Review Article

FTIR Spectroscopy: A New Technique In Cancer Diagnoses

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Abstract: Infrared spectroscopy of biological cells is a rapidly growing area of medical research. It involves the study of the difference of the spectra of normal and diseased cells such as cancer cells. The difference in spectra suggests that there are structural, chemical and metabolic changes occurring in the diseased cells. FTIR spectroscopy can be used in a myriad of clinical situations from inflammatory lesions to cancer diagnoses. In tumor diagnosis, it can be used in borderline, indeterminate or false negative cases as it detects early changes based on characteristic molecular vibrational spectra of cells, before morphological changes could be seen under light microscope. In microbiology, FT-IR could identify different strains of micro-organisms from viruses, bacteria, fungi and parasites. Although it has wide applications, it is yet to be widely used in routine clinical diagnostic practice. Many pathologists and other diagnostic laboratory physicians are somewhat reluctant to use this technique in routine practice, probably due to unfamiliarity with FT-IR micro-spectroscopy.

KeyWords: Fourier transform infrared spectroscopy (FTIR); Cancer; Diagnosis FTIR Spectroscopy

Fourier transform infrared (FTIR) spectroscopy measures the absorption of infrared radiation by chemical bonds in functional groups of molecules. The frequency range of absorption by these molecules is correlated with the structure of the molecules. FTIR spectroscopy is usually used in chemical, biochemical laboratories and in industries mainly to identify inorganic and organic materials. (1)

The development of FTIR spectroscopy began with the invention of two-beam interferometer by Michelson almost a century ago. The past decade has witnessed a revolution in the use of infrared spectroscopy. This progress is largely resulting from rapid commercial development of FTIR spectroscopy. (2)

In FTIR spectroscopy the spectra of both test and control samples are taken using the same number of scans and resolutions. The results are in the form of various spectra due to absorption of certain molecule at certain frequency. (3)

For the past thirty years, thorough researches on the spectroscopic features of biological molecules in live tissues have led to the application of FITR spectroscopy to study human biology. It is now possible to probe the biochemical changes of normal and abnormal cells by FTIR spectroscopy.

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This new technique provides a promising way to examine abnormal cells other than the conventional light or electron microscopy. All disease states are caused by changes in cellular and/or tissue biochemistry. We highlight in this paper previous and current ongoing researches on the clinical use of FTIR spectroscopy.

FTIR spectrometer is an effective tool for studying different molecular structures of living tissues. During the last years great discovery of data has been elaborated by FTIR spectroscopy relating to the structural properties of protein, (4,5) lipid, (2,6) and nucleic acids.(7) Chirgadze & Nevskaya in 1976 studied the difference between FTIR spectra of amide I and amide II,(8) Bendekar and Krimm in 1985 showed correlations between amide frequencies and gamma-turn structures,(9) and and Pande et al on metallothionein protein.(10)

In 2000 Bouchard used FTIR spectroscopy to study the structure of insulin and they reported that formation of amyloid fibrils by insulin requires substantial unfolding of the native protein.(11) Earlier in 1986 Bayler & Susi studied the secondary structure of proteins (12) and in 1977 Liquier and coleagues demonstrated that FTIR spectroscopy could allow scientists to identify the different conformations of DNA.(13) Since then there are many more complex studies on DNA and RNA structures.(14-16)

FTIR spectroscopy had been used to investigate lipid structure by several researchers. Among these, Goormaghtigh et all evaluated secondary structure of low density lipoprotein (LDL)(17) and Liu et all on serum cholesterol, HDL and LDL.(18) They concluded that FTIR spectroscopy had the potential to be used as a method of choice for quick and simultaneous determination of LDL and HDL cholesterol. Interactions of important molecules in complicated mixtures could be studied with improved instrumentation.

In almost all disease states, diagnostic tests depend on detection of the cellular abnormalities. This altered cellular state is due to al

terations in the biochemical and molecular constituents. Under light microscopy, these changes are seen in nuclear and cytoplasmic abnormalities. Identification of morphologic changes under light microscopy is subject to observer's experience. Since FTIR detects early changes, FTIR spectroscopy offers an interesting alternative to detect biochemical changes by non-subjective technique.

In general, FTIR spectroscopy has a number of advantages over other diagnostic techniques. It is relatively cheap. It requires no tissue/cellular fixation and it is not dependent upon human judgment to detect cellular/molecular changes. It can detect changes earlier than changes seen under light microscopy as cell biochemistry precede any morphological manifestations.

FTIR: Cells and Infrared Absorption

Cells contain glycogen, proteins and nucleic acids. The ratio of these structures to each other varies according to the state of the cells. When infrared radiation falls on these structures, quantum of specific energy is absorbed. The study of infrared bands in human cells rely on multiple studies of infrared spectra of these cellular constitutes in isolation and in complex mixtures. The results obtained are the summation of these different spectral types weighted according to relative concentrations.

