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Spectral Analysis of Breast Cancer is Conducted Using Human Hair Fibers Through ATR-FTIR

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Abstract: Hair serves as a protective barrier between the body and the environment. It contains biomolecules like proteins and lipids, similar to those found in blood and skin. By using the ATR-FTIR spectroscopic technique, these biomolecules can be replicated, allowing for disease diagnosis. The ATR-FTIR spectra of human scalp hair fiber samples were recorded in the mid-infrared region of 4000 – 450 cm^{-1}. In hair fibres collected from breast cancer patients, an increase in the intensity ratio of the C-H bending absorption bands in the region of 1446-1456 cm^{-1} of lipids was observed in the ATR-FT-IR spectra of a single hair fibre. Peak height ratios greater than 1.0 indicate the presence of breast cancer. The absorbance value at these specific vibration modes varied significantly from that of a normal person's scalp hair. The method of internal ratio parameters is used to characterize hair tissue quantitatively. This study highlights the potential of ATR-FTIR analysis of a hair fiber for the early detection of breast cancer and studies how hair acts as a biosensor for breast cancer without mentioning AI-powered assistance. The use of ATR-FTIR analysis allows for non-invasive and cost-effective screening of breast cancer, making it a promising tool for early detection. Additionally, studying hair as a biosensor for breast cancer opens up possibilities for developing more accessible and convenient diagnostic methods in the future.

Keywords: Spectral Analysis; Breast Cancer; Human Hair Fibers; Attenuated total reflectance (ATR); Fourier Transform Infrared (FTIR); C-H Bending Absorption; Convenient Diagnostic Methods.

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1. Introduction

Breast cancer affects many women worldwide,and detecting it can be difficult and costly. Early detection of breast cancer is crucial for successful treatment and improved survival rates [1]. However, limited access to healthcare facilities and financial constraints pose significant challenges in achieving timely diagnosis for many women. However, this project aims to make early detection easier by analyzing bio-molecular changes in human hair using ATR-FTIR spectroscopic techniques [2]. This non-invasive method has the potential to revolutionize breast cancer screening, especially in low-resource settings where mammography may not be readily available. By identifying specific biomarkers in hair samples, healthcare professionals can identify individuals at high risk for breast cancer and provide them with appropriate interventions and support. Ultimately, this project could help bridge the gap in breast cancer detection and save countless lives. Hair is highly suitable for detecting cancer cells, and FTIR spectroscopy allows for studying both primary and secondary structures of biological molecules. This non-invasive method of detecting breast cancer through hair samples can be particularly beneficial in areas where mammography services are limited or inaccessible [3].

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Additionally, the use of FTIR spectroscopy provides a detailed analysis of the molecular composition of hair, allowing for a more accurate identification of biomarkers associated with breast cancer. ATR techniques also enable non-destructive analysis of hair. Initial studies showed that hair from breast cancer patients had increased C-H bending absorption in the $1500^{-1}400$ cm^{-1} region compared to hair from non-cancer individuals [4]. This suggests that the presence of breast cancer may alter the molecular structure of hair, making it a potential non-invasive diagnostic tool. Furthermore, the use of FTIR spectroscopy in analyzing hair samples could potentially lead to the development of a cost-effective and easily accessible screening method for breast cancer in areas with limited healthcare resources [5]. This increased lipid material is located in the cuticle-cortex area. This work attempts to differentiate breast cancer patients from healthy individuals using ATR-FTIR techniques on a single hair fiber. The ATR-FTIR technique allows for the analysis of specific molecular components within the hair fiber, such as lipids, proteins, and nucleic acids. By examining the unique spectral signatures of these components, researchers can potentially identify biomarkers associated with breast cancer. This non-invasive approach has the potential to revolutionize early detection and improve patient outcomes in regions where traditional screening methods are not readily available.

2. Materials and Methods

General Hospital Chennai, Tamil Nadu, India. Received hair samples from two female Indian subjects who were both diagnosed with breast cancer and were between the ages of 45 and 54. The subjects consented, and two strands of hair were plucked from each subject. The hair colours ranged from white and brown to dark brown. The researchers collected each subject's cancer status and pertinent medical data. The hair samples were analyzed for biomarkers or abnormalities indicating breast cancer. The researchers also considered family history, lifestyle habits, and hormonal levels to understand the correlation between hair characteristics and breast cancer.

Human hairs were plucked from healthy volunteers aged between 20 and 50 years. Actively growing hairs with an intact bulb were carefully selected, and full-length single hairs of normal subjects with the root bulb intact were chosen. Before analysis, all full-length hairs were washed with distilled water and left to air dry. This ensured that the hairs being analyzed were in their natural state, without any external chemical interference. The use of distilled water as a cleaning agent helped eliminate any potential contaminants from the hair fibers.

To reduce the possibility of bias and guarantee an objective evaluation of the hair samples, the investigation was carried out as a double-blind study. After the ATR-FTIR analysis was complete, the investigator learned whether or not the hair samples had malignancy. Participants' privacy and confidentiality were safeguarded by not disclosing their personal information during the course of the inquiry.

The micrometre was used to measure hair diameter, and precise measurements were critical for obtaining accurate findings. Participants' hair diameters varied widely, from 38 to 53 m, indicating a wide variety of hair types.

Sample cards made specifically for the seagull ATR cell were used to hold the hair samples for analysis. The ATR cell's plunger pressure was calibrated to the zero line on the pressure applicator scale. Before each hair sample was spectrally collected, the IREs were cleaned with methyl ethyl ketone to remove any residue or impurities that could compromise the reliability of the data. Thus, the acquired spectra accurately reflected the hair samples and analytical noise was kept to a minimum.

3. Experimental

3.1. ATR-FTIR Spectra of Vibrational Band Assignments

The absorption bands of specific functional groups combine to form the infrared spectrum of a compound, which can function as a unique identifier by providing information about the position, shape and intensity of vibrational band assignments [6]. Table 1 provides a detailed breakdown of vibrational band assignments, including information on position, shape, and intensity. This information is essential for accurately identifying unknown compounds using their infrared spectra.

