P- ISSN 1991-8941 E-ISSN 2706-6703 Journal of University of Anbar for Pure Science (JUAPS) Open Access
2015, 9 (3) :27-34

Identifying Differences Between Normal and Invasive Ductal Carcinoma grades for Breast tissues 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

ARTICLE INFO

Received: 19 / 5 /2022
Accepted: 28 / 5 /2022
Available online: 19/7/2022
DOI: 10.37652/juaps.2015.127547

Keywords:
Cancer Grades Biomarkers,
FT-IR Diagnosis,
Vibrational Spectroscopy.

ABSTRACT

Breast cancer is one of the most important malignant forms of cancers and it represents a great threat to life for women. FT- IR spectra ware taken for 98 samples of breast tissues that were previously histopathologically identified by pathologist experienced as: 63 normal (N) samples and 35 invasive ductal carcinoma (IDC) samples that were classified in different grades 19 G1 samples,7 G2samples and 9 G3 samples in Mid-IR frequency range between 400 cm^{-1} and 4000 cm^{-1}. Many spectral differences were observed in the frequency regions N-H stretching, Amide bands, C-H vibrations, and 950-1400 cm^{-1}. The aim of this study is analyze different grades of IDC spectroscopically to evaluate the efficiency of FT-IR spectroscopy to differentiate between these grades. The results show considerable decrease in the lipid and carbohydrate content with the carcinoma grades (from G1 to G3), while, protein, collagen and nucleic acid (DNA) reveal slight increase with the carcinoma grade, that will be useful in classifying three different nuclear grades. This study demonstrates that FT-IR spectroscopy is a promising tool for accurate, rapid diagnosis of breast canser.

Introduction

Cancer is a multi-step process resulting from the accumulation of irreversible and transmittable genetic aberrations together with the concurrent presence of epigenetic alterations insusceptible cells[1].

The leading cause of cancer death of adult women in the world, is breast cancer[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.

* Corresponding author at: University of Anbar - College of Medicine .E-mail address: enasalani2@yahoo.com

The early detection of cancer can play a important role in its treatment, and increase in survival rates of cancer patients [3].

FT-IR spectroscopy has been used extensively in biology and biochemistry to study the composition of molecules, so this technique can be used to probe the vibrational energy levels of a molecule in a sample[4].

Lipids, proteins, collagen, nucleic acids (DNA), and carbohydrates are the important structural, and functional biomolecules in tissues.

The transformation from normal status to malignant status of cells induces not only changes in the amounts of biomolecules but also in their structures [5]. FT-IR is a simple, reliable, highly sensitive, specific , nondestructive technique [2,6]. FT-IR has shown as a sensitive diagnostic tool to distinguish cancerous from normal tissues as in colon [7], prostate [8], breast [9], cervical [10], gastric [11], oral [12] and esophageal [13], bladder [14] . All cancer grades are appear in fundamental changes in cellular morphology and/or tissue biochemistry. Proteins, lipid, and collagen, as a biochemical tumor makers can be detected by analyzing the differences in FT-IR spectra of normal and abnormal tissues [15], providing useful qualitative and quantitative information for tumor classification and grading, which are important in diagnosis and predicting the prognosis of cancer [5]. This study aimed to identify any chemical or structural changes based from the spectra of normal and cancerous tissues to IDC grading by FT-IR Spectroscopy.

Experimental Work

Normal and malignant (invasive ductal carcinoma) breast tissue specimens with different grades were obtained after mastectomy of female patients, The tissues were preserved in 10% formaldehyde solution and sampled immediately after the operations. Two pieces of the tissues, each of about 2cm in diameter, were taken. One was cut off from the center of the lesion (abnormal) and the other was from the distant edge (normal) of the removed tissues. Each ex vivo breast tissue is divided by a doctor into two parts, one goes to our lab, the second one goes to the pathology examination. So the samples for pathology examination undergo the standard procedure of preserving in 10% formalin, embedded in paraffin and cutting through the marked locations into 5-µm-thick sections, and staining with hematoxylin-eosin (H&E) stain to screen histological changes associated with tissue transformation [16]. The Normal and cancer tissues that goes to our lab, were cut into approximately 2 mm sizes 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, then they were 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 lesion) as shown in Table1

