Studies On The Spectral Lines of Salivary Samples Taken From Smokers And Non-Smokers

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Citation

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Abstract

The most common form of smoking in world is cigarette smoking, which is the leading cause of lung cancer, blood pressure, emphysema, bronchitis, heart attack etc[1] Studies show that smoking shortens the life of person by six minutes for every single cigarette he smokes. Saliva of smokers contains some harmful substances like nicotine which is not found in non-smokers[2]. Smoking tobacco destroys the molecules in saliva which are useful in protecting oral region from cancer. Under normal conditions saliva acts like a protective buffer between toxin and the lining of mouth, but in smokers this buffer nature and protective enzyme becomes non functional or less functional resulting in various oral diseases[3,4]. In the present study, Fourier-transform infrared spectro-microscopy was used for the analysis of saliva sample for the detection of marker for smokers. The analysis included 68 individuals; the samples were classified as 23 non-smokers saliva sample, 18 samples of active smokers and 27 samples of past smokers. Samples of active smokers were collected based on time intervals (15 minutes before smoking, immediately after smoking, 30 minutes after smoking and 1 hour after smoking). Several interesting peaks have been identified as the marker for the smokers from saliva. The peaks at 737cm^{-1}, 1659 cm^{-1}, 2146 $cm^{-1}$and 3414 cm^{-1} were significantly altered in the absorption level in active smokers and past smokers when compared with the non-smokers. Analysis based on the time scale suggests that about one hour time interval was required for regaining original spectral position of active smokers. Our study also indicates that the stable marker was possible for active smokers by analyzing the past smokers spectral pattern. Hence these parameters could be used as a basis for developing a spectral method in detection of active smokers using their salivary sample.

INTRODUCTION

Smoking is a practice in which a person most commonly uses tobacco, which is burned and smoke is inhaled[5]. Smoking greatly affects person's physical and mental health like it can cause wrinkles, cervical cancer, miscarriage and still birth in pregnant females, and also can cause problem with erection in males[6].Hence there is an increasing need in awareness on smoking and molecular level variation which happens due to smoking should be understood by the smoker for effective awareness[7].

FTIR is Fourier Transform Infrared Spectroscopy. FTIR is the most powerful tool for identifying chemicals that are either organic or inorganic in nature[8]. It can be utilized for identifying components of unknown mixture. It can also be applied to analyze solids, liquids, and gases. In this method IR radiation is passed through the sample, some of the IR radiation is absorbed by the sample and some of it is allowed to pass through. The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprinting of sample. FTIR Technique is applied in the field of biology for tracking the chemical changes in a live cell, studying the light induced reactions in photo biochemical systems such as rhodopsin, bacteriorhodopsin and photosynthetic reaction centres[9]. It is also applied to the in-vitro characterization of biomaterials. FTIR micro-spectroscopy has been used effectively to diagnose and characterize various types of disorders. Encouraging results were obtained previously in an attempt to diagnose leukemia, Gastric Inflammation, Alzheimers disease and kidney stone analysis. Applications of FTIR Spectroscopy over microbiology can give successful information in determining the strain and species difference between various microorganisms[10], detection of virus infected cell and fungi in wood. Besides that, FTIR's role in diagnostic aspects involving body fluids has been of growing importance in the last few years.

In our present study, we had examined the saliva samples

obtained from 23 non-smokers, 18 samples of active smokers taken 15 minutes before smoking, 18 immediately after smoking, 18 thirty minutes after smoking, 18 one hour after smoking, 17 past smokers who had left smoking two months before and 27 past smokers who had left smoking six months before. FTIR Spectroscopy was employed to detect stage wise diagnosis with specific Biomarkers pattern discrimination between non-smokers, active smokers and past smokers. The results showed several spectral peaks, which are useful biomarkers for detection of an active smoker and to find out time taken by him to reach to the normal state in the spectral line pattern.

MATERIALS AND METHODS

Saliva samples collected from of 68 individuals, the samples had been classified as 23 non-smokers, 18 samples of active smokers and 27 samples of past smokers. Samples of active smokers were collected based on time intervals 15 minutes before smoking, immediately after smoking, 30 minutes after smoking and one hour after smoking. To bring more clarity to the data, 27 past smokers were included in our study out of which 13 had stopped smoking before 2 months from our analysis and 14 samples of past smokers, who stopped smoking before 6 months. The samples were taken from individuals who were free from any infectious diseases and the body mass indexes of the 68 individuals were found to be normal. Participants gave written informed consent for the collection of samples. 2ml of saliva samples were obtained in an Eppendorf tube from each individual and freshly centrifuged for 5 min at 14,000 rpm in Eppendorf centrifuge. The supernatant was removed and then used for the spectral analysis.

