Human breast tissue cancer diagnosis by FT-IR spectroscopy

Enas S. Yousif*   Nabeil I. Fawaz**   Bilal J. Mohamed***
*University of Anbar - College of Medicine
**University of Anbar - College of Science
***University of Anbar - College of Education for Women

Abstract

Fourier transform infrared (FT-IR) spectroscopic study of normal, benign and carcinomal human breast tissues has been carried out. To our best knowledge, this work is the first statistical research on FT-IR spectroscopy-based diagnosis of breast cancers among the Iraqi women. Because of FT-IR sensitivity to the changes in the biomolecules in the tissues, it can be used for qualitative and quantitative analysis of cancerous breast tissues. FT- IR spectra ware taken for 91 samples of breast tissues that were previously histopathologically identified by pathologist experienced as: 63 normal (N) samples, 8 hyperplasia (H) samples, 10 fibro adenoma (F) samples, 10 ductal carcinoma (DC) samples. Several spectral differences were detected in the frequency range between 400 cm^{-1} and 4000 cm^{-1}. The ratio of intensities of the bands of A2925/A2854, A1653/A1545, A1343/A1450, A1083/A1236 and A1163/A1545 supplied conformational changes of lipids, protein, collagen, nucleic acids, and carbohydrates respectively in the breast tissues. There are remarkable differences in the spectral features between normal, benign and malignant tissues because of changes in molecules structure that accompany the transformation from a normal to a cancerous state during carcinogenesis, where the concentration of lipid is lower in cancerous breast tissues, as opposed to normal breast tissues, while the content of DNA, protein and collagen have been increased in benign and cancerous tissues . These differences in the spectral information may serve for the diagnosis of breast cancer.

Key words: FT-IR Diagnosis, Vibrational Spectroscopy, Cancer Biomarkers.

Introduction

Cancer is a complex genetic disease that is caused primarily by environmental factors. The cancer-causing agents (carcinogens) can be present in food and water and in the air, The resultant distorted cell conduct prompts extensive masses of strange cells that destroy encompassing normal tissue and can spread to vital organs resulting in disseminated disease, then commonly patient death [1]. Both in developing and developed countries, breast cancer is one of the major health hazards amongst women and stands fifth in death rate [2]. In Iraq breast cancer is one of the major causes of female death. According to the latest WHO (World Health Organization) statistics rankings data that published in May 2014, breast cancer in Iraq reached 1,962 or 1.33% of total deaths. The age adjusted Death Rate is 20.79 per 100.000 of population ranks Iraq #44 in the world. According to the increase incidence of cancer patients all over the world in general, and in Iraq specially, so the study of cancer diagnosis method is very important. Several techniques are currently used for breast cancer diagnosis. The histopathology, gold standard of diagnosis, has been shown to be subjective and depends on the experience of skilled pathologist [2], another systems incorporate X-beam mammography, ultrasonography (US), magnetic resonance imaging (MRI), optical coherence tomography (OCT), positron emission tomography (PET) and X-ray computed tomography (CT), followed by many tissue biopsies. However, they won't offer adequate evidence of the grade of malignancy of the tumor and the images acquired with these techniques give little information about the screened region [3]. Hence, there exists a need to develop alternate, rapid, objective , and cost effective methods for early diagnosis of malignancy. Fourier transform infrared (FT-IR) spectroscopy is a more suitable tool for biomedical applications, due to use of not harmful infrared radiation as excitation source; minimal sample preparation and adaptability to biomedical applications especially for *in vivo* applications [2] and is precise enough for both quantitative measurements of many components

