Brief Communication
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Valuable Modern Strategy (ATR-IR) Spectroscopy Technique to Distinguish Between Normal and Lung Cancer Tissue

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Abstract

Background: Diagnosis of lung cancer is often delayed because most of the patients are asymptomatic during the primary cancer stages. Infrared spectroscopy is an improved technique compared with the others for identifying abnormal tissue types because of its spatial and spectral capabilities. Therefore, this study is taking an advanced advantage of the physical and optical properties of the attenuated total reflection Fourier-transform infrared system.

Methods: The attenuated total reflection Fourier-transform infrared has been applied for cancer detection at infrared wavelengths that range from 4000-400 cm^{-1}. This technique may be a beneficial diagnostic method because it uses the principles of physics, as optics and photonics with a specific wavelength region, which can make an immense difference.

Results: The attenuated total reflection Fourier-transform infrared system spectra of normal lung tissue showed main peaks at 3321 and 1637 cm^{-1}, which has been assigned to the OH and C=O function group of amide I and has intensities of approximately 61% (OH) and 76% (C=O). That intensity has been shown to decrease in the cancer tissue. A new peak at 1545 cm^{-1} appeared in the cancerous tissue, which could be an amide II.

Conclusions: The identification of a biochemical component from either normal or cancerous lung tissue would help to evaluate malignant tissue. Thus, the obtained results indicated degradation of the biochemistry component (protein) of the tissue due to carcinogenic disease.

Keywords: Attenuated total reflection Fourier-transform infrared (ATR-FTIR), Cancer diagnosis, Carcinoma, Biochemistry component, Protein degradation

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Introduction

Detection of cancer is one of the challenges in medicine. Lung cancer is the most common cancer worldwide. Most often, if the tumor is diagnosed earlier and treated, the patient will have a better prognosis and much greater opportunity for a full recovery. The initial diagnosis of lung cancer is often delayed

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because up to 80% of patients are asymptomatic during the primary stages of lung cancer.[1]

Many recent technological innovations have used physics principles such as optics and photonics with specific wavelengths to improve early diagnostic and therapeutic procedures to moderate the morbidity and mortality of carcinomas.[2] One of the most challenging concerns in medical procedures, in order to identify or manage tumors, is the recognition of areas of inflammation because of the ambiguity between the cancerous and the adjacent non-cancerous tissues.

Physicians are faced with the problems of tumor detection and localization in order to determine the borders for resection and evaluate the resected bed after tumor extraction. Imaging techniques such as MRI, CT scan, and endoscopy are routinely used as diagnostic techniques for patients with suspected lung cancer. However, diagnosis is subjective and requires expert judgment.[3]

Numerous methods have been evaluated to find the most appropriate technique for cancer detection. The proposed methods include traditional excisional biopsy, laser induced fluorescence techniques which require injection of fluorescent dye, and CT with contrast which depends on X-ray radiation. These methods lack sensitivity and specificity as diagnostic tests because of their limitations.[4]

Infrared (IR) spectroscopy is an improvement compared with the above-mentioned techniques for identifying abnormal tissue types because of its spatial and spectral capabilities. IR spectroscopy is useful for identifying chemical composition, both qualitatively and quantitatively, of tissue components (e.g., water, lipid, collagen, carbohydrates) by measuring molecular absorption vibration bands of stretching and bending combination motions in these tissues.[5,6]

In this paper, we presented the properties of lung tissue that could be distinguished by attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR). We compared the differences between non-cancerous and cancerous tissues based on the components' physical properties. Molecular vibration alterations in the range of 4000 to 400 cm^{-1} wavelengths were measured.

The goal of this research was to develop advanced techniques for cancer diagnosis by taking advantage of the physical and optical properties of ATR-FTIR and highlight the differences in luminescent properties of malignant tumors versus non-cancerous tissues. This paper has set the basis for these goals by demonstrating the instrument's capabilities for lung cancer detection. We attempted to find an appropriate wavelength region to discriminate between cancerous and non-cancerous tissues.