Table 1: Major vibrational band assignment on human single hair fiber

S. No.	Wave number (cm^{-1})	Vibrational band Assignment
1	3280	N-H stretching/OH stretching
2	2930	υas (CH_2) stretching vibration of proteins abd lipids
3	2850	υs(CH_2) stretching vibration of proteins and lipids
4	1743	C=O groups of cholesterol ester (HDL)
5	1645	Amide I band mainly due to C=O stretching vibrations amide groups

6	1540	Amide II band due to the $\delta(N-H)$vibration strongly coupled to the υ(C-N) stretching vibration vibration of protein
7	1455	δas(CH_3) and δ(CH_2) of both lipid and protein groups (LDL)
8	1340	δ(CH_3), (CH_2) deformation vibration of amino acid tryptophan
9	1250	Amide III and vas(PO_2-) stretching mode of nucleic acids
10	1180	υas (S-O) groups of cystic acid and the C-O group of carbohydrate
11	1050	υs (S-S) stretching mode of cystic acid
12	930	C-H out of plane deformation of alkene group
13	650	C-H out of plane deformation
υ-stretching, δ-bending, s-symmetric, as-asymmetric		

The CH_2 and CH_3, fundamental modes of hair molecules (keratin, lipids), have been assigned based on the group vibrational concept (Table 1) [7]. Additionally, vibrational frequencies have been calculated using IRP methods. Both normal and breast cancer patients have provided the best representation of the mid-region IR spectrum. The comparison of IRP ratios from both patients indicates that the theoretically calculated value closely matches the experimental values obtained from the patients. This demonstrates the potential of hair molecule vibrational frequencies as a diagnostic tool for breast cancer detection. However, further studies are needed to determine the correlation between specific vibrational modes and different stages of breast cancer for more accurate diagnosis and monitoring of the disease.

3.2. ATR-FT-IR Spectral Measurements

The PerkinElmer spectrum two FTIR spectrometer was used to take the ATR-FTIR spectral observations; the accessory used was the Universal Attenuated Total Reflectance attachment, which uses a diamond as its internal reflection element [8]. There were 16 scans used to record the spectra, and each scan had a resolution of 4 cm^{-1}. The materials under study were placed on a 2-mm-square diamond crystal with a 350-cm^{-1} cut-off wavenumber; solids were subjected to appropriate pressure to ensure good optical contact with the diamond, while liquids received no such treatment [9]. The spectrum of air was used as a background, and these readings were removed. After each scan, a fresh backdrop of reference air was obtained and the crystal was cleaned with isopropyl alcohol-soaked tissue. This cleaning process removes any contaminants or residues on the crystal surface, allowing for accurate and reliable measurements. Additionally, the use of isopropyl alcohol helps to eliminate any potential interference from organic compounds that may be present in the crystal.

A representative AT-FTIR absorption Spectrum of human Hair tissue (Fig.1). Vibrational band assignments were carried out with the idea of group frequencies of the different.

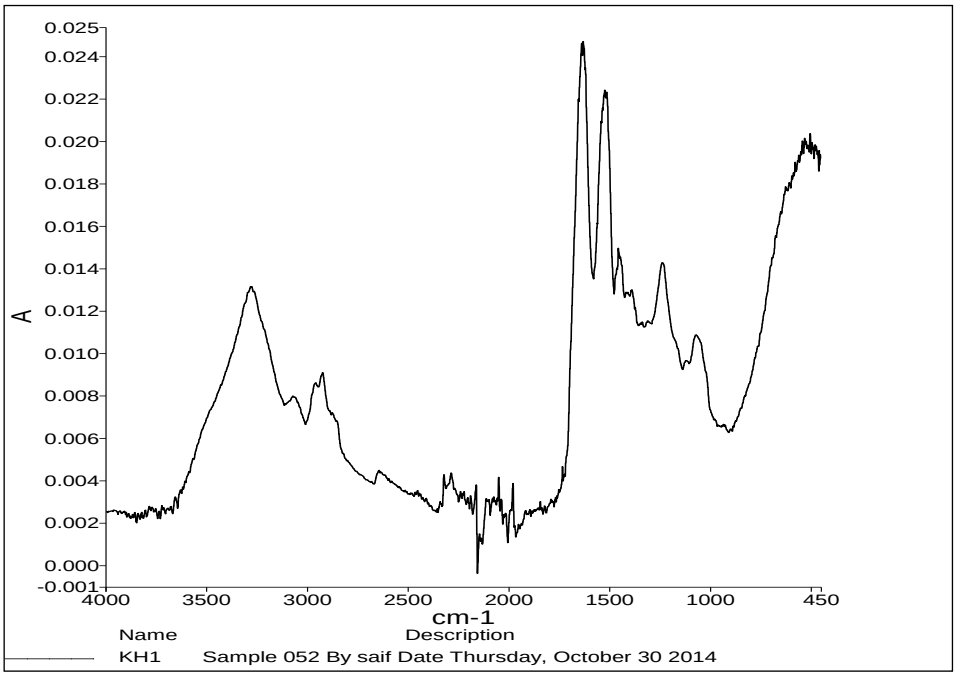

Figure 1: ATR-FTIR spectrum of a healthy Sigle human hair fibre

Analyses present in the sample. An N-H stretching region around 3280 cm^{-1} in the infrared spectrum usually strongly overlaps with the O-H stretching of hydrating H_2O molecules. To distinguish between the N-H stretching region and the O-H stretching of hydrating H_2O molecules, a deuterium oxide (D_2O) exchange experiment can be performed. This experiment involves replacing the hydrogen atoms in the sample with deuterium atoms, which have a different vibrational frequency [10]. By comparing the spectra before and after the exchange, it is possible to accurately differentiate between the N-H and O-H stretching regions and the fourth derivative spectrum in Fig. 2.