in a complex mixture and the qualitative identification of the conformational of outsider materials [4,5]. FT-IR spectroscopy involves the study of interaction of electromagnetic radiation with molecules. Electromagnetic radiation characteristic of the interacting system may be absorbed. The frequency and intensity of the absorbed radiation consist the experimental data. The interference pattern from a two beam interferometer as the path difference between the two beams is altered, when Fourier transformed, gives rise to the spectrum. The transformation of the interferogram into spectrum is carried out mathematically with a dedicated on – line computer [6], FT-IR has shown as a sensitive diagnostic tool to distinguish cancerous from normal tissues in colon [7], prostate [8], breast [9], cervical [10], gastric [11], oral [12] and and dried for 3 days for FT-IR investigation. This dried tissue was grinded and mixed with 200 mg of KBr powder. Then the samples were placed under a mechanical pressure about 20 tons to form the KBr disk of about 1 mm thick. These ex-vivo breast tissues investigated spectroscopically using Fourier Transform Infrared Spectrometer (FT-IR). The FT-IR that is used in our laboratory (FT-IR-84005,Thermo Scientific / Nicolet IR-100, USA). The FT-IR spectra of the samples were obtained in the spectral range 4000 to 400 cm^{-1} . Each spectrum was acquired with 32 scans and 4cm^{-1} resolution. For each patient, we have measured FT-IR spectra for normal tissue and abnormal tissue (malignant or benign lesion) as shown in Table 1.

esophageal [13], bladder [14]. The purpose of this study is to assess the possibility of using Fourier transform infrared spectroscopy (FT-IR) as a diagnostic tool for cancer detection by observing the biochemical changes between tumour and normal cells within a wavenumber range known as the "*fingerprint region*" (encompassing 1800 to 950 cm^{-1}) [15] to distinguish between normal (N) and abnormal breast tissues, and to classify the abnormal tissues in different subtypes such as hyperplasia (H) and fibroadenoma (F) as a benign tissues, and ductal carcinoma (DC) as a malignant tissues.

Experimental Work

In this study, the human normal, benign and malignant ex-vivo breast tissues samples were collected from four hospitals, Ramadi, AL-Kalidia and Heet hospitals in AL-Anbar and Dejla hospital in Baghdad.

After the operation, the removed tissues were preserved in 10% formaldehyde solution and sampled immediately. Two pieces of the tissues, each of about 2 cm in diameter, were taken. One was cut off from the center of the lesion (abnormal) and the other one from the distant edge (normal) of the removed tissues. Each ex vivo breast tissue is divided by a doctor into two parts, the first part goes to the pathology examination and undergo to the standard the pathology examination procedure. The second part of the ex vivo samples goes to our lab for spectral analysis, but we avoid (long) fixation because this process chemically alters the tissue, mainly proteins [16]. Normal and abnormal tissues were cut into approximately 2 mm sizes

Table 1. Number of cases studied with FT-IR.

No.	Histology	Total number of cases
1	Normal	63
2	H	8
3	F	10
4	DC	10
Total samples number	91	

Results and Discussion:

Histological Examination Results

In this study, normal and abnormal (benign and malignant) breast tissue specimens were obtained after mastectomy of female patients aged 30–76 years old (median age is 53 years old) showing different types of tumor.

Histological image of the cross section of the formalin-fixed human breast tissue stained with H&E as observed under a microscope is shown in (Figure 1). The histopathological analysis included 63 normal (N), 8 hyperplasia (H), 10 fibroadenoma (F), 10 ductal carcinoma (DC).

FT-IR Qualitative Analysis of Different Tissue Types

The FT-IR spectra of normal breast tissues (N), benign lesions (H and F), and cancerous breast tissues (DC) are shown in (Figure 2).

