

Infrared spectroscopic analysis of tumor pathology

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Infrared spectra of normal and malignant breast tissues were measured in the 600 cm⁻¹ to 4000 cm⁻¹ region. The measured spectroscopic features which are the spectroscopic fingerprints of the tissues contain the vital information about the malignant and normal tissues. Fourier Transform Infrared (FTIR) data on 25 cases of infiltrating ductal carcinoma of breast with different grades of malignancy from patients of different age groups were analyzed. The samples were taken from the tumor sections of the tissue removed during surgery. Infrared spectra demonstrate significant spectral differences between the normal and the cancerous breast tissues. In particular changes in frequency and intensity in the spectra of protein, nucleic acid and glycogen vibrational modes as well as the band intensity ratios for lipid/proteins, protein/nucleic acids, protein/glycogen were observed. This allows to make a qualitative and semi quantitative evaluation of the changes in proliferation activity from normal to diseased tissue. It was evident that the sample to sample or patient to patient variations were small and the spectral differences between normal and diseased tissues were reproducible. The findings establish a framework for additional studies, which may enable us to establish a relation of the diseased state with its infrared spectra.

Keywords: Ductal carcinoma proliferation, Fourier transform, Infrared spectroscopy

A rapid, economical, sensitive and standardized laboratory technique for early detection of cancer is highly sought after to reduce the alarming mortality rate due to cancer. The malignancy of a tissue, its invasive and metastatic potential, results from its failure in controlling proliferation and differentiation of cells in an integrated manner. These malignant transformations manifest themselves as cellular and subcellular biochemical changes. To distinguish a pathological tissue from a normal one, a technique needs to sense the changed biochemistry of the cell. Many efforts are being directed to improve the early diagnostic and prognostic methods. Nowadays high resolution techniques are being developed for investigating initial stages of the transformation processes resulting from a pathology. Among these methods vibrational spectroscopy has been employed successfully, as this technique offers possibility of qualitative as well as quantitative analysis on different chemical composition and molecular structures of

healthy and diseased tissues¹. Although the extraction of specific molecular information from such complex systems has not been fully achieved, assessment of spectra in terms of pattern recognition with sophisticated mathematical algorithms has produced promising results. Fourier Transform Infrared Spectroscopy (FTIR) is being extensively used to detect and monitor the characteristic changes associated with the transformation of a normal tissue into a cancerous tissue²⁻⁸. The development of an infrared spectroscopy based diagnostic method for detection of malignancy would have definite advantages over normal pathological methods such as X-ray transmission, ultrasound and computer tomography techniques. The histopathological examination of tissue samples by focusing on the bulk morphological features lacks quantitative accuracy and provides no information of the biochemical background of the changes that occur due to carcinogenesis.

In recent years several studies have been conducted on human cancers to examine, in detail, the spectroscopic properties of normal and carcinomatous cervix, colon, cervical, liver, lung thyroid, prostate, lymph system and endometrium and breast tissue⁹⁻¹⁵.

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These studies highlight the potential of infrared spectroscopy in identifying neoplastic tissues and differentiating them from the normal ones. Attempts have also been made to differentiate a normal and leukemic lymphocytes¹⁶. The spectral differences have been characterized and some markers have been established for identifying the disease in the tissue sample. IR based approach is powerful and sensitive to reveal changes in the structural and the biochemical properties that occur in healthy and abnormal tissues. Fingerprint region of FTIR spectra has been largely employed to highlight the characteristic differences both in the frequency and the intensity ratios. The studies have made use of rigorous mathematical analysis of the spectral data, like Fourier self-deconvolution and second order derivatization. The technique has enabled one to study the state of chemical bonds and relative concentration of lipids, proteins, carbohydrates, and phosphorylated molecules.

In the last few years incidence of breast cancer among women is increasing at an alarming rate. The causative factor of deaths due to breast cancer is its late detection. In most of the cases which suffer from cancer, the disease may start occurring at the age of around 30 years, while the detection of the disease becomes possible quite late. It takes almost 10 years to detect the disease as the detection has primarily been based upon the screening mammography followed by pathological examination of tissue samples (biopsies). More sophisticated techniques like magnetic resonance imaging (MRI), positron emission tomography (PET) and digital mammography have also come up. These techniques are not without shortcomings. Due to the existence of a diverse array of phenotypes for breast cancer, clinical distinction of adenomas (benign growths) from cancer of breast and from normal tissues using conventional histopathology is vague and difficult. The chances of error are substantial.

