan, FAD, NADH and porphyrins. Human Saliva also contains certain fluorophores such as NADH, tryptophan, FAD, DNA and RNA [23]. These fluorophores display changes with progress of cancer. Saliva is produced in between 1 to 1.5 litter daily and if needed, it can be easily collected several times a day from patients and volunteers without any complication. It may be an ideal choice for oral cancer detection due to direct contact of fallen cells in the oral cavity. It has been studied by several research groups over the last 20 to 25 years for various purposes [22-28]. Saliva has been used for forensic purposes in particular sexual assault cases, child abuse cases, to diagnose HIV infected patients and many more [29,30]. For breast cancer detection, saliva has also been studied [31]. Recently, human saliva is tested for the novel coronavirus detection [32]. Lung cancer detection was performed on cancerous patients and normal volunteers by the group of Li et al using the surface enhanced Raman spectroscopy (SERS) system. They achieved an overall accuracy of 80% [24]. Yuvraj et al utilized human saliva for the detection of oral cancer in cancerous and control groups. Using the ROC on the fluorescence data, they achieved sensitivity of 85.7% and specificity of 93.3% [25]. Patil et al. group used LIFS technique on saliva samples for the detection of oral cancer and achieved sensitivity and specificity values of 79% and 78% respectively [26]. Nager et al group performed a concentration-based study on oral carcinoma and normal saliva samples and found sensitivity and specificity values of 71 and 75 percent respectively [33]. Our group also examined human saliva on three groups (OSCC, OSMF and control) for oral cancer detection using the SS spectroscopy and achieved accuracy values of 93 %, 95 % and 92 % respectively [27]. Recently, I studied saliva for the detection of head and neck cancer on three groups (SCC, dysplastic and normal). Using the ROC on the fluorescence data, the three groups are differentiated with accuracies of 98 %, 93 % and 81 % respectively [28].

In the present study, I have established a comparison between two media i.e., human tissue and saliva for the detection of oral carcinoma using the DR spectroscopy. DR spectra were recorded from OSCC tissue & saliva samples, dysplastic tissue & saliva samples, and normal tissue & saliva samples. Differences in the spectra were observed. Blood valises are observed in the tissue samples and water absorption dips are found in tissue and saliva samples. For the classification of the different lesions, multivariate methods such as PCA, Mahala Nobis distance model and ROC are performed [18,34].

Materials and Methods

Measurements were performed on a total of 172 oral tissue and saliva samples. Tissue samples were collected from 61 patients, in which OSCC, dysplastic and normal are 34, 27 and 25 respectively. Normal tissues were taken from unaffected areas of OSCC and dysplastic patients. Saliva samples were collected from 61 patients (34 OSCC & 27 dysplastic) and 25 normal volunteers. The age of OSCC and dysplastic groups was 34 - 85 (mean age 47±13) and 22 - 65 (mean age 41±15) respectively. The age of the normal volunteers was 25-56 (meaning age 36±10). Patients were asked not to consume food and beverage and suggested to wash their oral cavity. Written consent was taken from all participating patients and volunteers. Sample collection was done in Hallets hospital affiliated to GSVM medical college Kanpur. Sterile containers (10ml) are used to collect saliva samples. After collecting the saliva samples from patients and volunteers, patients were sent for biopsy and tissue was collected. Before starting the experiment, tissue sample was cleaned by saline water then mounted on the quartz cuvette of size (1cm×1cm×5cm) and measurements were performed. Similarly, saliva sample was poured into a quartz cuvette and measurements were taken. After completing the measurement, tissue samples were sent for histopathology. Histopathology reports were later obtained and compared with DR results.

Diffuse reflectance (DR) measurements on tissue and saliva samples were taken on spectrofluorometer (Fluor log 3, Model FL (3-22). For DR measurements, slit width was fixed at 0.5 nm for both monochromators (excitation and emission) and spectra acquisition were done in 0.1s integration time in the interval of 1nm. Signals were recorded in the scan range 300 to 700nm.