Tests of cells and tissues can locate numerous diseases. Analysis of biopsy specimens by various testing methods is referred to as pathology. Not all irregular growths are cancers; other problems can cause growths that may resemble cancer. Numerous methods have been used to find the best for early cancer detection. Nevertheless, IR spectroscopy can be considered a useful tool for biological analyses[7] due to its sensitivity to the chemical composition and architecture of molecules.[2] It can penetrate much deeper into a sample than visible wavelengths.[2] Therefore, IR spectroscopy is a sensitive technique that can be very useful in early detection of cancers with no sample preparation. Visualization of the major biochemical components of tissue by ATR-FTIR spectroscopy has been utilized to determine the intensities of lipid and protein components for more accurate, useful, and faster diagnosis of lung cancer.

Materials and Methods

Sample preparation and physical properties

The Ethics and Research committee of Al-Muthanna University approved this study and all patients provided informed consent prior to the procedure at Al-Samawah Teaching Hospital. The 10 normal lung tissue biopsy specimens were obtained from the participants who also provided the lung cancer specimens. A total of 35 fresh specimens were obtained after surgery from

patients diagnosed with stage 1 lung cancer. All lung cancer tissue samples were obtained from patients who had previously confirmed diagnoses according to pathological inspections and biological tests performed as preoperative procedures. The lung specimens, each approximately 6-10 mm^2, were immediately cleaned in deionized water after resection, and then immersed in formalin (Figure 1). Finally, the tissue containers were placed on ice for transfer to the research laboratory where ATR-FTIR was performed on the samples.

The bulk of the lung tissues were exposed to ATR-FTIR to assess its effects on the tissue (Figure 2). The IR spectrum has been used to identify the functional chemical group properties of molecules by changing the rotational vibrational movements of excited electrons. The spectrum from the red edge that had a frequency of 430 THZ to 300 GHz was found to be useful for examining the absorption and transmission of photons by exciting the vibrational modes. Thus, we could distinguish tissues through changes in the dipole momentum. This enabled us to inspect the main tissue biocomponents of lipids, collagen, amide II, and amide I.

Figure 1. Lung tissue specimens immersed in formalin.

ATR-FTIR system and measurement technique

We collected the IR spectra of the analyzed data in transmission mode by using FTIR with an adjustable diamond crystal detector to select an area from which spectral data were obtained. The process was performed by placing samples of normal or cancerous tissues on a Platinum ATR single reflection diamond crystal. The measurements were performed at a 4000-400 cm^{-1} wavelength region by using a Bruker Tensor 27 FTIR that employed the advantage of a supplementary device (ATR) such that the procedure did not require the traditional technique that uses potassium bromide (KBr). The OPUS 7.5 program was used for data analysis.

Results

We applied a new strategy (ATR-FTIR) for early detection of lung cancer tissue that characterized the components of the cancerous tissue in comparison with normal tissue. Our result suggested that ATR-FTIR has a valuable potential for early identification of cancer tissue, which is dependent on the physical principle of specific wavelengths of the spectrum. However, the optical characterization of the normal tissue revealed marked absorption peaks at 3321, 2928, and 1637 cm^{-1}, which were potentially useful for a more accurate quantification of water and amide I. The cancer tissue spectra showed an additional new peak at 1545 cm^{-1}. The intensity of that peak reduced in the cancer tissue, which could be due to protein degradation.

These valuable accurate results that have been obtained by ATR-FTIR compared with conventional methods for histopathologic tissue diagnosis, which are time-intensive and can delay decision making during diagnostic and therapeutic procedure, can open the way for development of an automate and biocompatible handheld IR spectrometry device. In the near future, this device

