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# Optical Characterization of Breast Cancer using Fourier Transform Infrared Spectroscopy

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### ABSTRACT

**Background:** Breast cancer is the second most common cancer worldwide and the leading cause of cancer death among women. Early detection and diagnosis can significantly improve patient outcomes. Fourier transform infrared (FTIR) spectroscopy is a non-invasive diagnostic tool that can provide valuable information on the molecular composition of biological samples. It has been shown to be effective in diagnosing various diseases, including cancer. This proposal aims to investigate the potential of FTIR spectroscopy as a diagnostic tool for breast cancer.

**Objectives:** To identify differences in molecular composition between cancer tissue and normal tissue obtained from the breast cancer patients using Fourier transform infrared (FTIR) spectroscopy. To develop a diagnostic model for breast cancer using FTIR spectroscopy and assess its accuracy, sensitivity, and specificity.

**Methodology:** In present study thirty biopsy proven cases of carcinoma breast and 6 fibroadenoma patients as control were included after informed written consent. In the present study we have collect blood and the cancerous and normal tissue of breast cancer patients and characterize the tissue with Fourier Transform Infrared Spectroscopy (FTIR).

**Results:** Characterization of serum and tissue samples is shown in figures 1-figure 6. Figures indicate that there is a difference between the spectra of both groups. Absorbance of cancer tissue is more as compare to normal tissue sample. Discriminating wavenumber associated spectral difference in the range of 950-1200 cm<sup>-1</sup> (sugar), 2800-3000 cm<sup>-1</sup> (stretching motions of  $-CH_2$  and  $-CH_3$ ) and 3090-3700 cm<sup>-1</sup> (NH stretching) region.

**Conclusion:** There is spectral difference between the cancerous and non-cancerous group. This spectral difference is related to the difference in the protein conformation in the serum and tissue samples of two groups. It can be concluded that FTIR spectroscopy is able to discriminate between breast cancer and healthy serum sample.

Keywords: Breast cancer, FTIR spectroscopy, Molecular spectroscopy, serum

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## Introduction:

Cancer is the major cause of death worldwide and breast cancer is the commonest cancer among women worldwide [1]. Poor survival rate reflects that patients diagnosed at the advanced stage of the disease at which poor treatment response. Early diagnosis of the disease may delay or prevent further progression with suitable treatment and hence improve the survival rate of the patients. At present personal inspection and screening are the commonest and preferred method for the detection of breast cancer in asymptomatic women [1,2]. However, gold standard of imaging modalities is mammography which is not available in all medical centers and also low sensitivity in the young women due to dense breast. Moreover, no highlighted symptoms when the tumor is small and easily treatable [3]. Mammography screening may also miss up to 20% of underlying breast cancer [4] and 30% rate of overdiagnosis which increases unnecessary surgical procedure and patient anxiety [5]. Histopathology, a gold standard in cancer diagnosis which is still microscopic evaluation of stained sample by a trained pathologist which is performed when the precancerous or cancerous cells are observable. Moreover, histopathological diagnosis is invasive, time consuming and depends on the subjective judgment of pathologist that leads to intra and inter-observer variation. Therefore high false negative or false positive rate is common in tissue assessment [3a]. Infect method involves complex process of tissue staining for tissue sample.

FTIR is the powerful tool that can be used to provide the molecular composition, structure and interaction of the constituents' molecules of biological sample [6, 7]. Changes in the characteristics of biological fluids occurs in the disease can be detected by the spectral data and emerged tool in clinical studies over past few years [8]. Standard protocols are well established for the measurement of diagnostic medium and spectral data analysis [9, 10].Biochemical alteration in the serum could reflect the physiological changes due to breast cancer, enabling the disease diagnosis and treatment early [11]. FTIR spectroscopy has been successfully applied for diagnostic of breast cancer using blood components [12], breast tissue [13], and hair [14] to differentiate breast cancer samples [12]. Protein analysis is the promising technique for understanding the progression of cancers. FTIR spectral analysis is the holistic evaluation of protein structural content at molecular level in biological samples. Initiation, progression and response to the therapy depend on the interaction between the constituent of biomolecules such as protein, lipid, nucleic acid and carbohydrates. Our study is focused on the FTIR (Fourier Transform Infrared) Spectroscopic characterization of serum sample of breast cancer patients.