The FT-IR spectra are differentiated by 10 notable peaks at 1083, 1163, 1236, 1343, 1450, 1545, 1653, 1743, 2854, and 2925 cm^{-1}. The spectra show spectacular changes in peak heights, In this study, the main spectral contribution assigned to lipid peaks were 3010 cm^{-1} caused by   C-H stretching vibrations [17]; the peaks near 2925 and 2854 cm^{-1} originated from antisymmetric stretching of CH$_2$ and symmetric stretching of CH$_2$, respectively [11]; the peak at 1743 cm^{-1} is due to the C=O stretching vibration mode [18,19], and the peak at 972 cm^{-1} is due to the (CH$_3$)$_3$N$^+$ asymmetric stretching mode. Proteins peaks due to N-H stretching mode were   at 3374 cm^{-1} [15]; the protein amide I peak due to carbonyl (C=O) stretching was at 1653 cm^{-1}; and the protein amide II due to (N-H) bending was at 1545 cm^{-1} [3,20]. The bands near 1083 and 1236 cm^{-1} are generally assigned to the symmetric υ_s(PO$_2^-$) and antisymmetric υ_{as}(PO$_2^-$) phosphate stretching modes, respectively, which are caused by nucleic acids [15,18,21,22]. The major absorptions from carbohydrates are found in the 1000–1200 cm^{-1} region of the spectrum [23]. The band at 1163 cm^{-1} is attributed to the C-OH stretching vibration of carbohydrates [15]. The vibrational modes of collagen appear at 1343 and 1450 cm^{-1} peaks resulting from CH$_3$CH$_2$ wagging and CH$_3$CH$_2$ deformation modes, respectively [17,22].

The spectral feature of normal and cancerous tissues mutate because of the changes in molecular structures that join the transformation from a normal state to a cancerous state.

The differences in the spectra of the cancerous and noncancerous tissues in the intensity of the peaks at 1653 and 1545 cm^{-1} are notable. The spectrum of protein band from the cancerous tissue as shown in (Figure 2) were very increased compared with those of normal tissues and benign lesions. This result can explain the increased amount of protein in cancerous tissues during malignancy [24,25].

Although the benign breast lesions (H and F) represent an abnormal proliferation, structure of this tissue are more similar to those of normal breast tissue [3], therefore we noted that the spectrum of the normal and benign samples are almost similar. In contrast, cancerous breast tissue (DC) display compositional differences, gave spectral features that are noticeably different.

These results are consistent with previous findings [3,15,24,26], wherein some specific absorption peaks at 3400–950 cm^{-1} are possibly useful in distinguish normal from abnormal breast tissue.

FT-IR Quantitative Analysis of Different Tissue Types

The important spectral parameters in obtaining the quantitative information about the contents of biomolecules in the tissues are, the relative intensity of the major absorption bands of these tissues [21]. A change in peak shape and intensity indicates considerable biochemical changes [4]. To quantitatively discriminate the N, H, F and DC samples, we calculated five absorbance ratios by using the measured peak heights. These absorbance ratio were: A2925/A2854 that used to measure the ratio of lipid content [15,19], A1653/A1545 that used to measure the ratio of protein content [15,19,21], A1343/A1450 that used to measure the ratio of the collagen content [2,27], A1083/A1236 that used to measure the ratio of the nucleic acids (DNA) content [15,21], and A1163/A1545 is used to measure the ratio of the carbohydrate content [3,28].

Difference, in these ratios among samples of different tissue types was calculated as shown in Figure (3) and listed in Table (2).

In the spectra collected from normal tissue, an observed methylene   (-CH$_2$) peak grows sharply at 2925 and 2853 cm^{-1}, whereas the peaks in the same area of the spectra in cancerous tissues are apparently broader and less intense. This difference in band width and lower intensity in the spectra of cancerous tissues versus the spectra of normal tissues is possibly caused by the low concentration of lipid cells in cancerous breast tissues [29].

The ratio of band intensity at A2925/A2853 implies the total lipid content in the tissues. In this study, this ratio decreased in all the benign and malignant tissues as shown in Figure (3). This decrease in intensity or the disappearance of the lipid bands means a decrease in the relative number of (-CH$_2$) groups in the malignant tissue during carcinogenesis [15].

In the present study, the ratio between the bands at 1653 and 1545 cm^{-1} increased in benign and malignant tissues compared with that in normal tissues. The intensity of the amide I and amid II bands at 1653 and 1545 cm^{-1} increased in the spectra of carcinoma samples compared with those of the normal tissue possibly because of the increased proliferation of the tumor cells [15,20].