could enable rapid and nondestructive diagnosis of human cancer tissues. A handheld mass spectrometry device, MasSpec Pen,[12] has been introduced based on mass spectrometry. Disadvantages of this technique compared with IR spectrometry include: 1) high energy needed from the source; 2) the physical principle of mass spectrometry involving analysis of molecule fragments followed by the measurements of the mass-to-charge ratios of these fragments. Thus, the obtained fragments of ionized chemical species would not be easy to assign a corresponding structure; therefore, not easy to interpret the obtained data. However, IR measures the bond vibration frequencies in a molecule and is used to only determine the functional group; 3) completion of a scan in 3-5 min, whereas IR needs 1-2 s; 4) IR spectrometry takes several scans and averages them and has a laser beam that keeps the instrument accurately calibrated; and 5) the MasSpec pen is an expensive, complex machine compared with IR spectrometry. Previous studies focused on short wave IR spectroscopy (SWIR, ~100 to 2000 nm) associated with imaging techniques and/or visible and near-IR (~400 to 100 nm).[4,13,14] Near-IR have some disadvantages such as limitations of depth sensitivity where, in adults, this typically reaches 5 to 10 mm beneath the inner surface of the skull for brain activation. In addition, a single NIRS measurement is sensitive to a volume of tissue that falls between the source of light entering the tissue and the detector receiving the light that diffuses out of the tissue,[15] in contrast to ATR-FTIR, which overcomes these limitations. We have performed the first measurements on lung cancer tissue by ATR-FTIR at a wavelength range from 4000 to 400 cm^{-1}. It is expected that this new, simple, and faster method will lead to multiple studies on different types of cancer tissue. This could improve the early detection, enable a better prognosis and much greater opportunity for a full recovery by reducing the limitations of the other methods.

Discussion

An important challenge in today's medicine is the early diagnosis of cancer before it has the chance to grow and spread. In this study, we have used a newer, simple, less expensive, and faster ATR-FTIR technique, which increases the chances of detecting certain cancers. We monitored this technique during treatment rather than using common techniques such as laboratory analyses, imaging procedures, and tissue cultures. Figure 3 shows the ATR-FTIR spectra of normal and cancer lung tissues. The normal tissue spectra revealed a chemical structure of an amide I and an alkyl group. A typical broad band of approximately 3314 cm^{-1} is usually assigned to overlap OH and NH vibration for carbohydrate and protein components. This broad peak could be due to hydrogen bonding interactions between carbohydrates and proteins with water, which normally increase in cancer tissue. The stretching vibration adsorption band of aliphatic CH was observed at 2928 and 2853 cm^{-1}. These peaks began to disappear on the cancer tissue spectrum due to increased protein degradation.[8] The band centered at 1637 cm^{-1} could be assigned to the stretching vibration of the peptide C=O groups of amide I, which overlaps with the important OH bending band of the trapped water molecule within the tissue matrix.[9] This value was applicable to results from previous studies on differential protein folding of lung tissue by the FTIR microscopy technique.[10] On the other hand, the intensity of OH and C=O stretching vibration band showed high abundances of approximately 0.61% and 0.78%,

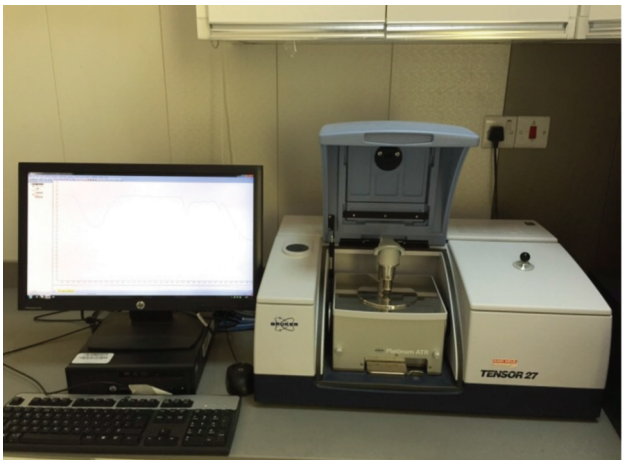

Figure 2. FTIR spectroscopy analysis of the lung tissues.

Table 1. Peak intensities of normal and cancer tissue components.