The absorption bands at 2930 cm^{-1}, 2850 cm^{-1}, and highlighted L (lipid) originate from the symmetric and asymmetric stretching vibrations of acyl CH_2 groups. The band around 1743 cm^{-1} is due to C=O cholesterol ester (HDL) groups [11]. The amide I (around 1645 cm^{-1}) is due to the stretching vibration of the C=O bond in the peptide backbone. The presence of deuterium atoms in the sample can also provide valuable information about hydrogen bonding interactions within the molecule.

The stretching of C=O is represented by the amide I band, while the amide II band (around 1540 cm^{-1}) represents the stretching of C-N and the bending of N-H in protein backbones. The band around 1454 cm^{-1} is due to bending CH_3 and bending CH_2 groups (LDL), while the band around 1340 cm^{-1} is due to stretching CH_3. The band around 1250 cm^{-1} is due to the contributions of amide III and stretching PO_2 stretching mode of nucleic acids [12]. The band around 1180cm^{-1} is due to the stretching S-S groups of cystic acid and the C-O groups of carbohydrates. The band around 1050 cm^{-1} is due to the contribution of the S-S stretching mode of cystic acid. These bands in the protein backbone spectrum provide valuable information about proteins' molecular structure and composition. These specific bands can help researchers identify and characterize different types of proteins based on their unique vibrational frequencies.

As the ATR-FTIR spectra exhibit vibrational band characteristics of the various group frequencies, the scope of a control human scalp hair and that of cancer patients' scalp hair are the same concerning the position of the peaks but different regarding the absorption levels of the peaks. Overlaid ATR-FTIR spectral features are the same, but 3280cm^{-1} and 1455cm^{-1} intensities are increased in cancer patients' scalp hair than in the control person's scalp hair. It is noticed, though, that the difference in the intensities of the band position at 3280cm^{-1} and 1455cm^{-1} is observed [13]. This difference in absorption levels suggests a potential alteration in the molecular composition of the cancer patients' scalp hair compared to that of the control individuals. Further analysis is required to determine the specific molecular changes responsible for these variations in intensity.

The protein and LDL structure changes for human scalp hair fibres are investigated. The ATR-FTIR indicates that the compositional ratio of 3280/1050cm^{-1} is due to the N-H stretching of proteins and the S-S stretching of cystic acid. For the control person's scalp hair, the absorbance ratio is> 1.0. The results reveal that the spectral absorption of the peaks is increased in breast cancer patients' scalp hair than that of the control person's scalp hair [14]. So, the alternations in regions 1455cm^{-1} and 3280cm^{-1} were essential in confirming breast cancer. These findings suggest that the changes in the absorbance ratio and spectral absorption peaks could serve as potential indicators for the presence of breast cancer [15]. Further research is needed to validate these results and explore their clinical applications in the early detection and diagnosis of breast cancer.

A non-invasive and potentially early diagnostic tool for breast cancer can be obtained through the ATR-FTIR spectroscopic technique. This technique allows for identifying and quantifying specific biomarkers associated with breast cancer, monitoring the effectiveness of treatments, and tracking changes in the molecular composition of the cuticle over time.

C-H bending absorptions at 1446 cm^{-1} and 1437 cm^{-1} have been demonstrated to be more pronounced in breast cancer. C-H bending absorption (-CH_2-) around 1446 cm^{-1} and C-H bending absorption (-CH_3) around 1456 cm^{-1} have different peak height ratios, which can be used to infer the relative amount of lipid material at the cuticle-cortex interface region. As can be seen from the comparisons in Figs.1 through 11 [16], this spectral analysis method has shown encouraging results in diagnosing breast cancer at an early stage.

The peak height ratio of 0.1435/1043 cm^{-1} in the LDL/Glucose region suggests a lower amount of lipid material in the hair sample from an average person compared to the initial condition hair sample from a breast cancer patient [17]. Additionally, a peak height ratio of 0.2 in the LDL/Glucose region indicates a significant difference between the hair sample of an average person and a breast cancer patient. These findings suggest a potential correlation between the observed peak height ratios and breast cancer in patients.