FTIR spectroscopy has been applied for analysis of breast cancer tissues. A comparative study of breast tumors and breast cell xenographs using infrared spectroscopy was reported by Fabian⁸. Their work has pointed out that variation in the collagen content of the tissue or heterogeneity of the sample may lead to spectral differences between normal and malignant tissues. Alterations in breast DNA leading to cancer type phenotype have also been characterized using FTIR¹⁷. However the technique involves isolation of DNA.

In the present study we have analyzed few samples of infiltrating ductal carcinoma, lobular carcinoma and adenomas of breast and have made their comparison with the normal tissues to obtain more information and comprehension of breast cancer using FTIR spectroscopy technique.

Materials and Methods

Tissue sampling—Samples from 25 cases of breast carcinoma (infiltrating type, Grade II) and 5 cases of Grade III disease were collected from patients under treatment at Rajeev Gandhi Cancer Research Institute, Delhi. After the lumpectomy/mastectomy of breast, the samples of cancerous tissue and normal tissue (2-3 cm away from the tumor) were taken. For each case two sections were cut, one was put on the glass slide and was used for histology review. The other part of the tissue was frozen (-27.8°C) to obtain cryostat sections (3-4 μm), which were taken on Zinc Selenide crystal plates, 1.5cm \times 2.5cm \times 0.2cm. The tissue sections were simply attached to the plates without any fixative.

Spectral measurements—FTIR measurements were performed in the transmission mode. Bio-Rad 175 C FT-IR spectrophotometer equipped with a deuterated triglycine sulphate detector was used to record the spectra. The spectra were scanned in mid-IR range from 600 to 4000 cm^{-1} , with a resolution of 4 cm^{-1} . Sixty-four scans were coded for each spectrum and the spectra were ratioed against the background spectrum. The spectra were normalized after the baseline correction of the entire spectrum. Second order derivatives of all the spectra were also calculated.

Results and Discussion

The spectra of the normal breast and the malignant breast tissues were recorded in frequency range 700 cm^{-1} - 3850 cm^{-1} . The spectra of the normal and the malignant tissues of different patients were compared and one of the typical cases is shown only for brevity in Fig. 1. The malignant cells show appreciable biochemical deviations from their normal forebears in all the cases. The spectra of the normal tissues showed well defined spectral features, while the spectra of the malignant tissues appeared to be more complicated. In some easily identifiable regions, the differences between the normal and the carcinomatous tissues, were highly marked. In order to isolate broad overlapping absorption bands and the baseline difference due to the change in the optical properties of biological tissue, calculation of second

derivative spectra becomes important. Calculation of the second derivative enhances spectral features and also compensates for baseline shifts. The Savitzky-golay second order polynomial was used with 21 data points to obtain second order derivative spectra.

The spectral assignments were based on earlier data^{9,18-21}. Figure 2a shows the overlaid, derivative spectra of the normal and the malignant breast tissues in 700 to 1900 cm^{-1} region. This region is associated with some characteristic bands of cellular constituents, nucleic acids^{18,19}, phosphates, proteins and breast tissue carbohydrates mainly glycogen. Changes in the overall biochemistry of the cells during carcinogenesis were quite evident from the spectra. The spectrum of the malignant tissue showed a strong peak at 1076 cm^{-1} which was present only as a shoulder at 1097 cm^{-1} in the spectrum of the normal tissues. The absorption was attributed to symmetric phosphate ($\nu_s \text{PO}_2^-$) stretching vibrational modes of phosphate (PO_2^-) groups^{18,19}, contributed largely by nucleic acids in the breast tissues. The strong anti-symmetric phosphate ($\nu_{as} \text{PO}_2^-$) stretch vibration evident at 1238 cm^{-1} in the spectrum of the malignant tissues appeared to have undergone a low frequency shift from 1253 cm^{-1} in spectrum of the normal tissues. The absorption is usually influenced by the changes due to coupling between CH_3 rocking vibrations and phosphodiester stretching vibrations within the nucleic acids¹⁹. The spectral difference indicated a change in coupling of PO_2^- groups with alkyl groups in malignant tissues. Another obvious difference between the spectra of the normal and the malignant tissues was in the glycogen associated band at 1157 cm^{-1} . The absorption was almost absent in the malignant tissues suggesting a marked decrease in the glycogen level of the breast tissues during malignancy. The spectral features were indicative of increased DNA and decreased protein content in the malignant samples. These changes were prominent and were common to other types of malignant tissues^{9,18,19}. The protein region of the spectra (1300-1800 cm^{-1}) housing the three conformational sensitive amide I, II and III regions showed significant variations in the profiles of the normal and the malignant tissues. The malignant tissue showed highly intense protein bands at ~ 1543 and 1653 cm^{-1} indicating high concentration of protein in the malignant samples unlike the normal samples where lipid concentration appears to be high as indicated by ester carbonyl absorption at $\sim 1746 \text{ cm}^{-1}$. The band