# Methods:

In the present study we have enroll total 36 cases which include 30 cases of biopsy proven breast cancer patients and 6 as control from the surgery OPD of AIIMS New Delhi after written informed consent. We have collect blood and the cancerous and normal tissue of breast cancer patients and characterize the tissue with Fourier Transform Infrared Spectroscopy (FTIR). Serum was extracted from the blood sample and analyzed using FTIR (Fourier Transform infrared) spectroscopy technique. In the FTIR analysis we use on 1  $\mu$ l serum sample only using machine Parkin Elmer UATR two. The spectra will be recorded in the mid-infrared region (4000-400 cm<sup>-1</sup>) using a high-resolution detector. Each sample will be measured in triplicate to ensure the reproducibility of the results.

# **Results:**

Characterization of serum and tissue samples is shown in figures 1-figure 4. Figure 1 and figure 2 indicates the FTIR spectra of breast cancer and normal tissue sample in absorbance and transmission mode. Figures indicate that there is a difference between the spectra of both

groups. Absorbance of cancer tissue is more as compare to normal tissue sample. Discriminating wavenumber associated spectral difference in the range of 950-1200 cm<sup>-1</sup> (sugar), 2800-3000 cm<sup>-1</sup> (stretching motions of  $-CH_2$  and  $-CH_3$ ) and 3090-3700 cm<sup>-1</sup> (NH stretching) region.

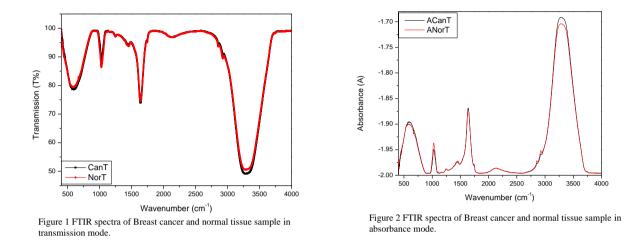


Figure 3 and Figure 4 are the FTIR spectra of serum samples of breast cancer patients in transmission and absorbance mode. Spectra of serum sample of breast cancer patients are similar as spectra obtained from the tissue sample except peak observed in the spectral region from 950 to 1200 cm<sup>-1</sup>.

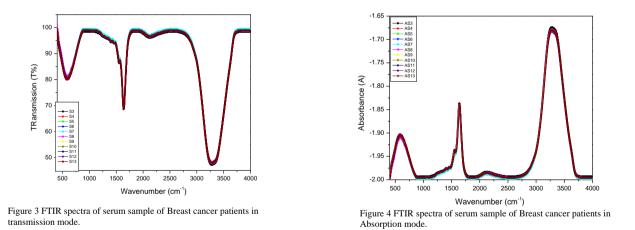
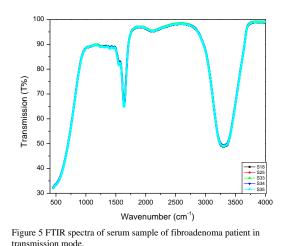
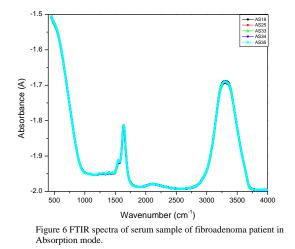


Figure 5 and Figure 6 are the FTIR spectra of serum samples extracted from fibroadenoma patient blood in Transmission and absorption mode. Spectra look similar as spectra shown in figure 3 and figure 4 except peak intensity.