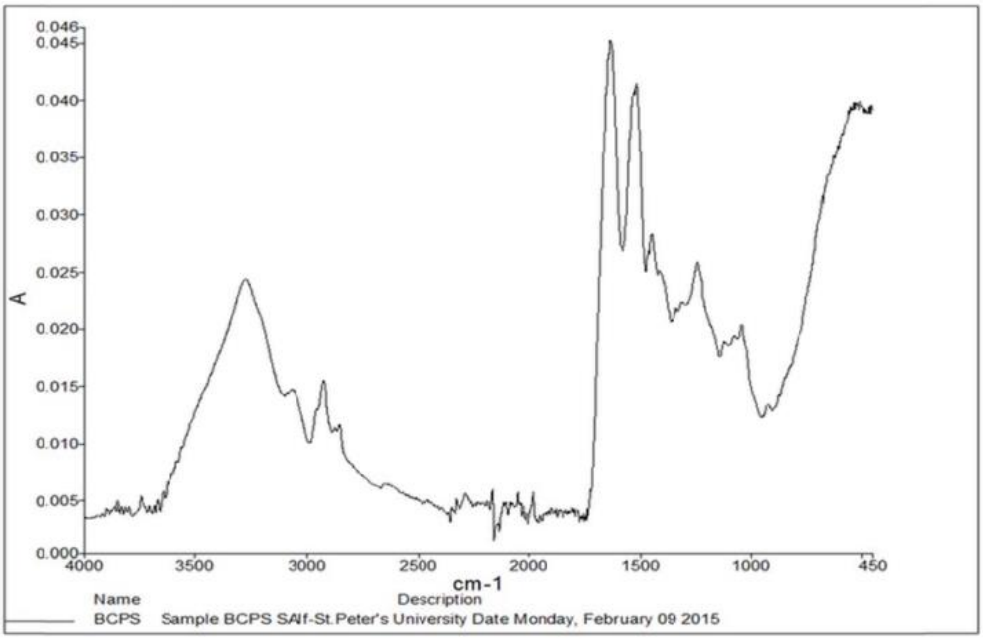

Figure 2: ATR-FTIR Spectrum from initial stage breast cancer patient

The LDL/Glucose peak height ratio may serve as a potential biomarker for breast cancer detection, as seen in the hair sample of a breast cancer patient after therapy and ration treatment (Fig. 2). However, more studies involving larger sample sizes and diverse populations are required to confirm these findings and establish the clinical utility of this ratio in breast cancer diagnosis. Changes in secondary structure of the lipid due to increased lipid content, rather than production of new lipid material, are likely responsible for the little shift in the C-H bending absorption peaks in cancer individuals. Positive breast cancer samples had peak-to-average height ratios more than 1.0. Breast cancer risk may be related to lipid content, as indicated by the LDL/Glucose peak height ratio of 2.1786 [18]. Changes in the secondary structure of lipids may account for the observed shift in the C-H bending absorption peaks in cancer patients, suggesting an influence of the increased lipid content on this phenomenon. These results emphasise the significance of lipid analysis in breast cancer patients' diagnosis and follow-up [19].

Breast Cancer Patient 1 and the Sample Initial Condition Breast Cancer Patient were found to be real cases of breast cancer after all [20]. Indicators of breast cancer in their early stages may be gleaned through ATR-FTIR studies. Additional medical evidence is required to confirm that the Sample Initial Condition breast cancer patient is not a false positive but rather an early indicator of cancer [21].

After analysing the cancer status of the individual from whom we collected the hair sample for this investigation, we determined that Sample Breast Cancer Patients 1 was taken from a person who had breast cancer [22]. Based on these results, ATR-FTIR analysis has the potential to serve as an early diagnostic tool [23] by detecting any lingering cancer cells in the hair sample. Validating these findings and understanding the full scope of ATR-capabilities FTIR's in identifying breast cancer will require additional research and access to follow-up medical data. This led to the conclusion that the sample was not cancerous [24].

After cancer surgery, the hair grew at a rate of around 0.35mm per day, so it was estimated that roughly 21mm of the proximal region of the hair fibre had formed [25]. Likewise, the terminal end of the hair follicle was already in existence before the malignancy was excised [26]. This indicates that malignant cells may have altered the hair fiber's makeup while it was developing. Research into whether or not hair composition changes can be employed as a marker for breast cancer recurrence or treatment response is warranted [27-39].

After analysing the original spectrum [30-32], we found that it was taken from a sample of the normal person's hair roughly 26mm from the proximal end. The sample had been placed on the sample card at an angle that exposed a portion of the hair fibre further from the IR source than the usual 10mm. This distal region was already there before the cancer treatment [33].

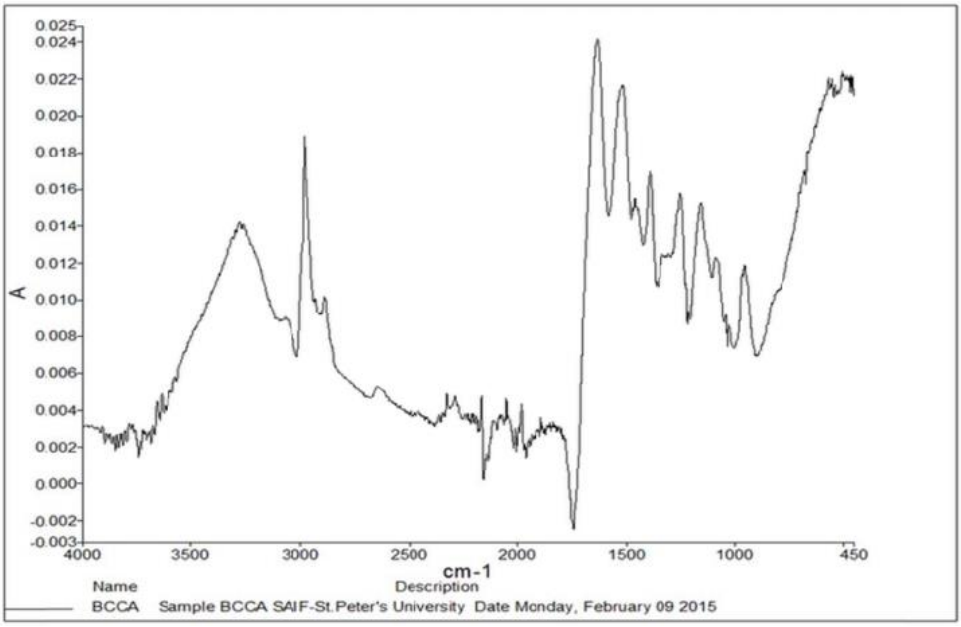

Figure 3: ATR-FTIR Spectrum from after-therapy breast cancer patient

A second spectrum (fig. 3) of Breast Cancer Patients 1 of this sample had shown a C-H bend in peak ratio of (LDL/Glucose) 2.17, indicating cancer. A first spectrum (fig. 2) of the Initial Condition in a Breast Cancer Patient was then obtained from the same hair fibre at about 10mm from the proximal end [34-36]. This portion of the hair formed during the initial stage of cancer patients showed a C-H banding peak ratio of (LDL/Glucose) 1.5, indicating breast cancer. Obtaining ATR-FT-IR spectra from the hair fiber as close to the proximal end is crucial for accurate results [37]. This ensures that the spectrum obtained represents the initial stage of breast cancer in patients, as indicated by the C-H banding peak ratio (figs. 1-11) [20].