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

No.	Histology	Total number of cases
1	Normal	63
2	IDC G1	19
3	IDC G2	7
4	IDC G3	9
Total samples number		98

G1 is grade1, G2 is grade2 and G3 is grade3

Results and Discussion:

Histological Examination Results

Upon removal during the operation, normal and malignant (IDC_S) breast tissue specimens of female patients aged 30–76 years old (median age is 53 years old) showing different grades 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), 19 IDC G1, 7 IDC G2 and 9 IDC G3.

FT-IR Qualitative Analysis of Different IDC Tissue Grades

The FT-IR spectrum of normal and cancerous breast tissues (IDC) is shown in Figure2. The intensity was higher in N tissue than in the malignant IDC tissue because of the structural changes in breast tissue during carcinogenesis, in which the tissues become disorganized and display different optical properties [17]. we found that the spectra generated for each sample type were highly reproducible. The FT-IR spectrum are characterized by 10 distinguished 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, protein and nucleic acids peaks is listed in Table (2). 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 methylene ($-CH_2-$) group band in the 2925 and 2854 cm^{-1} peaks which belongs to lipids, is enhanced in the spectra of normal (N) tissue, as shown in Figure (2), while they are markedly diminished in the spectra of cancerous (IDC) tissues.

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 tissues as shown in (Figure 2) were greatly increased compared with those of normal tissue lesions. This result can explain the increased amount of protein in cancerous tissues during malignancy [25,26].

Table 2. Peak positions and assignments of Breast Tissue Spectra[α]

Peak position (cm^{-1})	Major assignment
3374	N-H stretching mode (protein)
3010	C-H stretching vibrations (lipid)
2925	CH_2 antisymmetric stretching (lipid)
2854	CH_2 symmetric stretching (lipid)
1743	C=O ester stretching mode (lipid)
1653	(C=O) carbonyl stretching, Amide I (protein)
1545	N-H bending mode, amide II (protein)
1450	CH_3CH_2 deformation mode
1343	CH_3CH_2 wagging mode
1236	$\upsilon_{as}(PO_2^-)$ antisymmetric phosphate stretching(DNA)
1163	C-OH stretching vibration mode
1083	$\upsilon s(PO2-)$ symmetric phosphate stretching (DNA)
972	$(CH_3)_3N^+$ asymmetric stretching mode (lipid)

[α] [3,5,11,18,19,20,21,22,23,24]

The 1743 cm^{-1} band is a reliable marker, which is assigned to the vibration of the ester group (C=O), and present in normal tissues, It was realized that this band is weak or absent in IDC tissue. The decrease of lipid cells in IDC tissue may be accounted to two reasons. Normal tissues, are excluded by the proliferating malignant tissue during tumor growth, Which leads to the absence of fat cells in the tissue. The fat is consumed in the region of the malignant tissue because of the increased nutritional and energy requirement of the developing carcinoma [27].

These results are consistent with previous findings [3,20,,23,25,27], 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 IDCs Different Tissue grades.

In this study, We analyze different grades of IDC spectroscopically to evaluate the efficiency of FT-IR spectroscopy to differentiate between these grades. And to understand any biochemical changes that occur. Our samples (IDCs) were classified by an accredited pathologist who has an experience in breast pathology . The structure of breast cancer samples and normal tissue sample was determined through FT-IR spectroscopy.

For the purpose of carcinoma grading G1, G2, G3 of IDC samples with quantitative analysis, we plotted the variations in the bellow mentioned five absorbance ratios with tumor grades (G1,G2 and G3) as shown in Figures (3 to 7). 19 samples were classified as IDC G1, Seven and nine samples were classified as G2 and G3, respectively.