The ratio of band intensities at 1083 and 1236 cm^{-1} reflected a measure of the changes in cellular nucleic acids (DNA) in the tissues. In the present investigation, this ratio increased in the benign and malignant tissues compared with that

in normal tissue, demonstrating the higher activity of the malignant cells [22,29].

The ratio of the band intensities at 1343 and 1450 cm^{-1} represents the collagen content in the normal, benign, and malignant tissues. In this work, this ratio increased in the benign and malignant tissues compared with that in normal tissue.

The ratio of the band intensities at 1163 and 1545 cm^{-1} indicates the carbohydrate content in the tissues. In the present investigation, the carbohydrate content decreased in the benign and malignant tissues compared with that in the normal tissue. The results in Table (1) show a obvious uniqueness between the FT-IR spectra collected from normal and cancerous tissues in the spectroscopic region of 2950–900 cm^{-1} by the peak intensity ratios of the spectral peaks characteristic of the cellular components, and they are consistent with previous findings [2,15,30]. Several reports have indicated the facility of FT-IR spectroscopic method in classifying breast cancer types [4,21,24].

Conclusions

The FT-IR results for normal, benign, and malignant breast tissue presented in this study can be summarized as follows but more extensive studies are required to confirm these findings.

The spectrum of carcinoma is remarkably different from the normal, and benign, where the spectral changes indicate alterations in content of lipids, proteins, nucleic acids, collagen, and carbohydrates. The concentration of a lipid cells and carbohydrate is lower in cancerous breast tissues than in normal breast tissues, while the content in the patterns of DNA, protein, and collagen have been increased in benign and cancerous tissues. These results are consistent with the histopathological results. The present study shows that FT-IR spectroscopy can be used to recognize cancerous from noncancerous tissues.

References

1- Denise Warren, Prevention and Treatment of breast cancer: new rays of hope, U.S.A. (2012).

2- M. V. P. Chowdary, K. Kalyan Kumar, Stanley Mathew, C.Murali and Jacob Kurien, Biochemical Correlation of Raman Spectra of Normal, Benign and Malignant Breast Tissues: A Spectral Deconvolution Study ,Biopolymers, 91(2009) 540.

3- C.-H. Liu, Y. Zhou, Y. Sun, J. Y. Li, L. X. Zhou, Resonance   Raman and Raman Spectroscopy for Breast Cancer Detection, Technology in Cancer Research and Treatment, 12 (2013) 371-377.

4- Shazza Rehman, Zanyar Movasaghi, Jawwad A. Darr, and Ihtesham U. Rehman, Fourier Transform Infrared Spectroscopic Analysis of Breast Cancer Tissues; Identifying Differences between Normal Breast, Invasive Ductal Carcinoma, and Ductal Carcinoma In Situ of the Breast, Applied Spectroscopy Reviews, 45(2010)355-365.

5- Quan Liu, Role of optical spectroscopy using endogenous contrasts in clinical cancer diagnosis, World J Clin Oncol , 2 (2011) 50-63.

6- G. Devi, T.S. Renuga Devi and S. Gunasekaran, FTIR Spectroscopic Study on Benign and Cancerous Human Breast Tissues- A Run Chart Analysis, International Journal of Pharmaceutical Sciences Review and Research, 2(2010);74-8.

7- Shmuel Argov, Jagannathan Ramesh and Igor Sinelnikov, Diagnostic potential of Fourier-transform infrared microspectroscopy and advanced computational methods in colon cancer patients, Journal of Biomedical Optics 7(2002) 11-18.

8- MJ Baker, E Gazi, MD Brown, JH Shanks, P Gardner and NW Clarke, FTIR-based spectroscopic analysis in the identification of clinically aggressive prostate cancer, British Journal of Cancer, 99(2008) 1859 – 1866.

9- Gao T, Feng J, Ci Y: Human breast carcinomal tissues display distinctive FT-IR spectra: implication for the histological characterization of carcinomas, Anal Cell Pathol, 18(1999) 87-93.