SAMPLE LAYER PREPARATION

High IR radiation transparent thallium bromide iodide crystals were used as the slide material and a drop of 1 µl of the saliva sample was placed on a certain area on the thallium bromide iodide crystal, air dried for 30 minutes at room temperature and examined by FTIR micro spectroscopy. Five experimental replicates of each sample were subjected to FTIR micro spectroscopy to standardize our spectral data[11].

FTIR DATA ANALYSIS

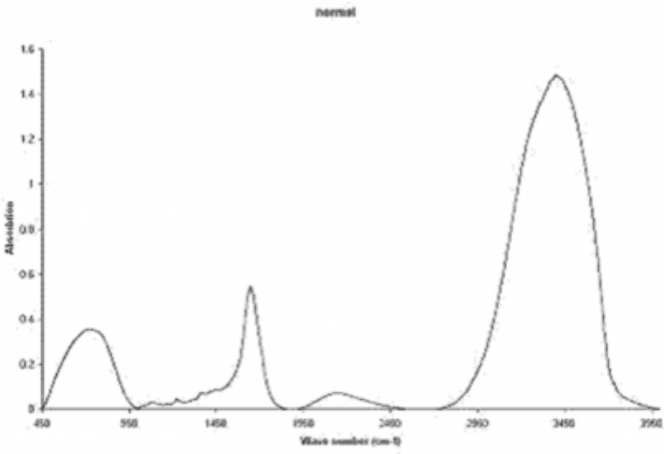
FTIR measurements were performed in absorption mode with FTIR Spectro-microscopy coupled to the FTIR spectrometer Panorama software[12]. The spectra were obtained in the wave number range of 450–4000 cm^{-1}. Baseline correction and normalization were performed for all the spectra by Panorama software. Baseline correction was done by line Algorithm. Data smoothing was done at 9 points smoothing window with polynorm of order 3 and the spectral resolution was at 4 cm^{-1}. Peak Normalization was performed to bring the y value to zero. Derivative and peak finding were performed using Panorama software[13].

RESULTS & DISCUSSION

Careful inspection of the obtained spectra from the FTIR data analysis gives an exclusive spectral variation among non-smokers, active smokers and past smokers with dissimilarity in the absorbance bands at 737cm^{-1} ,1659 $cm^{-1,}$,2146 cm^{-1}, 3414 cm^{-1} of spectral region. The spectral line pattern using FTIR analysis for non-smoker was found to be having the common peaks at conventional places (Fig.1)

Figure 1

Fig.1. FTIR analysis of non-smokers



The spectral analyses were performed to the active smoker's samples and it was found that one hour time duration is required for regaining the original spectral position. The spectral position of an active smoker 15 minutes before smoking is found to have minimum variation as far as peaks are concerned (Fig.2). The FTIR analysis was also done immediately after smoking (Fig.3), 30 minutes after smoking (Fig.4) and 1 hour after smoking (Fig.5). The clear stable variation has been identified by comparing non-smokers with past smokers. The results for past smokers (Fig.6, 7) suggested that the spectral variation had been retained for a period of 6 months.