1)) The absorbance ratio A2925/A2854 is used to measure the ratio of lipid content [3,5]. The mean values of this absorbance ratio are 1.77, 1.27, 1.03 and 0.75 for the N, G1, G2 and G3 of IDC samples respectively. The percentage rates of change in G1, G2 and G3 from N sample are −28.2%, −41.8%, and −57.6%, respectively, where (−) means decrease and (+) means increase.

2)) The absorbance ratio A1163/A1545 is used to measure the ratio of the carbohydrate content [20,28]. The mean values of this absorbance ratio are 1.98, 0.39, 0.31 and 0.21 for the N, G1, G2 and G3 of IDC samples, respectively. The percentage rates of change in G1, G2 and G3 from N sample are −80.3%, −84.3% and −89.4%, respectively.

3)) The absorbance ratio A1653/A1545 is used to measure the ratio of protein content [3,5,22]. The mean values of this absorbance ratio are 1.07, 1.31, 1.34 and 1.45 for the N, G1, G2 and G3 of IDC samples, respectively. The percentage rates of change in G1, G2 and G3 from N sample are +22.4%, +25.5%, and +35.5%, respectively.

4)) The absorbance ratio A1343/A1450 is used to measure the ratio of the collagen content [29,30]. The mean values of this absorbance ratio are 0.65, 0.81, 0.82 and 0.84 for the N, G1, G2 and G3 of IDC samples, respectively. The percentage rates of change in G1, G2 and G3 from N sample are +24.6%, +26.1% and +29.2%, respectively.

5)) The absorbance ratio A1083/A1236 is used to measure the ratio of the nucleic acids (DNA) content [3,22]. The mean values of this absorbance ratio are 1.04, 1.54, 1.61 and 1.73 for the N, G1, G2 and G3 of IDC samples, respectively.

The percentage rates of change in in G1, G2 and G3 from N sample are +48%, +54.8% and +66.3% respectively.

Figures (3and 4) show a clear reduction in lipid and carbohydrate contents with carcinoma grades, and the percentage rate of change in content from N sample decreases (as the cancer progresses from G1 to G3). So the differences between the invasive cancer grades indicate that, as the tissue increases in malignancy, the lipid and carbohydrate content decreases at a high grade [31]. By contrast, the absorbance ratios for protein, collagen, and nucleic acid (DNA) (Figures 5, 6 and 7), respectively, slightly increased with carcinoma grade; the percentage rate of change in content increased as the cancer progresses from G1 to G3 compared with that in N. Variation in intensity of carcinoma grades confirms that higher nuclear grade (G3) is rich in protein and nucleic acid content compared with the lower nuclear grade cancer (G1 and G2). These results are consistent with previous findings [2,31] and contradict with those results [22], who found obvious decrease in the collagen level with breast cancer grades by FT-IR spectroscopy.

Conclusions

w lipid and carbohydrate contents relative to the high contents of total protein and nucleic acid in malignant tissues. Therefore, combining the five parameters, namely, lipids, proteins, collagen, DNA, and carbohydrate, is useful in more precise and consistent classification of the three different nuclear grades (high, intermediate, and low). This study can illustrate that, this method can be used as a diagnostic tool, complementary to histopathology.

References

1-Giuseppe Bellisola, Claudio Sorio, Infrared spectroscopy and microscopy in cancer research and diagnosis, Am J Cancer Res, 2(2012)1-21.

2- 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.

3- 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.

4- 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.

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

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

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- Le-Petross HT, Shetty MK, Magnetic resonance imaging and breast
ultrasonography as an adjunct to mammographic screening in high-risk
patients, Semin Ultrasound CT MR, 32(2011)266–272.

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- M. Dimitrova, D. Ivanova, I. Karamancheva, A. Milev, I. Dobrev, Application of FTIR-Spectroscopy for Diagnosis of breast Cancer Tumors, Journal of the University of Chemical Technology and Metallurgy, 44(2009) 297-300.