shift in this region may also suggest a changed configuration of protein and lipids. The normal tissues exhibit two peaks at 1359 and 1454 cm^{-1} out of which the former shifts to 1396 cm^{-1} and the latter disappears in the cancerous tissues.

Remarkable changes in the lipid and N-H region 2600-3850 cm^{-1} are depicted by the overlaid derivative spectra in Fig. 2b. Two main peaks at ~ 2849 and 2932 cm^{-1} resulting from stretching vibrations of the CH_2 and CH_3 groups in acyl chains of the lipids or in the proteins were similar in two types of tissues. The relative intensity of 2849 to 2932 cm^{-1} peak increased for malignant tissues. Increased methylation has usually been associated with gene inactivation or diseased state. The unsaturated C-H stretch peak was at $\sim 3007 \text{ cm}^{-1}$ in the normal tissue and shifted to a higher frequency, at 3057 cm^{-1} in the malignant tissue indicating a change in the configuration of acyl chains or proteins. The strong N-H stretch peak at $\sim 3292 \text{ cm}^{-1}$ in the cancerous tissues was not present in the normal tissues.

Representative spectra of the tissues with infiltrating ductal carcinoma of Grade II and Grade III type were compared with the normal tissue (Fig. 3). It can be clearly seen that spectral features that were abundant in the normal tissues appeared diffused as the disease progresses. The variations in the nucleic acid and carbohydrate region were markedly significant. The intensity of glycogen band at 1163 cm^{-1} decreased with the advancement of the disease. In grade III carcinomatous tissues, the band totally disappeared. Another noteworthy feature was the increase in the intensity of nucleic acid bands at 1097 and 1244 cm^{-1} in Grade III tissues. Absorption at $\sim 1365 \text{ cm}^{-1}$, not present in normal tissues, appeared in Grade II tissues and become quite significant in Grade III tissues.

The relative intensity of major absorption bands was also important spectral parameter in obtaining the semi-quantitative information about the relative contents of biomolecules among these tissues. The change in the absorbance ratios of these bands was observed in several studies on cancerous tissues. The spectral data indicated an increase in the protein and nucleic acid content in the malignant samples. The relative intensity of (1657/1539) band, the ratio of amide I/amide II were higher in malignant tissues in all the samples. This may be due to the change in type and extent of hydrogen bonding to protein amide groups that occurs during malignancy. The intensity

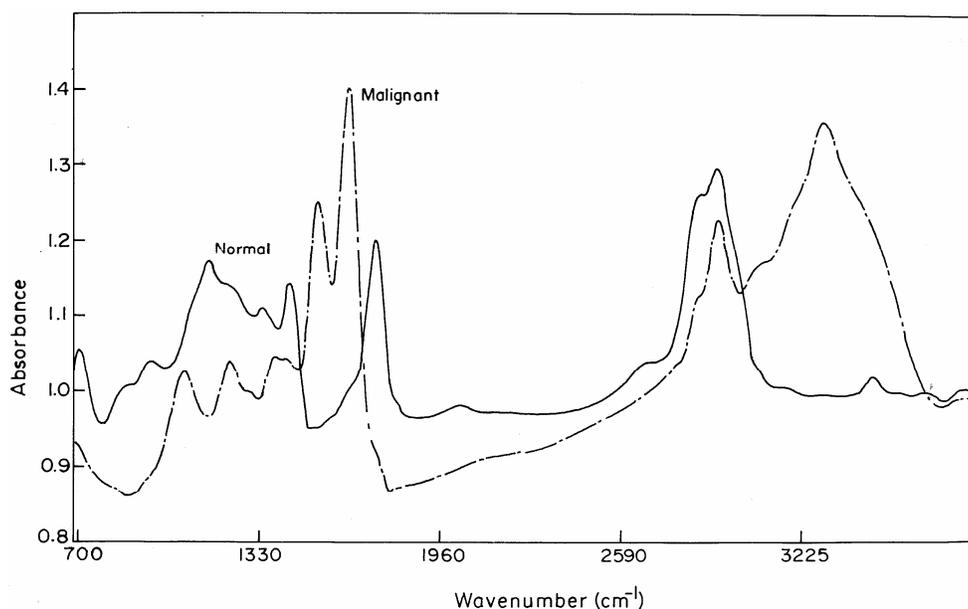


Fig.1—Infrared spectra of normal and malignant breast tissue of a patient.