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### **Discussion:**

Breast cancer is the most frequent kind of cancer among the women. Breast cancer disease trend have asymptomatic pattern especially in the initial stage which significantly complicate its detection, prognosis for treatment and consequently survival. Survival rate of breast cancer patients directly depends on the timely detection of the disease. This is the main reason why a fast and early diagnosis is of enormous importance. Therefore development of new accurate methods for early diagnosis of disease is the need of time. Current diagnostic methods are more or less complicated and highly subjective. Standard diagnostics methods such as ultrasound (US), X-ray mammography, magnetic resonance imaging (MRI), computed tomography (CT) are resorted to when the disease began to progress. Histopathology is the gold standard method for diagnosis of the disease in which biopsy samples are taken from the breast cancer patients. This method is time consuming, subjective opinion of the doctor and pathologist needs atleast 0.5 hr for diagnosis of the disease.

Infrared (IR) spectroscopy is the rapid, sensitive, non-destructive and fairly inexpensive analytical method. Fourier Transform infrared (FTIR) spectroscopy is simple, rapid and reagent free biochemical tool which can provide molecular composition of biological samples. Organic compound absorbed infrared light energy corresponding to the nature of bonds between its atoms yielding a "fingerprint" spectrum of the biological sample. FTIR spectrum is widely used for differentiating two samples and locates the bands and possible molecules which may contribute the spectral difference. FTIR spectroscopy is found to be useful in detection and characterization of cancer cells and tissue. The region from 800-1300 cm-1 is due to vibration of functional group such as PO1-, CO and CC present in proteins, amino acids, carbohydrates and phospholipids. Region corresponds to 1450-1700 cm-1 is due to amide I and amide II absorption.

In the present study we have characterize total of 37 samples which include 30 samples of breast cancer patients and remaining 7 samples as control. Figure 1 and figure 2 indicates the FTIR spectra of tissue sample of breast cancer and normal tissue sample. Spectrum obtained from the tissue samples have spectral peaks in the spectral region 950-1200 (sugar), 1190-1350 cm-1 (collagen), 1475-1710 cm-1 (Protein), 1710-1760 cm-1 (ester), 2800-300 cm-1 (stretching motion of –CH2 and –CH3) and 3090-3700 cm-1 (NH stretching). The main difference between the normal and tumour tissue in the fingerprint area for the protein, nucleic acid and lipid have been reported. Figure indicates the spectral difference between

the spectra in the two groups in the spectral region of sugar (950-1200 cm-1), proteins (1475-1710 cm-1) and lipids (2800-3000 cm-1). The range of 3090 -3700 cm-1 is the most relevant criterion for the separation of serum sample of breast cancer from the healthy one. This according to the literature is due to protein modification. It is also found that protein part of the spectrum is the most discrimination part especially collagen could serve as a potential biomarker that could be used for distinguishing the cancerous and normal tissue. Statistically significant difference found in the spectral range of 1200-1100 cm-1between the cancer and non cancer group, this may be due to progressive structural changes in the DNA of normal female breast leading to precancerous or cancerous phenotype in major portion of women. Figure 3 and figure 4 are the FTIR spectrum of serum samples of breast cancer patients. Whereas figure 5 and figure 6 are the FTIR spectrum of serum samples of breast cancer patients and fibroadenoma patients looks similar except the peak intensity. In case of cancer patients absorption peaks intensity is high as compared to fibroadenoma cases. FTIR characterization of tissue sample is the new paradigm for understanding the etiology of breast cancer and predicting its occurrence in the early stage of cancer.

### **Conclusion:**

Results indicate the major spectral difference between the cancerous and non-cancerous group. This spectral difference is related to the difference in the protein conformation in the serum and tissue samples of two groups. It can be concluded that FTIR spectroscopy is able to discriminate between breast cancer and healthy serum sample.

### **Outcomes:**

Breast cancer is the most frequent type of cancer among the women. Therefore early diagnosis has a special value in the treatment and survival rate of the patients. We have found that FTIR spectroscopy is the simple, sensitive and rapid method for the detection of breast cancer. This method needs only  $1\mu$ l of serum sample or very tinny tissue sample without the need of any reagent. Every disease leaves fingerprints in the IR spectrum. This typical fingerprint can be used to identify the different patients groups.

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