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

19- 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.

20-. 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

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

22- 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.

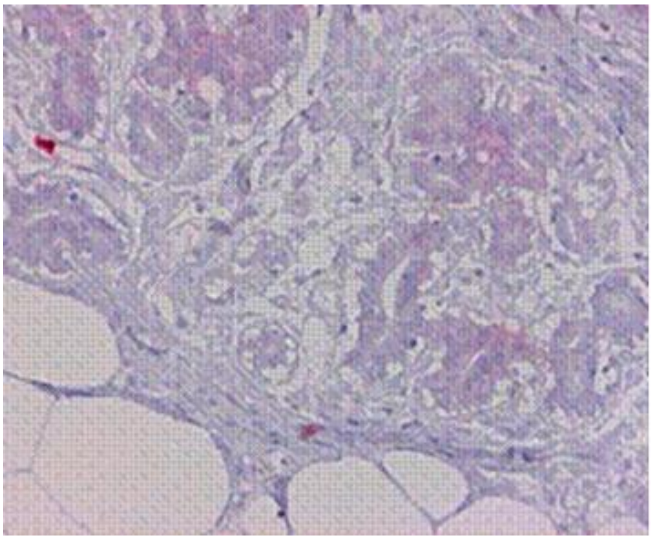
23- Heinz Fabian, Ngoc Anh Ngo Thi , Michael Eiden , Diagnosing benign and malignant lesions in breast tissue sections by using IR-microspectroscopy, Biochimica et Biophysica Acta 1758 (2006) 874–882.

24- 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.

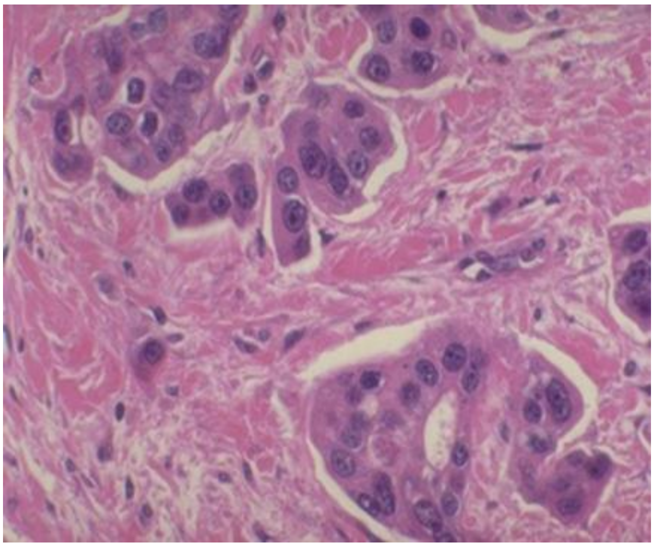
25- Jakub Surmacki, Jacek Musial, Radzislaw Kordek and Halina Abramczyk, Raman imaging at biological interfaces: applications in breast cancer diagnosis, Molecular Cancer, 12(2013)48.