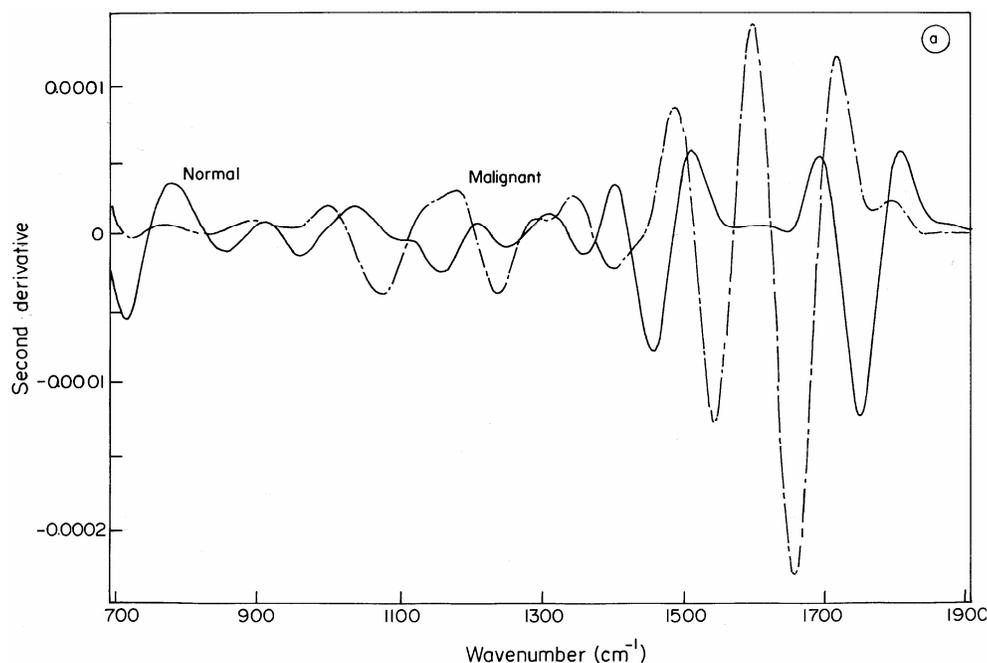


Fig. 2a—Second order derivative of infrared spectra of normal and malignant breast tissues in 700 to 1900 cm^{-1} region.

ratio (1657/1244) band was higher in most of the malignant samples. Ratio (2928 / 2864) band was significantly higher in the malignant tissues which indicate increase in the number of CH_3 groups compared to CH_2 groups in the malignant tissue, which is in agreement with increase in number of protein fibers.

It emerges from the above discussion that the IR spectral features hence the chemical composition of normal breast tissues was significantly different from cancerous tissues. These changes can be correlated with the changes in some of the biological activities during breast malignancy and hence can be used for differentiating normal tissues from malignant ones.