Results and Discussion

Diffuse Reflectance Spectroscopy (DR) for human oral tissue and saliva

Averaged diffuse reflectance (DR) spectra and intensity normalized spectra of OSCC, dysplastic and normal oral tissue samples in the scan range of 300 - 700nm are shown in the figures 1a & 1b. respectively. DR spectra of tissue samples of all the three groups (OSCC, dysplastic & normal) show valleies near 410, 537 and 577nm, which are due to blood absorption and a kink near 397nm may be Raman band due to presence of water. A dip near 505nm also present in the spectra of all the three groups, which may be due to absorption by water. As seen clearly in figure 1b. blood absorption dips and water absorption dips are slight higher in OSCC samples than the dysplastic and normal tissue samples. Averaged spectra of saliva samples displayed in figure 2a. shows significant difference in intensity in between OSCC to normal. DR spectra of saliva show a valley near 513nm, which may be due to water absorption and a kink near 397 nm may be Raman band of water. Intensity normalized DR spectra is shown in figure 2b. In the normalized DR spectra as well as conventional spectra, it can be seen that water dip is higher in OSCC than the other two groups. Over all spectra of tissue and saliva samples are composed due to prescence of scatters (cells, nucleous, mitrokaniya etc,) and absorbers (hemoglobin, water). DR spectra also show some noisy signal in the range 450 to 490 nm as seen in spectra of tissue and saliva are due to leakege of Xeon light.

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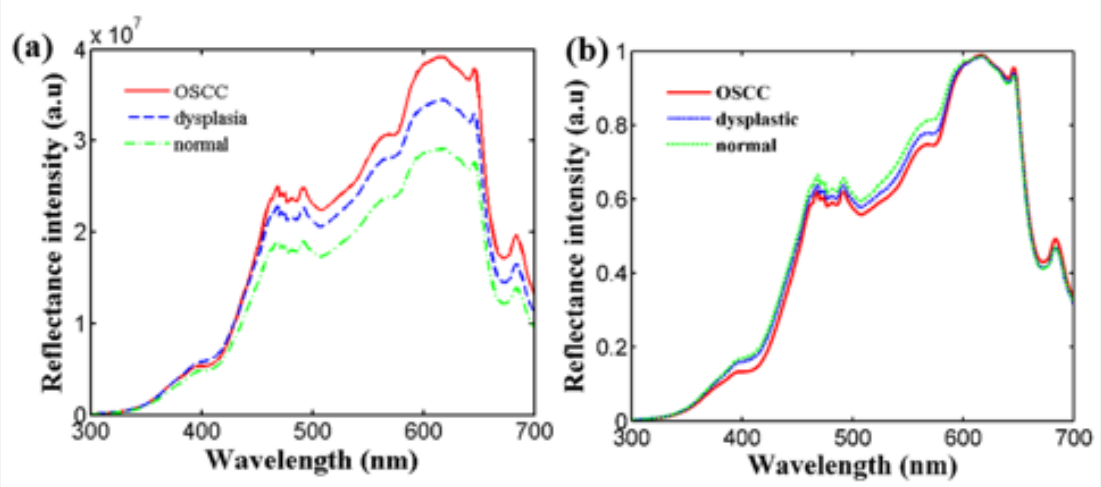

Figure 1: Averaged diffuse reflectance (ADR) spectra of OSCC, dysplastic and normal tissue samples in the scan range 300- 700 nm (a) averaged spectra (b) intensity normalized averaged spectra.