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

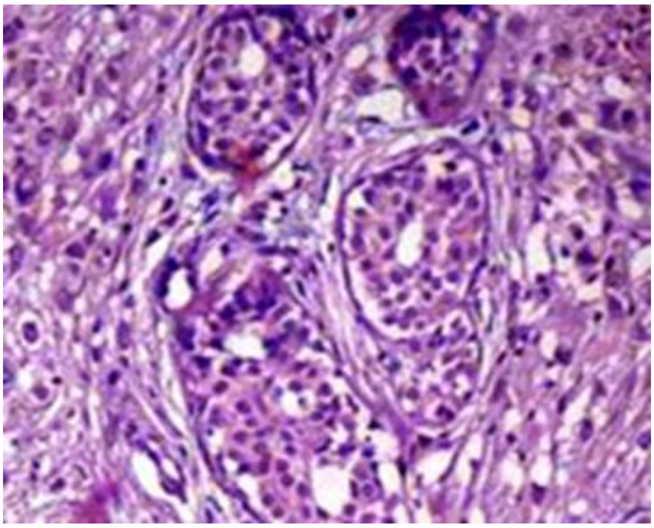
27- Jin Guang WU, YI Zhuang XU, Cuan Wen, Distinguishing Malignant from Normal Oral Tissues Using FT-IR Fiber Optic Techniques, FTIR Fiber Optics, 23(2001)186-197.
28- Bird RE, Wallace TW, Yankaskas BC: Analysis of Cancers Missed
at Screening Mammography. Radiology, 184(1992) 613–617.
29- M. V. P. Chowdary, 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.
30-- Shazza Rehman ,Vibrational Spectroscopy for Tissue Analysis, U.K., (2013).
31- R Baker, KD Rogers, N Shepherd and N Stone, New relationships between breast microcalcifications and cancer, British Journal of Cancer, 103(2010)1034 – 1039.



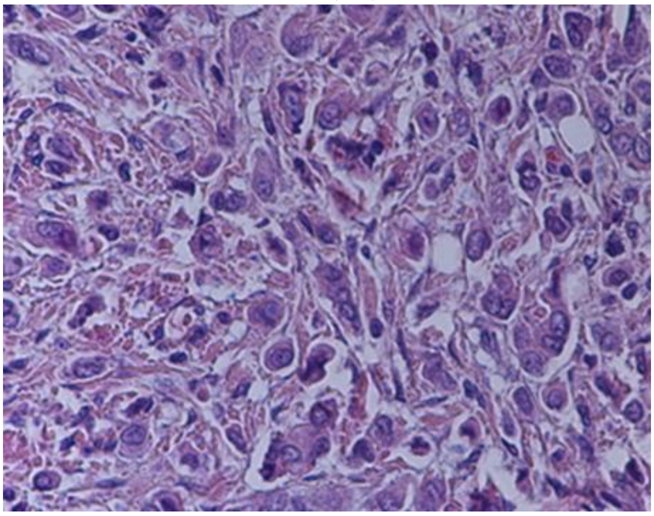
N



IDC (G1)



IDC (G2)



IDC (G3)

Figure(1):Histological image cross section of formalin-fixed human breast tissue with hematoxylin-eosin (H&E X 100). (N):normal breast tissue and (IDC: invasive ductal carcinoma grades G1G,2G,3.

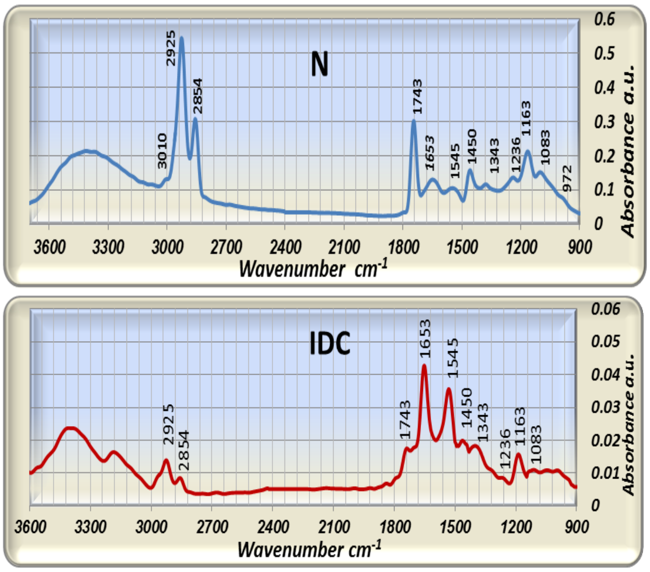


Figure (2) : FT-IR spectra for normal (N)and invasive ductal carcinoma (IDC)tissue.