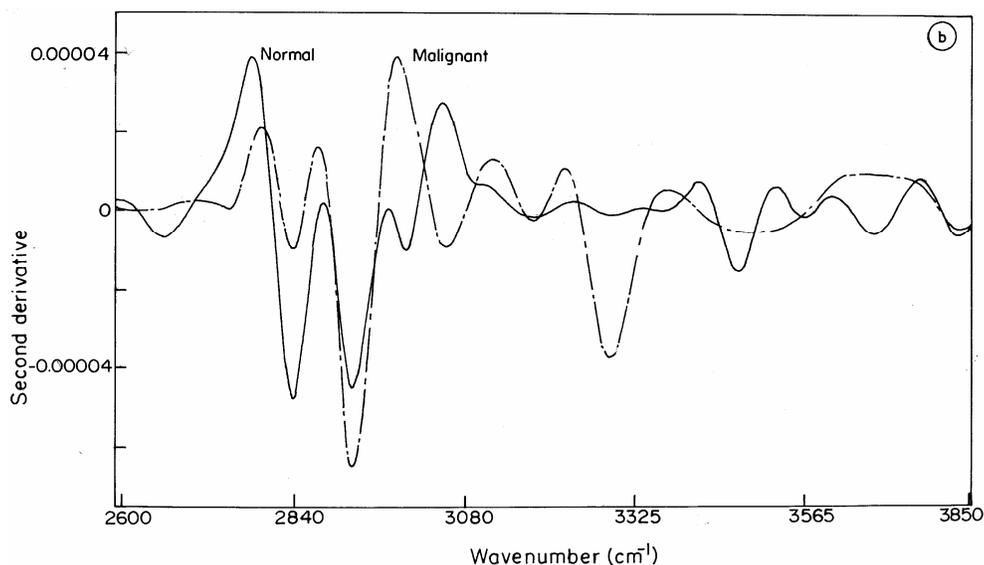


Fig. 2b—Second order derivative of infrared spectra of normal and malignant breast tissues in 2600 to 3850 cm^{-1} region.

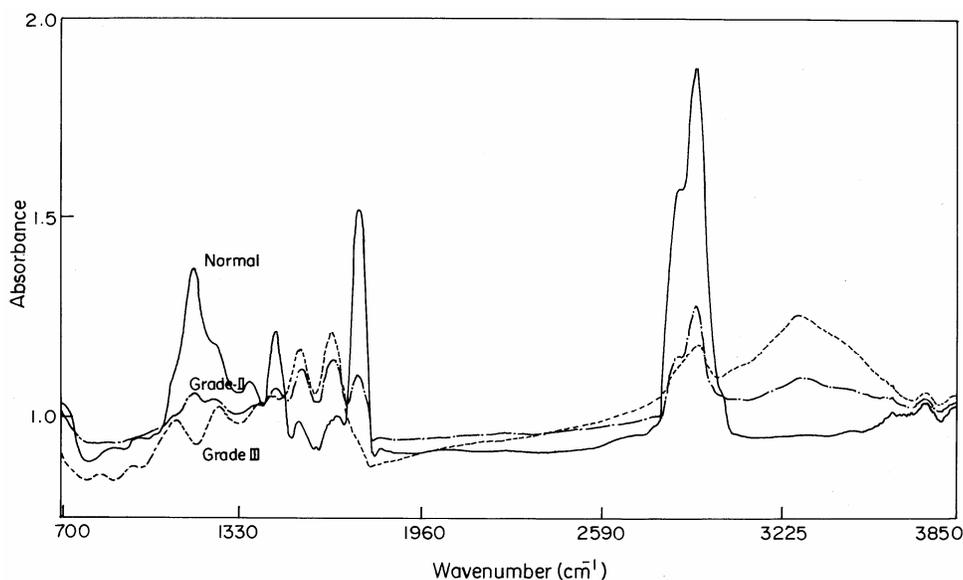


Fig. 3—Infrared spectra of normal and malignant Grade II and Grade III breast tissues.

Conclusion

The onset and development of cancer is a multistep process that is controlled by many genes that regulate the biochemical reactions undergoing at the molecular level inside the body tissues. These are affected by many factors and the process of disease involves changes in concentration of protein, nucleic acid, sugar and fat in cells. FTIR spectroscopy is a powerful technique for providing useful information regarding biochemical changes occurring in the tissue and hence in diagnosing cancer.

The results of the present study have shown that remarkable differences exist between the spectra of

the normal and the infiltrating ductal carcinoma tissues in terms of spectral profiles, absorption frequencies and absorbance ratios of prominent absorption bands. The spectral differences reflect the changes in the content, conformation and composition of the nucleic acid, protein and fats in the cells. The results are in accordance with histopathological observations such as nucleus condensation in cancerous tissues. In addition to the detection of malignancy, the technique can be applied to the identification of the diseased tissue states. Differences in the intrinsic biochemical composition are reflected in their mid-IR spectra and the spectra are highly

characteristic of the tissue's state. With the use of statistical techniques, these signatures may form a basis for staging the disease. With further studies, the technique can be envisioned as a rapid and sensitive diagnostic tool to help the pathologist in detecting the breast cancer.

So far the method is based on an invasive approach requiring removal of a part of the tissue which can itself contribute to the progression cancer, researchers are not far from possibility of developing a non-invasive technique in real time using infrared spectroscopy. Whether the method would become a powerful tool in differentiating different stages of the disease, detailed analysis of a number of tissue samples will be required.

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