Components	Peak area (cm^{-1})	Normal tissue (%T)	Cancerous tissue (%T)
Lipids and Carbohydrates	2866, 2928,	0.97, 0.99	N/A
Protein Amide I	1645, 1025	0.78, 0.91	0.76, 0.91
Amide II	1547	N/A	0.95
Water and OH (Sugar)	3321	0.61	0.67

respectively, in normal lung tissue (Table 1), whereas there was a decrease in intensity of the C=O peak (0.76%) and increased water (OH peak; 0.67%) in the lung cancer tissue (Table 1). We observed a new peak at 1542 cm^{-1} in the cancer tissue, which we attributed to the NH bending vibration coupled with C-N stretching vibration of amide II absorbance.[11] The appearance of that new peak accompanied by decreased intensity of all the functional group peaks was a good indication of carbohydrate and protein degradation, which was due to the existence of cancer cells in the tissue. However, this degradation of biochemical components could increase with time and lead to decreased curve of ATR-FTIR intensity peaks. In general, changes in ATR-FTIR spectra between normal and cancer tissue might arise from secondary cellular events such as increases or decreases in substances (DNA, RNA, lipids, proteins, and carbohydrates). These results confirmed the successful diagnosis of lung cancer by ATR-FTIR.

Conclusion

The IR spectrum has many applications for medical purposes. The diagnosis of cancer diseases is one of the best medical applications by studying changes in IR spectra between normal and abnormal tissues, which might be raised for various reasons. Specific changes in the genetic code (DNA, RNA) of cancerous cells contribute to spectral changes. In addition to the increases or decreases of components such as lipids, proteins, and carbohydrates cause distinct IR patterns. Finally, the variations between healthy and cancer samples could be observed from different intensities of the transmitted IR spectra for different bending bands and functional group peaks. In the future, we intend to manufacture a portable IR spectrometry device sensor that will have high accuracy and enable early diagnosis.

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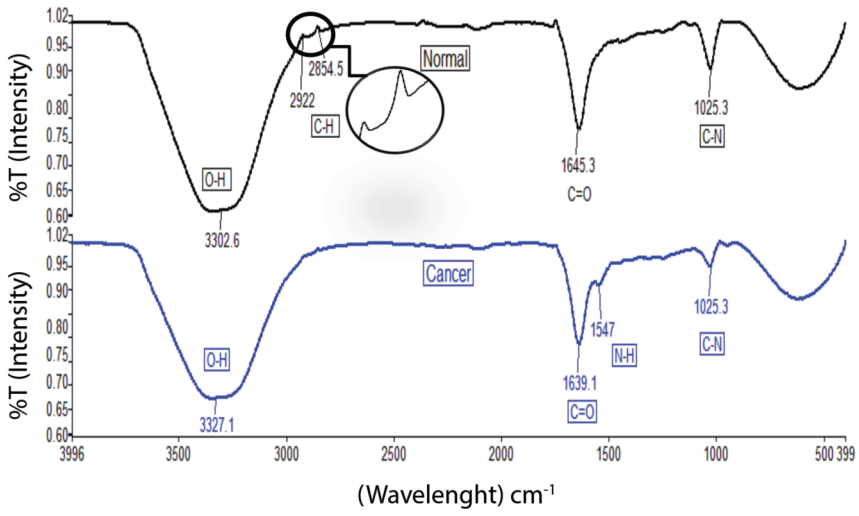

Figure 3. Overlap of ATR-FTIR spectroscopy spectra showed a decrease in the biochemical component of lung cancer tissue compared to normal tissue.

supplying the lung tissue samples.

Ethical statements

The Ethics Committee of the College of Pharmacy at Al-Muthanna University approved this research in accordance with the Declaration of Helsinki and guidelines of the Iraqi Ministry of Education and Scientific Research.

Statement of human rights

The study was approved by the appropriate Institutional Review Board (IRB) and was conducted in accordance with the ethical standards written in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

This article does not contain any studies with animals performed.

Informed consent was obtained from all individual participants included in the study.

Conflict of Interest

None declared.

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