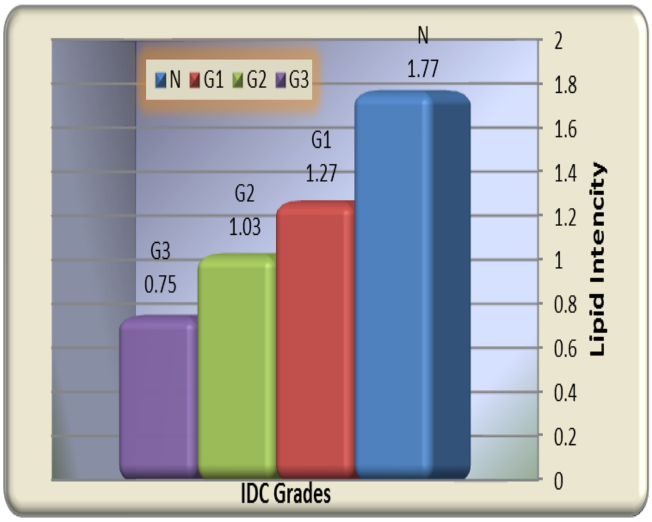


Figure (3): Variations of absorbance's ratios of lipid with the different grades of Invasive ductal carcinoma.

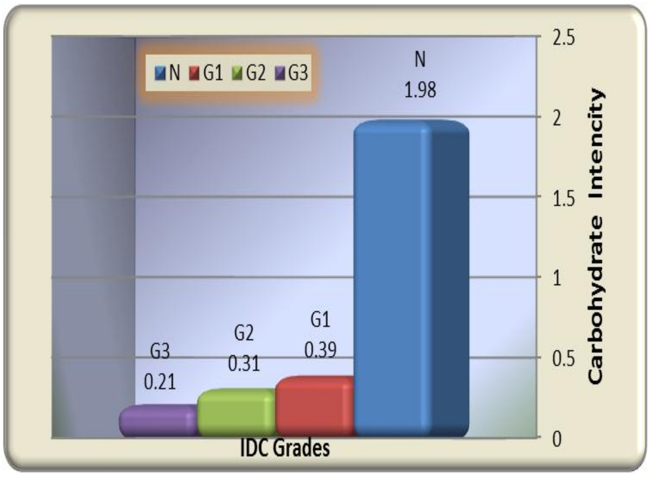


Figure (4): Variations of absorbance's ratios of carbohydrate with the different grades of Invasive ductal carcinoma.

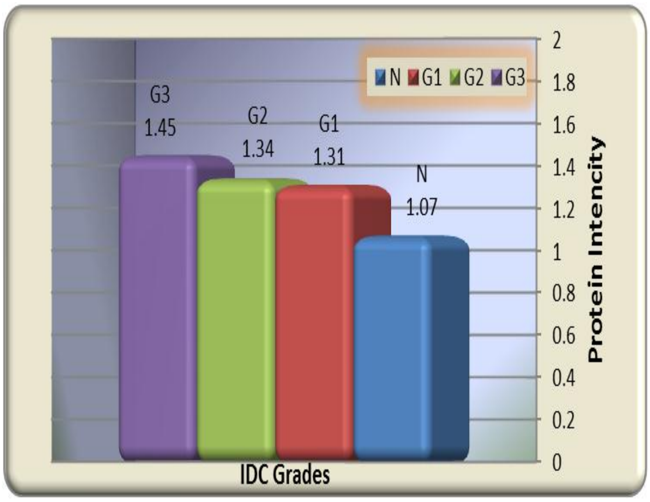


Figure (5): Variations of absorbance's ratios of protein with the different grades of Invasive ductal carcinoma.