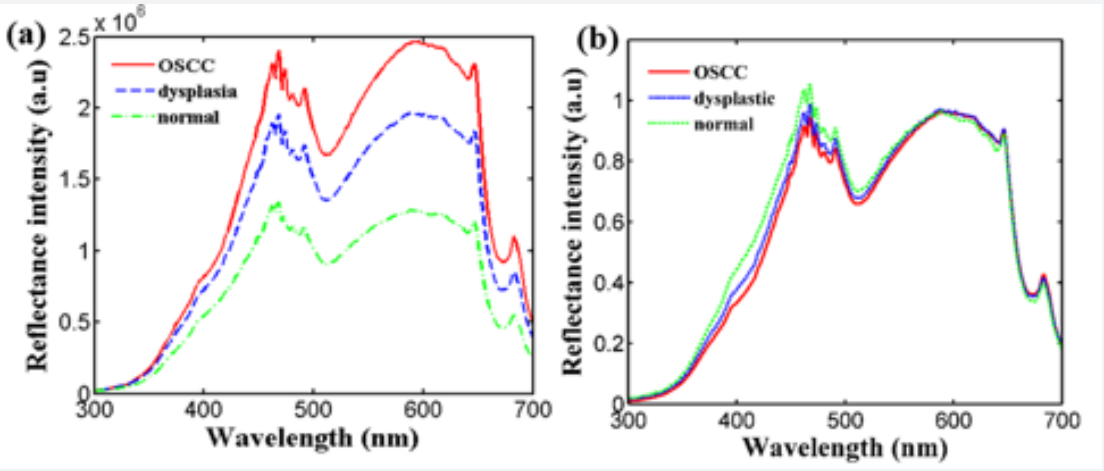

Figure 2: Averaged DR spectra of OSCC, dysplastic and normal saliva samples in the scan range 300- 700 nm (a) averaged spectra (b) intensity normalized averaged spectra.

Data Analysis

Principal component analysis (PCA) has been applied on the DR spectra of oral tissue and saliva in scan range of 300 to 700 nm. Data sets of DR spectra have dimensions of 401. PCA is a statistical method used to convert a large data set (higher dimension) into a small data set (lower dimension) called principal components (PCs). These higher dimensional data sets are reduced to lower dimensional data sets by computing the principal components of correlation matrix. After computing correlation matrix, principal component scores are estimated by projecting the original spectral data along the principal components (PCs). The first seven PCs (PC1 to PC7) having variance ≥ 98% are chosen for both data sets. Scatter plots of the first three PC scores obtained from DR spectra of oral tissue and saliva are plotted as shown in figures 3a & 3b respectively. It can be seen in figure 3a. that clusters formed by the normal group is separated from the overlapped clusters formed by dysplastic and OSCC groups of tissue samples. Similarly scatter plot of first three PC scores obtained from DR spectra of saliva are plotted and shown in figure 3b. Here the cluster of normal groups overlap with OSCC group while dysplastic group is well separated from other two groups.

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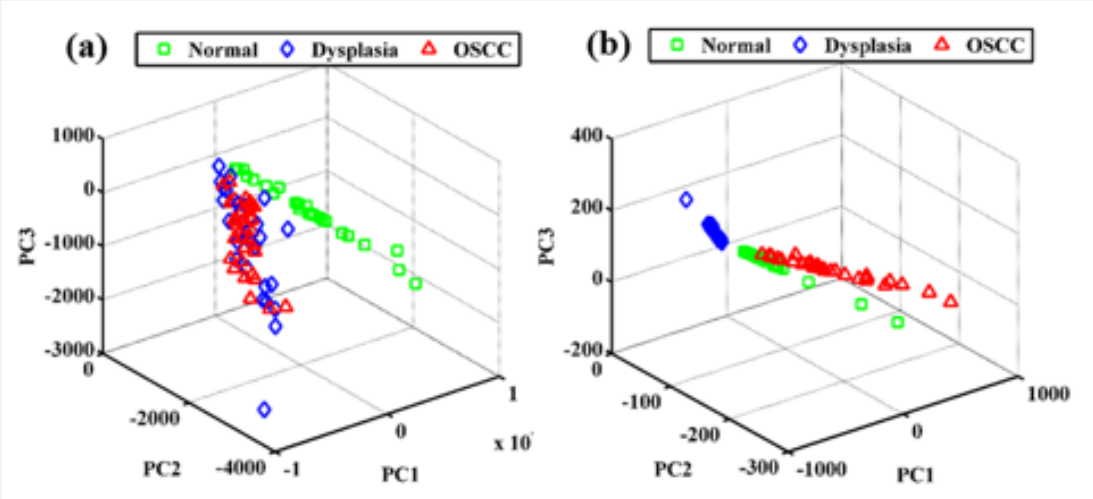

Figure 3: Scatter plot of first three PC scores (PC1, PC2, and PC3) in three-dimensional space for normal, dysplastic and OSCC groups for (a) oral tissue samples (b) saliva samples.