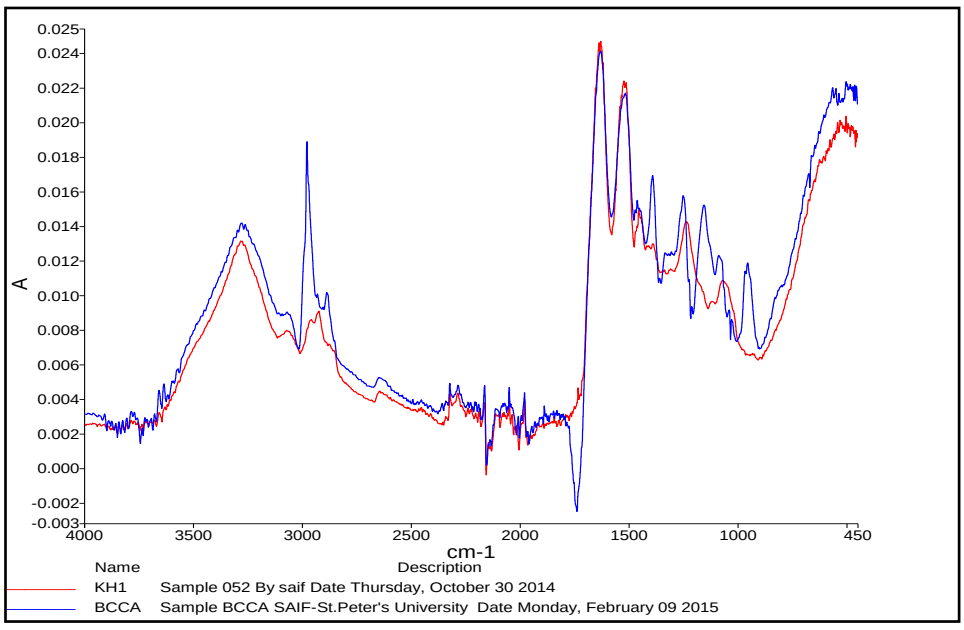

Figure 4: An overlaid ATR-FTIR spectra of the hair tissues from a normal person's and breast

C-H bending peak heights of 1.000 or less were seen in the spectra of both cases, ruling out breast cancer. The ATR-FTIR study confirmed the participants' lack of cancer diagnosis in the clinic. One patient was diagnosed with breast cancer after undergoing treatment, and the other was diagnosed with breast cancer in the early stages of the disease [38-41]. Peak-to-base ratios of 2.7 and 1.5 are associated with an increased risk of breast cancer. Peak height ratios of (LDL/Glucose) 0 to 0.01 were associated with non-breast cancer. All patients with breast cancer showed an increase in C-H bending absorption intensities,

indicating that this might be used as a biomarker in ATR-FTIR analysis for the diagnosis of breast cancer [42]. Additional studies are required to confirm these results and investigate their therapeutic consequences [43].

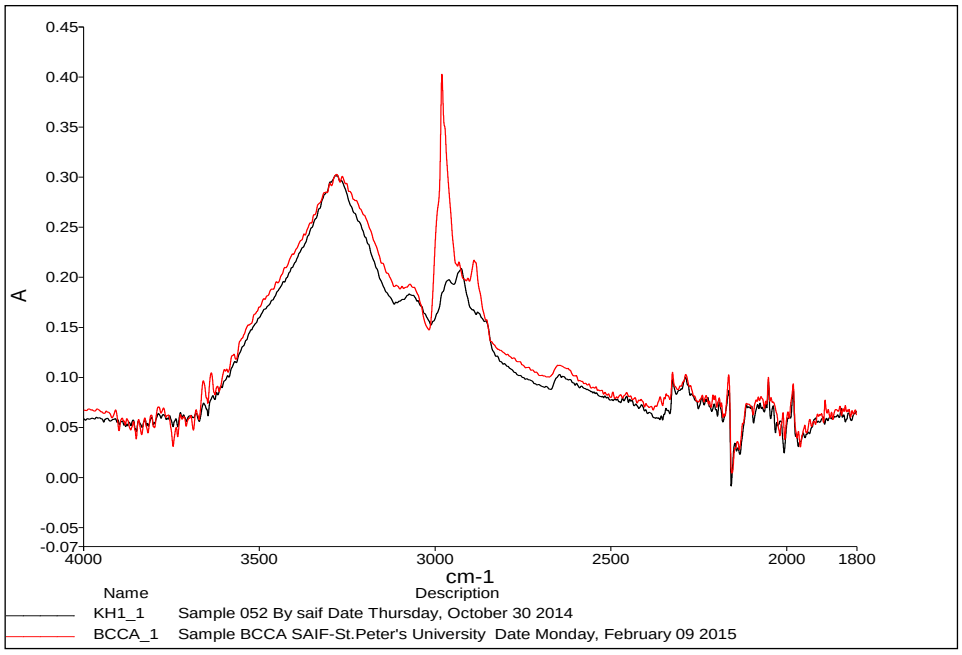

Figure 5: ATR-FTIR spectra of control and breast cancer scalp hair fiber ($4000^{-1}800cm^{-1}$)

From the ATR-FTIR analysis, we compared the spectrum of breast cancer-affected persons' hair with that of normal persons' hair. Normally, the CH_2 stretching vibrations of proteins and lipids are observed at 2930 cm^{-1}. In the case of breast cancer-affected persons, that particular CH_2 stretch vibration has sharp absorption at that specific wavelength and a slight change in frequency [44]. Additionally, the CH_2 and CH_3 bending vibrations of protein and lipid group (LDL) have high absorption at 1455 cm^{-1}. In the fingerprint region, these vibrations are more pronounced when compared to a normal person. These changes in vibrational frequencies can be attributed to the altered molecular structure and composition of proteins and lipids in breast cancer-affected individuals [45]. The observed differences in absorption patterns at specific wavelengths provide valuable insights for detecting and diagnosing breast cancer using infrared spectroscopy.