10- El-Tawil SG, Adnan R, Muhamed ZN, Othman NH, Comparative study between Pap smear cytology and FTIR spectroscopy: a new tool for screening for cervical cancer, Pathology, 40(2008) 600-613.

11- Abasalt Hosseinzadeh, Mohammad Javad and Taerreh Khadjvand, Fourier transform infrared microspectroscopy as a diagnostic tool for distinguishing between normal and malignant human gastric tissue, J. Biosci., 36 (2011) 669–677.

12- Fukuyama Y, Yoshida S, Yanagisawa S, Shimizu M, A study on the differences between oral squamous cell carcinomas and normal oral mucosas measured by Fourier transform infrared spectroscopy, Biospectroscopy, 5(1999)117-26.

13- Jian-Sheng Wang, Jing-Sen Shi, and Xiao-Yi Duan, FT-IR spectroscopic analysis of normal and cancerous tissues of esophagus, World J Gastroenterol, 9 (2003) 1897-1899.

14- The Use of Fourier Infrared Spectroscopy and Laser – Raman Spectroscopy in Bladder Malignancy Diagnosis, A comparative Study, Applied Physics Research, 2(2010)108-115.

15- Venkatachalam, L. Lakshmana Rao, N. Kiishna Kumar, Anupama Jose and Shaiju S.Nazeer, Diagnosis of Breast Cancer Based on FT-IR Spectroscopy, American Institute of Physic, 8(2010)144-149.

16- H. Abramczyk, I. Placek , B. Bro˙zek-Płuska , K. Kurczewski, Human breast tissue cancer diagnosis by Raman spectroscopy, Spectroscopy, 22 (2008) 113–121.

17- Barbara Stuart, Infrared Spectroscopy: Fundamentals and Applications, U.S.A., (2011).

18- Abigail S. Haka,1 Zoya Volynskaya,1 Joseph A. Gardecki, In vivo Margin Assessment during Partial Mastectomy Breast Surgery Using Raman Spectroscopy, Cancer Res, 66(2006) 3317-3330.

19- Tiyu Gao, Yunxiang Ci , Fourier Transform Infrared Spectroscopic features of human breast benign and malignant tissues, Vib. Spec. J., 3(2004) 35-42.

20- E.B. Hanlon, R Manoharan, T-W Koo, K E Shafer, Prospects for in vivo Raman spectroscopy, Phys. Med. Biol., 45 (2000) 6-59.

21- Safaa K. H. Khalil, Mostafa M. Khodeir, Rasha Abd El-Hakam, Rezq Abd El-Monem Rezq, Spectroscopic Study for Detection and Grading of Breast Carcinoma In vitro, Australian Journal of Basic and Applied Sciences, 3(2009) 2419-2428.

22- Heinz Fabian, Ngoc Anh Ngo Thi , Michael Eiden , Diagnosing benign and malignant lesions in breast tissue sections by using IR-microspectroscopy, Biochimica et Biophysica Acta 1758 (2006) 874–882.

23- Essam G. Ahmed, The Use of Fourier Infrared Spectroscopy and Laser – Raman Spectroscopy in Bladder Malignancy Diagnosis: A comparative Study, Applied Physics Research, 2(2010) 108-1017.

24- Jakub Surmacki, Jacek Musial, Radzislaw Kordek and Halina Abramczyk, Raman imaging at biological interfaces: applications in breast cancer diagnosis, Molecular Cancer, 12(2013)48

25- Ceren Aksoy and Feride Severcan, Role of Vibrational Spectroscopy in Stem Cell Research, 27 (2012) 167–184.

26- Jin Guang WU, YI Zhuang XU, Cuan Wen, Distinguishing Malignant from Normal Oral Tissues Using FT-IR Fiber Optic Techniques, FTIR Fiber Optics, 23(2001)186-197.

27- Shazza Rehman ,Vibrational Spectroscopy for Tissue Analysis, U.K., (2013).

28- Bird RE, Wallace TW, Yankaskas BC: Analysis of Cancers Missed
   at Screening Mammography. Radiology, 184(1992) 613–617.