Figure 2

Fig.2. FTIR analysis of an active smoker 15 minutes prior to smoking

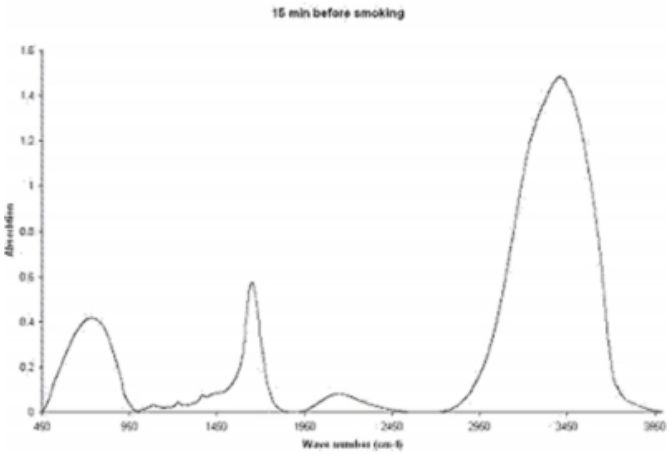


Figure 3

Fig.3. FTIR analysis of an active smoker immediately after smoking

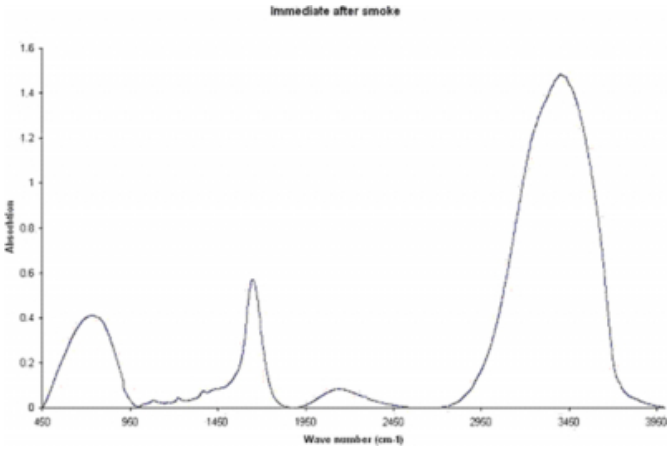


Figure 4

Fig.4. FTIR analysis of an active smoker 30 minutes after smoking

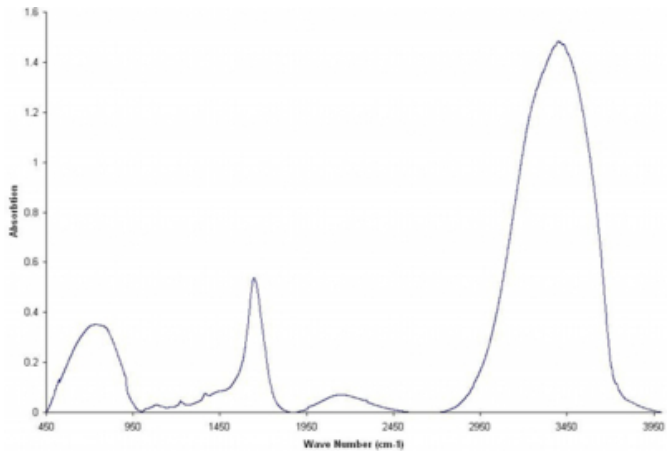


Figure 5

Fig.5. FTIR analysis of an active smoker 1 hour after smoking

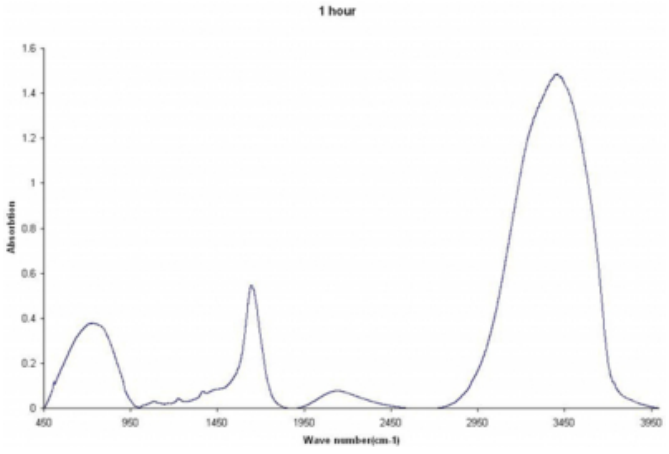


Figure 6

Fig.6. FTIR analysis of a past smoker (2 Months)

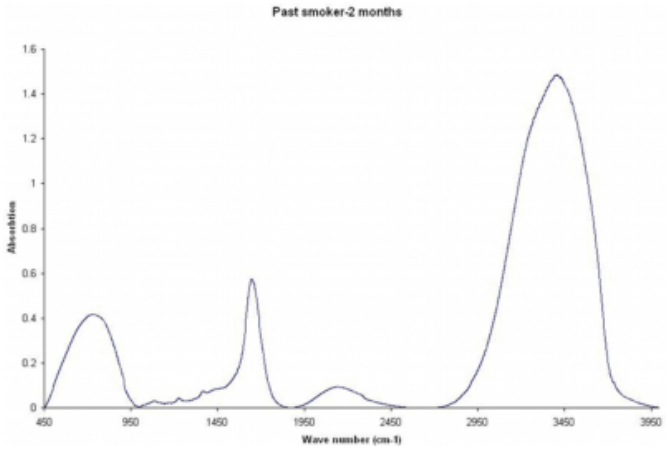
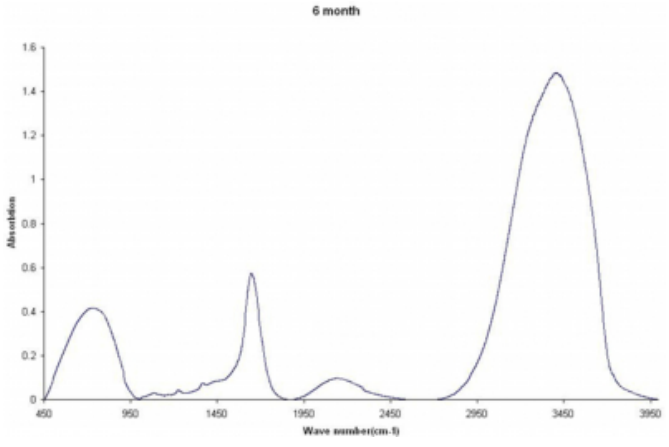


Figure 7

Fig.7. FTIR analysis of a past smoker (6 Months)