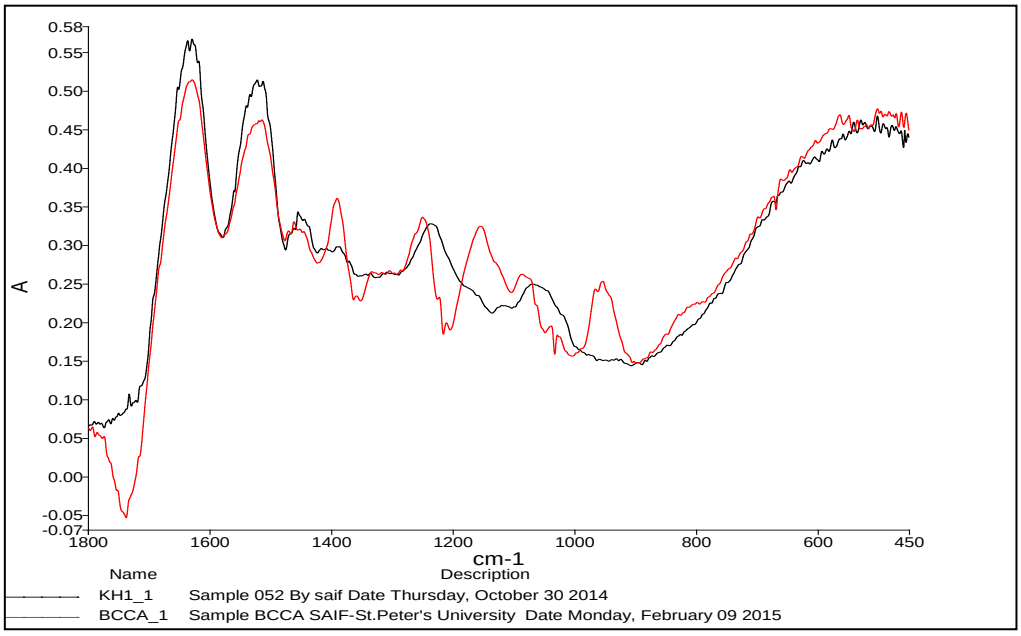

Figure 6: ATR-FTIR spectra of control and breast scalp hair fiber ($1800\text{-}450cm^{-1}$)

It appears that an increase in lipid material in the hair fibre occurs during the formation of breast cancer and reverses itself when the tumour is eliminated [5], [6]. This indicates that signalling molecules, or biomarkers, from the developing breast cancer are expressed into the blood and transported to the papilla of the hair follicle, where they change the hair biosynthesis in the fibroblasts, resulting in enhanced lipid material formation.

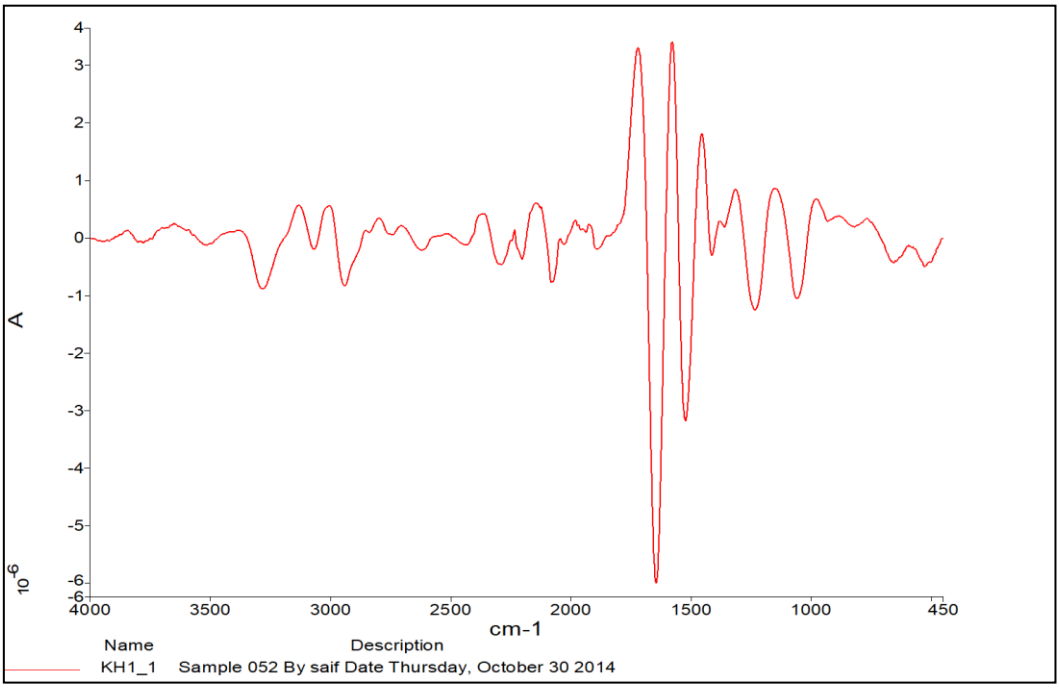

Figure 7: Atypical controlled fourth derivative ATR-FTIR spectrum of hair fiber spectra

Thus, the hair fibre acts as a selective chemical sensor for specific breast cancer signaling molecules, enabling early and accurate detection of breast cancer. This discovery opens up new possibilities for non-invasive and cost-effective breast cancer screening methods.