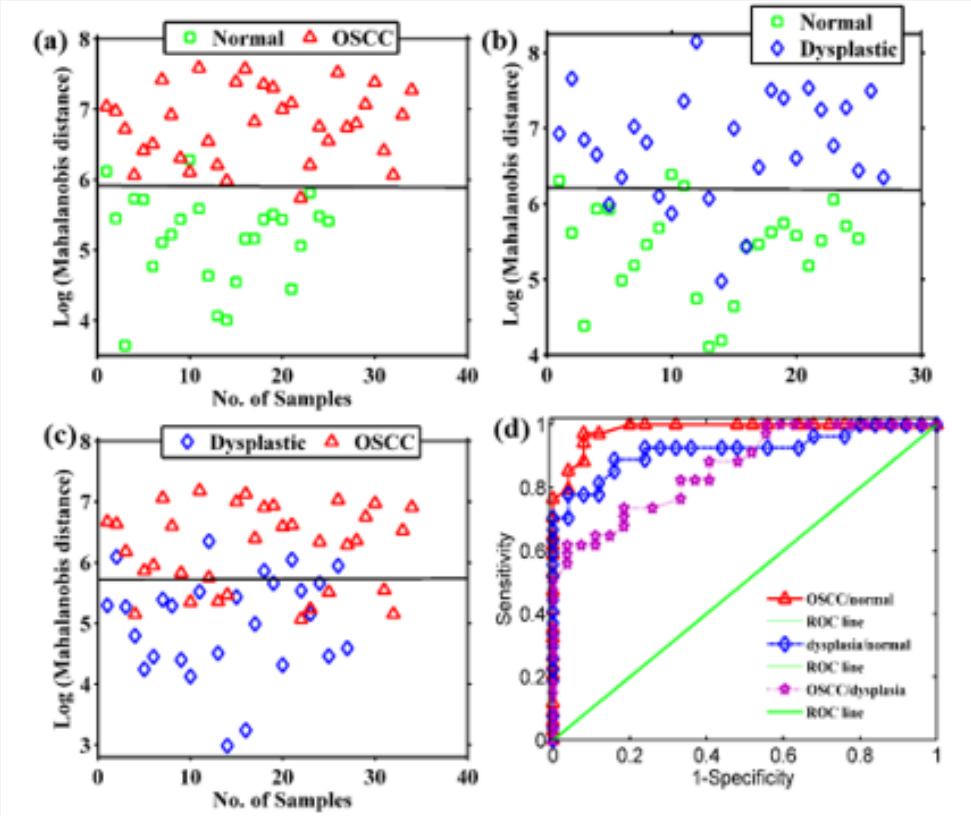

Figure 4: Scatter data plots of Mahala Nobis distances and ROC curves among the three groups of oral tissue samples (a) Normal to OSCC plot (b) Normal to Dysplasia plot (c) Dysplasia to OSCC plot (d) ROC curves.

After computing PCs, principal component scores of tissue and saliva samples are calculated and loaded in MD model and Mahala Nobis distances are computed. Prior to calculating Mahala Nobis distance, PC scores data are divided into two sets i.e., training (known) and validation (unknown) and MD distances are computed. Mahala Nobis distances among the three groups i.e., distances from OSCC to normal, dysplasia to normal, OSCC to dysplasia and vice versa of the DR data of tissue are plotted

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against the total number of samples and displayed in figures 4a-4c. Similarly, Mahala Nobis distances of saliva data are computed and plotted against the number of samples, which are displayed in figures 5a-5c. ROC employed on the MD distances of tissue samples differentiate OSCC to normal, dysplasia to normal and OSCC to dysplasia groups with sensitivities of 97% (33/34), 77.78% (21/27), 73.52% (25/34) and specificities of 92% (23/25), 88% (22/25), 81.48% (22/27) with the overall accuracies of 94.91% (56/59), 82.69% (43/52) and 77% (47/61) respectively. ROC on the MD distances of saliva samples differentiate the respective groups with sensitivities of 88% (30/34), 74% (20/27), 67.65% (23/34) and specificities of 80% (22/25), 88% (22/25), 88.88 % (24/27) with the overall accuracies of 88% (52/59), 80.76% (42/52) and 77 (47/61) respectively. ROC curves obtained from the data of tissue and saliva are displayed in figures 4d & 5d. respectively. Training and validation data sets are chosen randomly from each group several times and Malinois distances are computed but significant change in sensitivity and specificity values are not observed.

