Assessment of bound water of saliva samples by using FT-IR spectroscopy

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Abstract:

The objective of the present work is to show the relationship of the high-wavenumber spectral region concern OH vibrations, which show in a way how bound water can be altered in different sample groups.

1. Introduction

The objective of the present work is to show the relationship of the spectral region of the high wave numbers with the OH vibrations, which show in a way how confined water can be altered in different sample groups, in the present study inverted smokers, sporadic smokers and non-smokers.

It is important to show how this spectral region, sometimes not addressed as much as the fingerprint region, can be important not only in the discrimination of sample groups, but also to elucidate how the water confined by OH vibrations (region 3000-3600 cm^{-1}) behaves in different pathological states.

2. Material and methods

2.1 Clinical protocol and research ethics

By using the spit/expectoration method, saliva samples of non-smokers and smoker patients were collected in a sterile universal collector, homogeneized and stored at -20ºC until analysis. All procedures performed in this study received approval from the Research Ethics Committee of the University of Taubaté under protocol number 19436919.7.0000.5501. All volunteers are over 18 years-old and no gender distinction was considered before saliva samples were collected.

2.2 Data collection

FTIR measurements were performed by using a Thermo Scientific Nicolet 6700 ATR FT-IR Spectrometer to measure dried saliva samples. By completely drying every saliva sample, we ensured that the remaining water was bound to organic saliva constituents such as proteins. In addition, lipids and carbohydrates remaining on dried samples are not affected by water absorption which contaminates FTIR signals. The drying procedure consisted of placing 1 µl of each saliva sample on the crystal with no additives and waiting for complete drying for times between 2-7 minutes (average time of 5 minutes). Contamination was avoided by cleaning the crystal with 92.8% alcohol by using absorbent paper.

2.3 Data analysis

The high-wavenumber spectral region from 2800-3600 cm^{-1} was selected for Fourier-transform infrared (FTIR) spectral analysis. In order to evaluate the existence of statistically significant differences between saliva samples of control (n=11), smoker (n=9), and occasional smoker (n=8) groups, ANOVA and post hoc tests were used to evaluate intensity at the center of FTIR absorption bands and/or area under the curve for the wavenumber range of specific bands. Metrics of CH, OH, and NH vibrations of water and proteins were considered coming from the 3050-3600 cm^{-1} band, whereas CH_2 and CH_3 vibrations of lipids and proteins were assumed to originate

from the 2,800–3,050 cm^{-1} spectral region. Classification performance metrics were calculated by building classification models and evaluating their performance by using leave-one-out cross-validation.

3. Results

Fig. 1 shows that the spectral mean of each study group. overlap considerably. The most prominent and broad peak is the OH vibration of bound water, which is confined on molecules such as proteins of the dried saliva samples. Other characteristic saliva bands are present at 2850 cm^{-1} (CH3 symmetric stretching; vsCH3), 2875 cm^{-1} (vsCH3), 2930 cm^{-1} (CH2 asymmetric stretching; vasCH2), 2959 cm^{-1} (CH3 asymmetric stretching; vasCH3) and correspond to lipids, fatty acids and DNA. Less prominent bands appear as a shoulder of the broad OH spectral band. These bands are centered at 3070 cm^{-1} (vsCH3), 3207 cm^{-1} (NH symmetric stretching; vsNH), 3288 cm^{-1} (NH stretching of amide A and OH stretching of carbohydrates), and 3410 cm^{-1} (OH asymmetric stretching).

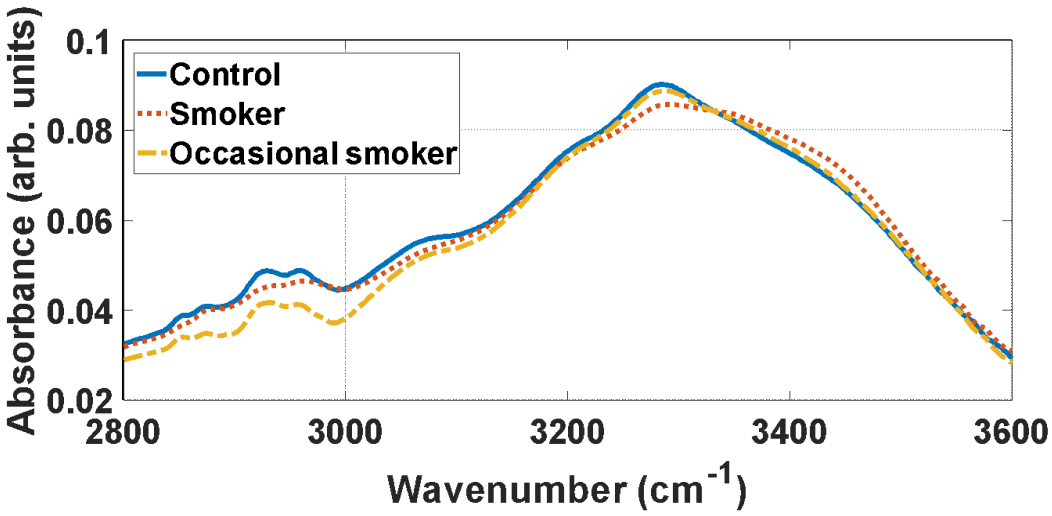

Fig. 1: Mean of the high-wavenumber region of the FTIR spectra collected in this study.

4. Conclusions

This is a pilot study with a small number of samples to evaluate the efficacy of the technique in the discrimination between the groups. We concluded that differentiating normal and smoking individuals can be performed by using high-wavenumber FTIR spectral analysis. In addition to this observation, we can show the relationship of water molecules bound to saliva biomolecules for control, smoker, and occasional smoker groups. In future studies, we aim to expand the number of patients and potentially use FTIR spectroscopy as a fast analytical technique to biochemically characterize saliva samples for early-stage oral cancer detection, as smoking is the main cause of oral cancer.

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