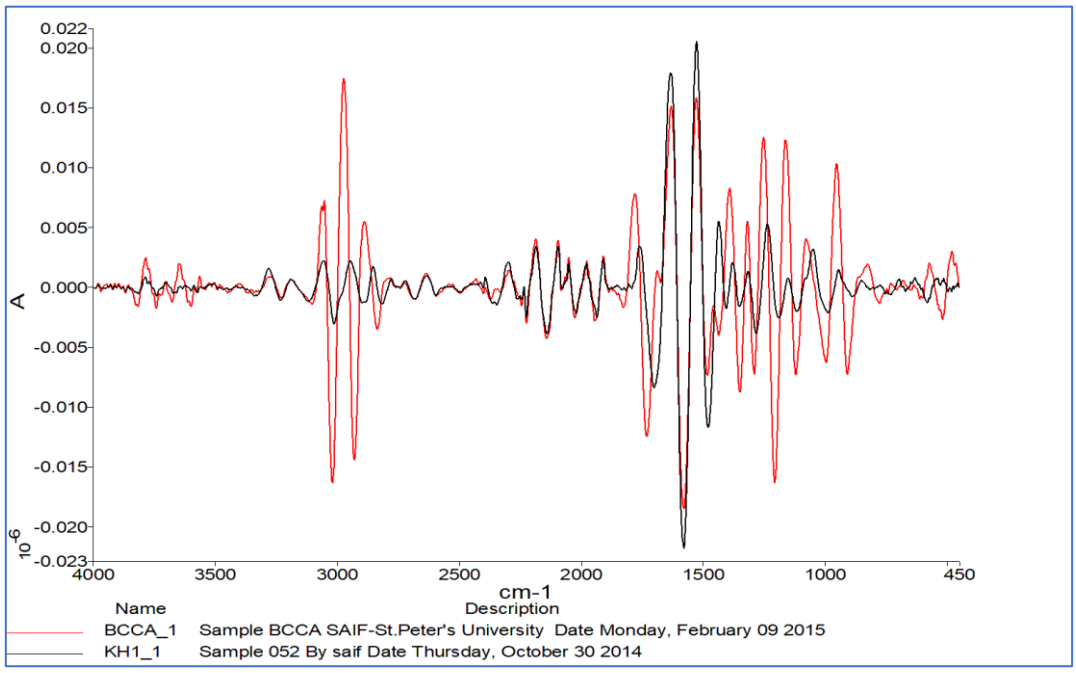

Figure 8: Fourth derivative spectra of normal and breast cancer hair fiber spectra

By analyzing the changes in hair composition, doctors may be able to detect breast cancer at its earliest stages, increasing the chances of successful treatment and survival rates. Additionally, further research is needed to determine if this hair-based detection method can be applied to other types of cancer as well.

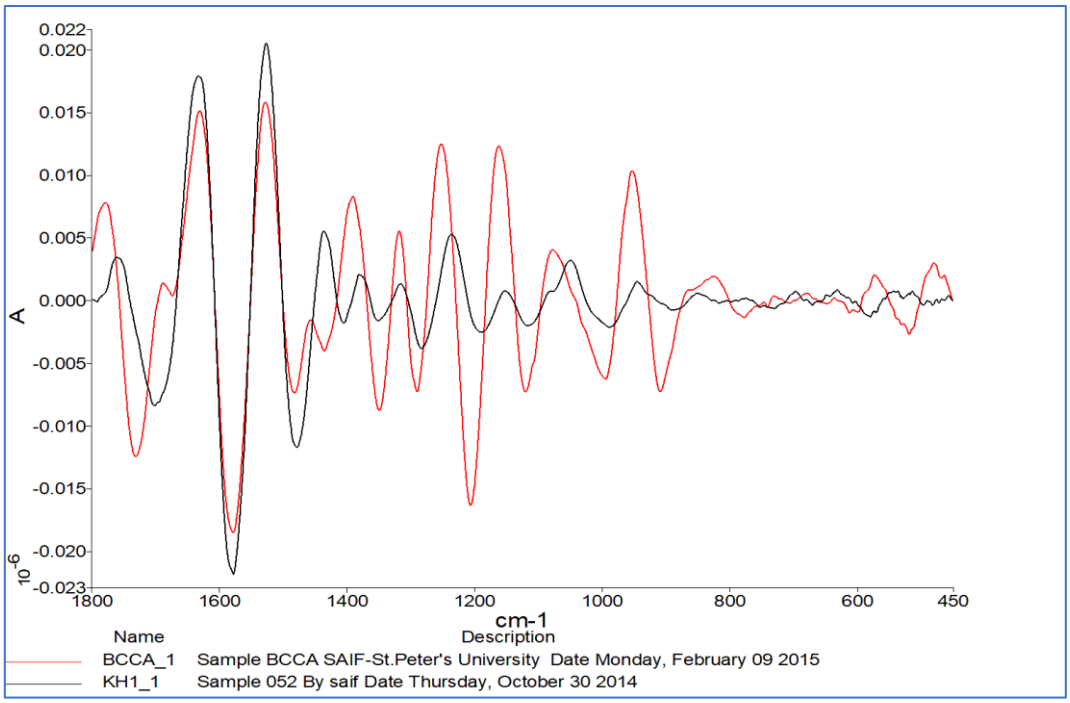

Figure 9: ATR-FTIR Spectra of control and breast cancer scalp hair fiber spectra (1800-450cm^{-1})

There is no difference in the composition of the newly generated lipid material, as measured by the C-H bending absorptions in the hair of those with breast cancer and those without the disease. Breast cancer biomarkers increase the amount of lipid components in the cuticle-cortex CMC's delta layer and the orthocortical cells that are flattened next to the cuticle.