FTIR spectrums of lipids (e.g. phospholipids) are found in the region 2800-3000cm-1. They are due to the asymmetrical and symmetrical stretching vibrations of CH3 (2956 and 2874 cm-1) and CH2 (2922 and 2852 cm-1) groups of alkyl/acyl chains. Since there are a greater number of methylene groups in phospholipids, the intensity of the CH2 absorptions is 10-20 times that of the corresponding CH3 absorption. The region from 1800- 2800 cm-1 is free of infrared absorptions in biological cells. Bands in the region from 1600-1800 cm-1 are related to C=O stretching vibrations. (19,20)

Amide I, which is one of the major components of proteins, has absorption bands in the region between 1600-1800 cm -1 arising from the C=O stretching vibration of the amide C=O. It is sensitive and can be used to study secondary structure of proteins.(4) Amide II absorption is due to N-H bending vibration coupled to C-N stretching. It is seen at 1500-1560 cm-1. Amide III absorption is due to C-N stretching and N-H in-plane at 1250-1350 cm-14.

The region from 1000-1250 cm-1 has absorption bands from vibrational modes of phosphate groups. Nucleic acids are composed of phosphodiester bonds which have two infrared bands; symmetric (1087 cm-1) and antisymmetric (1224 cm-1) PO2 stretching vibrations. The absorptions for carbohydrates (including glycogen) are found in the region between 1000-1200 cm-1 and are attributed to C-O stretching vibrations. Carbohydrate absorption bands are only seen in tissues with high carbohydrate content (e.g., cervical smears).(21)

FTIR and Cancer Detection

In normal cells, absorption at 1240 cm-1 is attributed by the PO2 asymmetric stretching vibration of phosphate groups in nucleic acids. In malignant cells there is a shift to lower wave numbers, which arise from an increased absorption at around 1225 cm-1. It has been suggested that this spectral changes may indicate changes in the hydrogen bonding pattern of nucleic acids, which in turn indicates structural alterations in the DNA of malignant cells. Features common to all types of cancers observed in many studies are reduced glycogen level, increased hydrogen bonding of the phosphodiester groups of nucleic acids, decreased hydrogen bonding of the C-OH groups of proteins, increased hypomethylation, and increased disorder of the methylene chains of membrane lipids.

When cells become malignant, the changes in chemical structure of the cells include reduction in glycogen content, increase in hydrogen bonding of the phosphodiester groups of nucleic acids, decrease in hydrogen bonding of the C-OH groups of proteins, increase in hypomethylation and increase in disorder of the methylene chains of membrane lipids. These changes could be detected with FTIR spectroscopy. This technique now paves new ways in tumour diagnoses.

Biochemical changes precede nuclear and cytoplasmic morpho logic changes, and hence FTIR spectroscopy could detect the chemical changes during cancer development before morphologi cal cytological changes are detectable under light microscope.

Many studies have shown that FTIR spectroscopy could differentiate the biochemistry of normal and neoplastic cells significantly. Many cancers have been studied using FTIR spectroscopy such as carcinoma of the cervix,(22,23) colon,(24) breast,(25,26) esophagus,(27) stomach,(28,29) prostate,(30,31) and pancreas.(32)

Malins D.C et al found significant differences in FT-IR spectral properties of DNA of normal and cancerous breast tissue with a sensitivity and specificity of 83% proposing it could be used to de tect very early lesions. (25) Later on this group of researchers showed that Benign Prostatic Hyperplasia had distinct spectral characteristics than adenocarcinomas of prostate and suggested the change from benign to malignant prostate epithelial cells was due to hydroxyl radicals.(31) Both studies gave better understanding on carcinogenesis of breast and prostate cancers. Neviliappan S. et al evaluated and compared the infrared spectral features of normal and malignant exfoliated cervical cells and they found spectral bands of malignant cells were markedly different from those of ex foliated normal cervical cells. (21) FTIR could also differentiate adenomatous polyp from colon cancer (33) and normal crypt from abnormal ones in colonic mucosa which appear normal histologi cally.(34) Sadhu et al proposed FTIR could be used in false nega tive biopsies as it detect biochemical changes before morphological changes is seen.(34)

Salman A. et al studied inflammatory bowel diseases (IBD) and its association with colon cancer using FTIR spectroscopy. (24) They noted FTIR could differentiate IBD from colon cancer, some times these two diseases pose a diagnostic challenge to pathologists. Researchers have also studied other cancers such as

esophageal cancer,(27) melanoma,(35) stomach(28) and non-solid tumour; leukemia.(36) In these tissues, significant differences have been seen between FTIR spectra of normal and malignant tissue. Li et al concluded that FT-IR could differentiate normal gastric epithelium, gastric atrophy, superficial gastritis and gastric cancer with high sensitivity (range from 82.5 to 91.5%) and specificity (89.5 to 97.3%).(28)