29- Renata A. Bitar Cartera, Airton A. Martina, Mario M. Nettob, FT-Raman Spectroscopy Study of Human Breast Tissue, Biomedical Vibrational Spectroscopy, 5321(2004)190-198.

30- Tiyu Gao, Jun Feng and Yunxiang Ci, Human breast carcinomal tissues display distinctive FTIR spectra: implication for the histological characterization of carcinomas, Analytical Cellular Pathology, 18 (1999) 87–93.
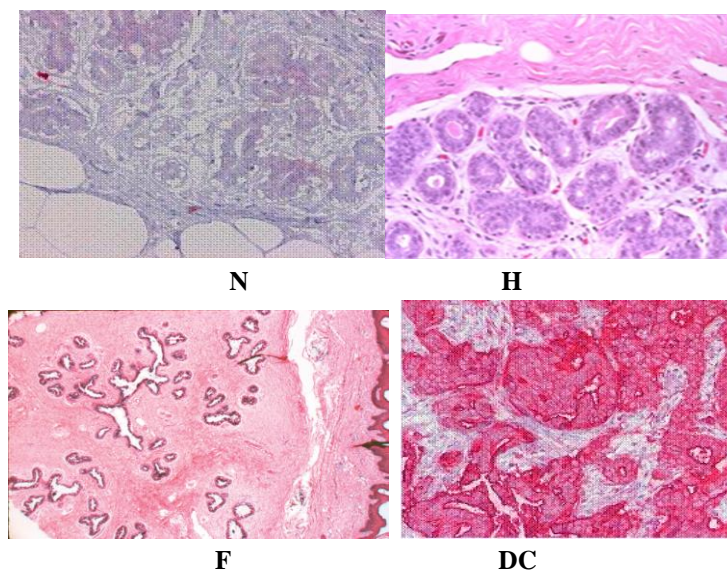