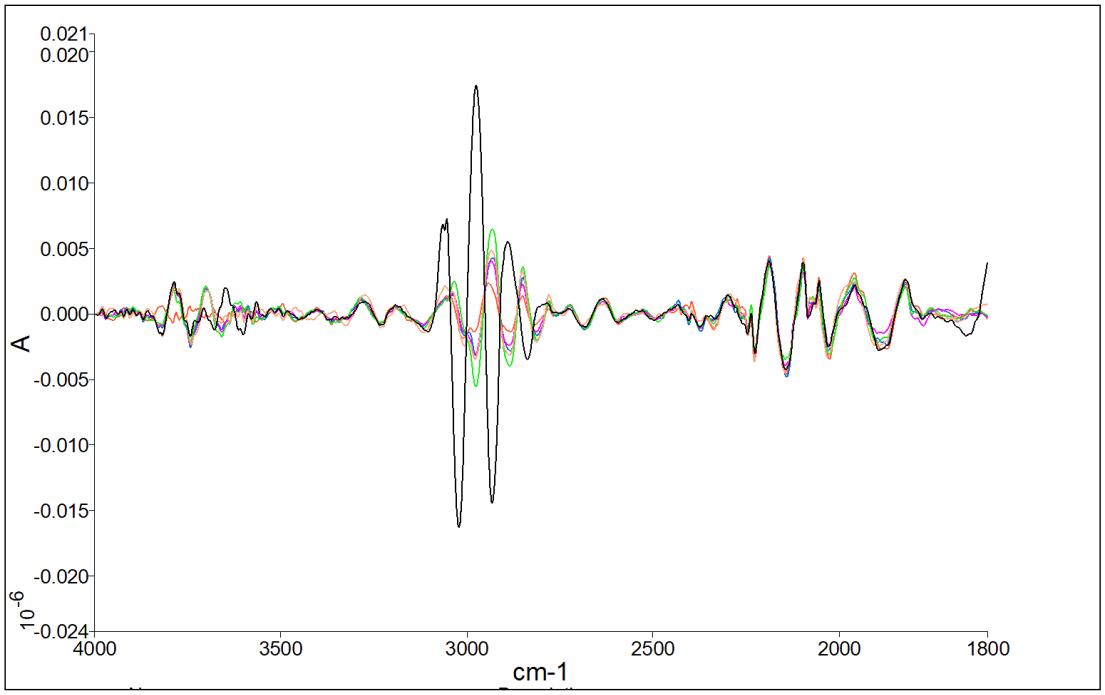

Figure 10: FTIR-ATR Spectra of control and breast cancer scalp hair fiber spectra (1800-450cm^{-1})

The lipid substance that is generated remains unchanged. Peak height increases in absorption bands often linked with lipids (such as lipid esters, C-H lipids, C-O stretches, C-O-C stretches, and cholesterol) provide further evidence for this. Studies of lipid rafts in the hair of women with breast cancer show enhanced palmitic acid, cholesterol, and/or mermaid interactions.

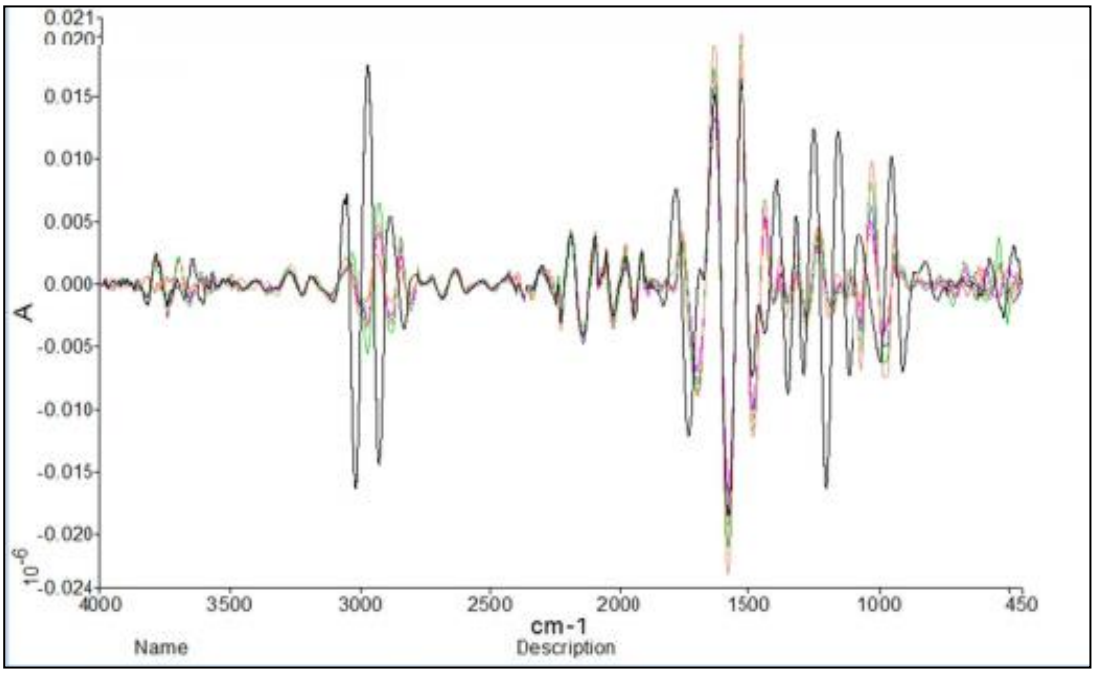

Figure 11: FTIR- ATR spectra of control and breast cancer scalp hair fiber spectra (4000-450cm^{-1})

Research on the composition and structure of the cuticle-cortex interface region of hair fibres is ongoing. The purpose of this study is to shed light on whether or not cholesterol and other lipids play a part in the onset or progression of breast cancer. The identification of novel biomarkers for early diagnosis or targeted therapies in breast cancer treatment may also be aided by a better understanding of the content and structure of the cuticle-cortex interface area.

4. Conclusion

A recent study has shown that ATR-FTIR spectroscopy is a useful tool in distinguishing intact hair fibers of breast cancer patients from those of controlled individuals. This non-invasive technique offers an efficient way to analyze the molecular composition of hair samples. By comparing the spectra of hair fibers from both groups, researchers were able to establish a unique fingerprint that can differentiate between the two. This discovery highlights the potential of ATR-FTIR spectroscopy in early breast cancer detection and monitoring. The study also demonstrates the role of this technique in analyzing hair samples from both control individuals and breast cancer patients. By providing insights into the molecular composition of hair fibers, ATR-FTIR spectroscopy can potentially identify specific biomarkers that may be indicative of breast cancer progression or treatment response. This could lead to the development of more targeted and personalized therapeutic approaches for breast cancer patients. Additionally, the non-destructive nature of ATR-FTIR spectroscopy ensures that hair samples can be repeatedly analyzed without causing damage, making it a valuable tool for longitudinal studies. The complexity of the fingerprint region can be resolved using fourth derivative spectroscopy, allowing for the identification of specific biomarkers and chemical changes in hair fibers that can be indicative of breast cancer. Overall, ATR-FTIR spectroscopy provides valuable insights into the structural differences between control and patient hair samples, which can aid in the development of potential diagnostic tools for the early detection of breast cancer.

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Data Availability Statement: This research contains data related to employee demographics and performance and collected surveys related to operational excellence and work environment-related factors. The research also contains diagnostic information to aid in answering the research questions presented.

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Conflicts of Interest Statement: The authors declare that they have no conflict of interest.

Ethics and Consent Statement: The consent has been obtained from the organization and individual participants during data collection and has received ethical approval and participant consent.

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