We recently concluded a study comparing Pap smear cytology and FTIR spectroscopy. FTIR spectroscopy could differentiate normal from abnormal cervical cells in 800 samples examined, more samples that studied by other researchers previously. The sensitivity was 85% , specificity 91% , positive predictive value 19.5% and negative predictive value of 99.5%.(37,38) Such observation implies that FTIR spectroscopy is a good alternative for cervical cancer screening. In countries which have limited number of cytopathologists, FTIR spectroscopy offers a reasonable alternative.(37,38)

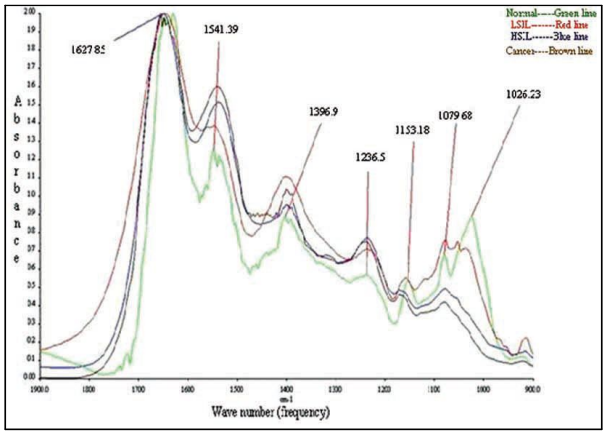

Fig. 1. The difference of spectra of normal, LSIL, HSIL and cancer using FTIR spectroscopy [Ref 38].

FTIR Spectroscopy and Other Clinical Diagnosis

FTIR spectroscopy has also been used for diagnoses of arthritis,(1,39) B-Thalassemia,(40) G6PD deficiency,(41) cardiomyopathy.(42) In addition FTIR could discriminate different phenotypes and genotypes of bacterial and fungal species and provides a reliable and reproducible rapid technique for differentiation, classification and identification of micro-organisms.(43) van der Mei and colleagues differentiated different strains of oral streptoccoci (44) and Kirschner, C et al on species of enteroccoci.(45) Tintelnot et al differentiated Candida dubliniensis from C. albicans (46) and Cryptococus neoformans from Cryptococcus gattii using FT-IR spectroscopy combined with hierarchical clustering.(47) FT-IR combined with artificial neural network can provide rapid diagnosis in identifying bacteria in mixed isolates.(48) Cells which are infected by viruses could also be identified.(49)

FTIR spectroscopy has also been used in screening of neonates and babies. Lin H.L. et al demonstrated that FTIR micro spectroscopy is a rapid and effective noninvasive diagnostic method to diagnose hypothyroidism using scalp hair roots of neonates.(50) Liu et al found that IR spectra revealed changes in the secondary structure of hemoglobin in ?-Thalassemia patients, inferring FTIR spectroscopy could be an adjunct tool in the diagnosis and screening of ?-Thalassemia.(51) David-Vaudey, E. et al used FTIR micro-spectroscopy to measure proteoglycan and collagen contents in superficial, mid, and deep zones of cartilage and found there was significant difference in these compounds between healthy and arthritic joints.(52) Wang and colleagues used FTIR spectroscopy to differentiate healthy cardiac muscles from cardiomyopathy. They attributed the difference is due to biochemical components especially collagen fibers.(42)

Limitations of FTIR Spectroscopy

The use of FTIR spectroscopy as a non-invasive diagnostic instrument is limited by the difficulty in interpreting subtle changes between the spectra. This could be overcome by improving the capability and imaging technique of FTIR spectroscopy. Such difficulty is also seen in histology or cytology where subtle early changes could be missed. FTIR instrument and its spare parts are expensive, it is sensitive to water vapor, difficult to be used for thick samples or water rich samples and produce overlapping absorption bands in mixed chemical components.

Many pathologists and other diagnostic laboratory physicians are somewhat reluctant to use this technique in routine practice, probably due to unfamiliarity with FT-IR.

Conclusion

FTIR spectroscopy is used in a myriad of clinical situations from inflammatory lesions to cancer diagnoses. In tumour diagnosis, it can be used for borderline or indeterminate cases or false negative case as it detects early changes based on characteristic molecular vibrational spectra of cells, before morphological changes could be seen under light microscope. In microbiology, FT-IR could identify different strains of micro-organisms from viruses, bacteria, fungi and parasites. Although it has wide applications, it is yet to be widely used in routine clinical diagnostic practice.

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