Figure(1):Histological image cross section of formalin-fixed human breast tissue with hematoxylin-eosin (H&E X 100). (N):normal breast tissue, (H):hyperplasia, (F):fibro adenoma, and (DC): ductal carcinoma.
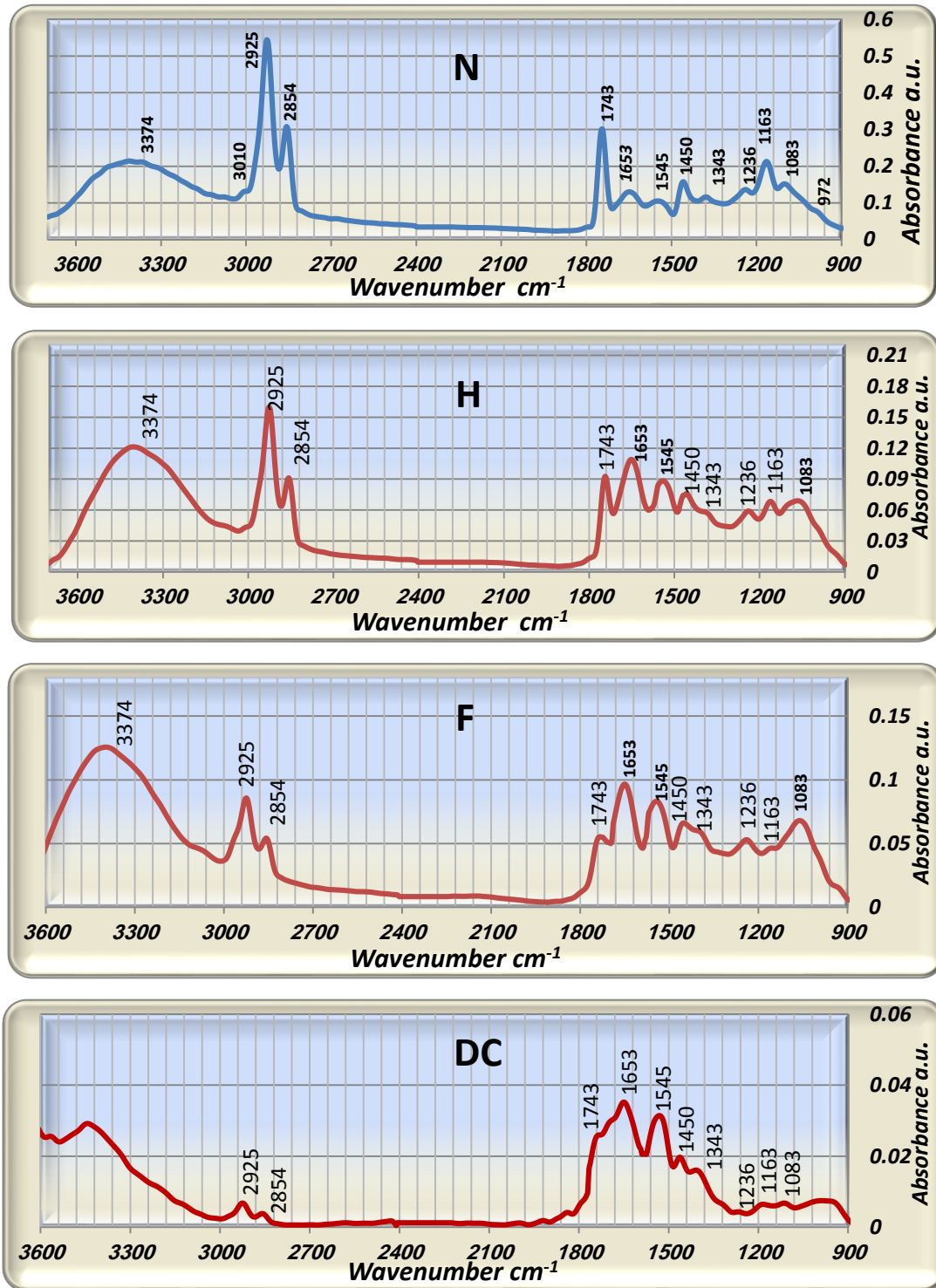
Figure (2) : FT-IR spectra for normal (N), hyperplasia (H), fibroadenoma (F) and ductal carcinoma(DC) .
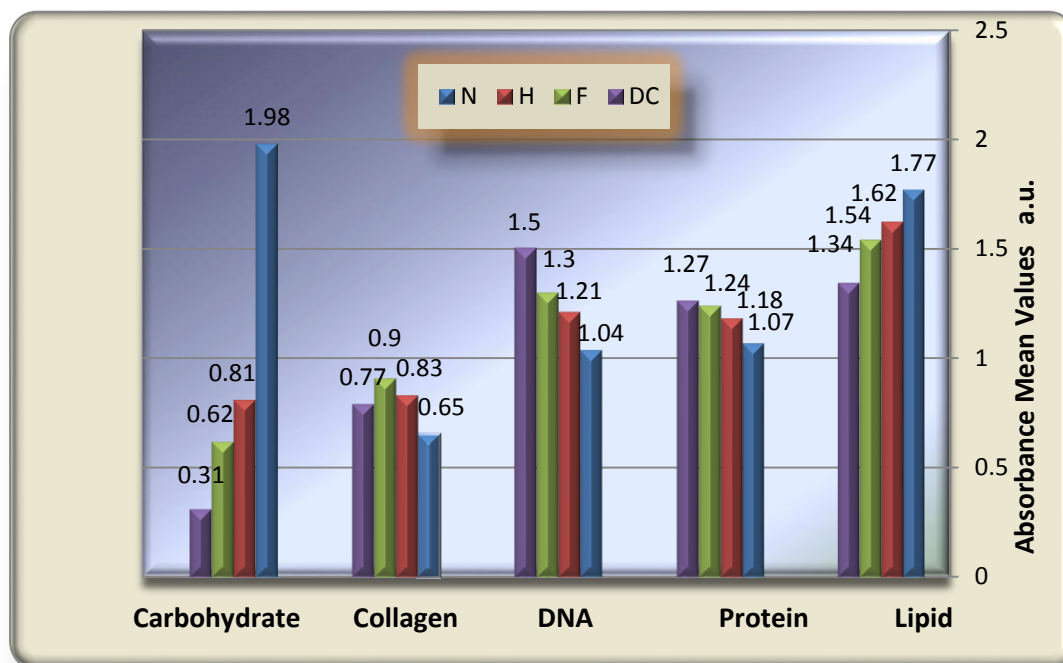
Figure (3): Variation of standard absorbance's ratios (functional biomolecules) with different breast tissue pathologies.