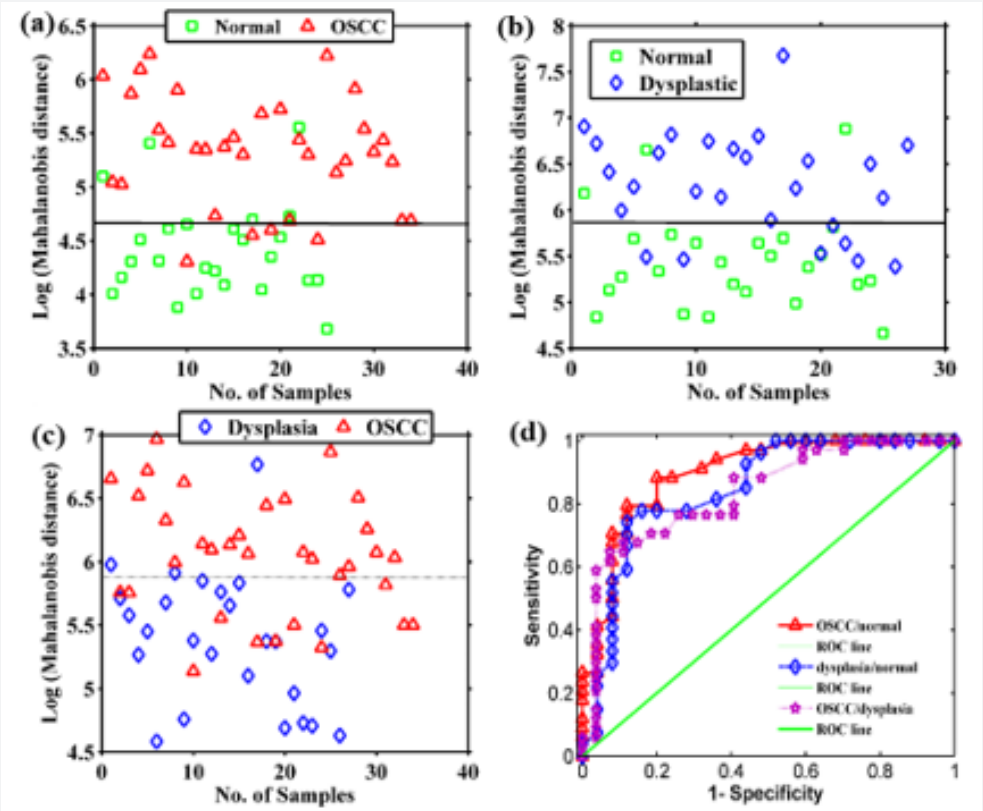

Figure 5: Scatter plots of Mahala Nobis distances and ROC curves of saliva for (a) Normal to OSCC (b) Normal to Dysplasia (c) Dysplasia to OSCC (d) ROC curves.

Conclusion

Detection of oral malignancy using the human oral tissue and saliva samples as a diagnostic tool and DR spectroscopy as a diagnostic technique was studied in the presented work. Three groups of patients, i.e., OSCC, dysplastic and normal of different age groups were included. A total of 172 samples (tissue samples = 86 and saliva samples = 86) were taken. In the measured DR spectra of tissue and saliva, blood absorption dips, water absorption dips and Raman bands were found. Difference in fluorescence intensities among the three groups in human saliva samples were found higher than the tissue samples. It was due to absorption by the hemoglobin in the tissue samples. To classify among the group's multivariate analysis such as PCA, Mahala Nobis distance model and ROC were employed one by one and sensitivity, specificity and accuracy values are computed. It was found that DR spectroscopy was able to differentiate among the three groups with sensitivities of 97%, 78%, 74 % for oral tissue and 88%, 74%, and 68% for saliva. These values indicate that discrimination among the three groups using human tissue is slightly better than saliva. But saliva may still be used to differentiate OSCC and dysplastic patients from normal volunteers. This study thus reveals that human saliva may be used as an alternate medium and DR spectroscopy is seen to be an excellent tool for the detection of oral cancer at an early stage.

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