Analysis of variance (ANOVA) was performed to validate the statistical significance of the work. The results suggested

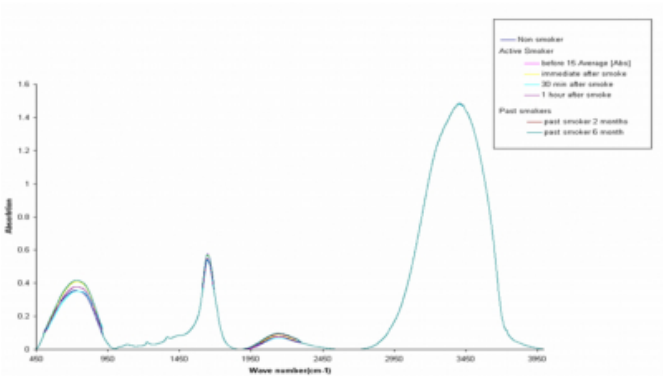
that the peaks show variability in the active smoking and past smoking cases when compared with the non-smokers. It was established as statistically significant with $p < 0.001$. Hence these results are biologically and statistically significant and they can be considered as a valid platform to detect the active, past and non-smokers.

STATISTICAL ANALYSIS

Analysis of variance was performed to identify the spectral variations in smoking candidates who were statistically significant. Cluster analysis is one of simplest and most rapid procedure for classification of biological data. In the present study, these two techniques were used to classify certain regions of the FTIR spectra of the examined healthy and patient samples. Statistical analysis was performed by Gene spring GX7.3 Micro array software PCA for entire spectrum from 500 to 4000 cm^{-1} show the full successful classification to distinguish healthy and Parkinson's patient samples. Cluster analysis were performed for the selected region 1543 cm^{-1} with similarity measurement of Pearson correlation and average linkage algorithm[14]. The results indicated excellent classification for the region 1543 cm^{-1} (Fig.8) for classical classification of stages.

Figure 8

Fig.8. Overall graph for statistical analysis



CONCLUSION

In this present stage, diagnosis of Parkinson's disease is very crucial and no diagnosis method is available from blood yet[15]. We examined the salivary samples of 129 subjects by FTIR spectro-microscopy technique for detecting and early diagnosis of Parkinson's disease by rapid and simple methodology. Although the results obtained in this study could be considered only as preliminary results, but still several interesting and consistent spectral differences between patient and healthy spectra may be considered as a promising basis for a future study including large number of samples. These spectral differences could be useful as biomarkers for Parkinson's disease diagnosis. Furthermore, this technique is more feasible, it required small amount of plasma (1 l), which can be easily obtained from any patient, and the final results could be obtained during very short time and ANN can be further implemented as the tool for the rapid diagnosis of Parkinson's disease.

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References

1. Shopland DR, Eyre HJ, Pechacek TF: Smoking-attributable cancer mortality in 1991. J. Nail. Cancer Inst. (Bethesda); 1991; 83: 1142-1148.
2. WHO: Histological Typing of Lung Tumors. Geneva: WHO, 1981.
3. Doll R: Uncovering the effects of smoking: historical perspective. Stat. Methods Med Res; 1998; 7: 87-117.
4. Doll R, Hill AB: Smoking and carcinoma of the lung, Preliminary report. BMJ; 1950; ii: 739-748.
5. Wynder EL, Graham EA: Tobacco smoking as a possible etiologic factor in bronchogenic carcinoma. JAMA; 1950; 143: 329-336.
6. Mills CA, Porter MM. Tobacco smoking habits and cancer of the mouth and respiratory system. Cancer Res;1950; 10: 539-542.
7. IARC: Tobacco Smoking. Monographs on the Evaluation of the carcinogenic risk of Chemicals to Humans. IARC Sci. Publ; 1986; 386-394.
8. Nakamoto K: Infrared and Raman Spectra of Inorganic and Coordination compounds. Wiley; New York, 1997.
9. Argade PV, Rothschild KJ: Biochemistry; 1983; 22: 3640-3646.
10. Bock CW, Trachtmann M, GeorgeP: J. Mol. Spectrosc; 1980; 84:243-255.
11. Braiman,M. and Mathies,R. (1980) Biochemistry, 11, 5421-5428.
12. CurryB: Thesis, University of California, Berkeley; 1983.
13. Mix G, Schweitzer-Stenner R, Asher SA: J. Am. Chem. Soc; 2000; 132: 9028.
14. Jentzen W, Unger E, Karvounis G, Shelnutt JA, Dreybrodt W, Schweitzer-Stenner RJ: Phys. Chem; 1996; 100:14184.
15. Lockhart DJ, Dong H, Byrne MC, Follettie MT, Gallo MV, Chee MS, Mittmann M, Wang C, Kobayashi M, Horton H, et al: Nat. Biotechnol;1996; 14: 1675–1680.

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