Table (2): **Comparison of standard ratio among four breast cancer type.**

Standard Ratio	N	H	Rate of Change From N	F	Rate of Change From N %	DC	Rate of Change From %
A2925/A2854 Lipid	1.77	1.62	−8.5	1.54	−13	1.34	−24.2
A1653/A1545 Protein	1.07	1.18	+10.3	1.24	+15.9	1.27	+18.6
A1083/A1236 DNA	1.04	1.21	+15.4	1.30	+25	1.5	+44.2
A1343/A1450 Collagen	0.65	0.83	+27.7	0.9	+38.4	0.77	+18.4
A1163/A1545 Carbohydrate	1.98	0.81	−59	0.62	−68	0.31	−84.4

(+) means increase , (−) means decrease

تشخيص سرطان نسيج الثدي البشري بإستخدام مطياف FT-IR

إيناس سليمان يوسف   نبيل إبراهيم فواز   بلال جاسر محمد

E.mail : enasalani2@yahoo.com

الخلاصة:

لقد تم انجاز هذه الدراسة الطيفية لتحويل فورييه بالأشعة تحت الحمراء لنسيج الثدي البشري السليم والحميدي والخبيث. على حد علمنا، هذا المشروع هو اول بحث إحصائي للتشخيص القائم على التحليل الطيفي باستخدام **FT-IR** لسرطان الثدي بين نساء العراق. في هذه الدراسة تم استخدام جهاز **FT-IR** الطيفي للتحليل النوعي والكمي لأنسجة الثدي السرطانية بسبب حساسيته للتغيرات في الجزيئات الحيوية في الأنسجة. لقد تم تسجيل طيف الأشعة تحت الحمراء لعدد 91 عينة من أنسجة الثدي التي سبق تحديدها نسيجيا من قبل مختصين بعلم الامراض بشكل مسبق الى : 63 عينة (**N**) سليمة، 8عينات تضخم حميدي (**H**) ، 10 عينات ورم ليفي حميد (**F**) و 10 عينات سرطان الأقنية (**DC**). لقد تم الكشف عن العديد من الاختلافات الطيفية في نطاق الترددات بين 400 سم $^{-1}$ و 4000 سم$^{-1}$. ان الشدة النسبية للحزم **A2925/A2854** و **A1653/A1545** و **A1343/A1450** و **A1083/A1236** و **A1163/A1545** تمثل التغيرات المتعلقة بمحتوى الدهون ، والبروتين ، والكولاجين ، والأحماض النووية ، والكربوهيدرات على التوالي في أنسجة الثدي البشري. لقد تبين لنا ان هناك اختلافات واضحة في الخصائص الطيفية بين الأنسجة السليمة والغير سليمة (الحميدة والخبيثة) بسبب التغيرات في تركيبة الجزيئات و البنية التي تصاحب التحول من الحالة الطبيعية الى الحالة السرطانية. حيث يمكن أن نميز أن تركيز محتوى الدهون أقل في ألانسجة السرطانية ، على العكس من الأنسجة السليمة للثدي ، وايضا فان محتوى الحمض النووي والبروتين و الكولاجين قد ازداد في نسيج الورم الحميدي والخبيث. وتشير هذه الاختلافات في المعلومات الطيفية الى اهميتها و فائدتها في تشخيص سرطان الثدي والتي يمكن أن تفيد كأساس لمعرفة التغييرات في مكونات الأنسجة أثناء عملية التسرطن