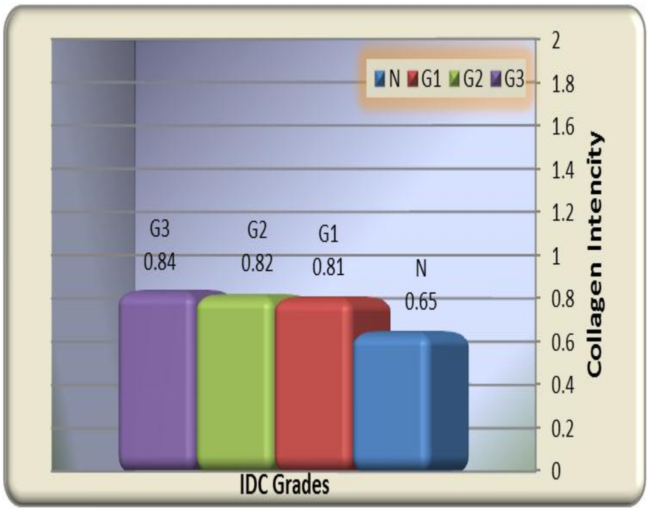


Figure (6): Variations of absorbance's ratios of collagen with the different grades of Invasive ductal carcinoma.

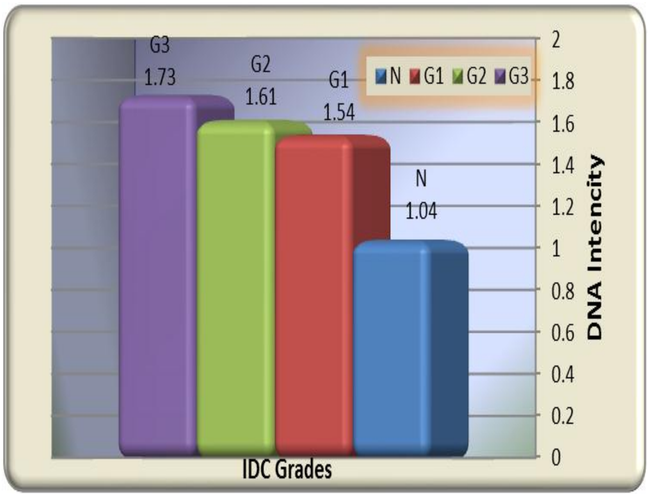


Figure (7): Variations of absorbance's ratios of nucleic acid with the different grades of Invasive ductal carcinoma.

P- ISSN 1991-8941 E-ISSN 2706-6703 *Journal of University of Anbar for Pure Science (JUAPS)* Open Access
2015, 9 (3) :27-34

تحديد الاختلافات بين نسيج الثدي السليم و درجات سرطان الأقنية الغازية باستخدام مطياف FT-IR

إيناس سليمان يوسف نبيل إبراهيم فواز بلال جاسر محمد

E.mail : enasalani2@yahoo.com

الخلاصة:

سرطان الثدي هو احد أهم أشكال السرطانات الخبيثة التي تشكل تهديدا كبيراً لحياة للنساء في العالم . لقد تم تسجيل طيف الأشعة تحت الحمراء لعدد ٩٨ عينة من أنسجة الثدي التي تم تحديدها نسيجيا من قبل مختصين بعلم الامراض بشكل مسبق الى: ٦٣ عينة **(N)** سليمة و٣٥ عينة سرطان الأقنية الغازية **(IDC)** والتي تم تصنيف الاخيرة الى مختلف الدرجات وهي: ١٩ عينة درجة اولى **G1**، و٧ عينات درجة ثانية **G2** و٩عينات درجة ثالثة **G3**، في نطاق الترددات بين ٤٠٠ سم$^{-1}$ و٤٠٠٠ سم$^{-1}$. لقد تم الكشف عن العديد من الاختلافات الطيفية في مناطق التردد **N–H** المطية واهتزازات **C–H**، وحزم الأميدات ومنطقة ٩٥٠-١٤٠٠ سم$^{-1}$. لقد تم تحليل وتصنيف درجات مختلفة من سرطان الأقنية الغازية **IDC** بهدف التقييم لامكانية جهاز **FT–IR** الطيفي أن يفرق بين هذه الدرجات. إن النتائج تكشف عن انخفاض كبير في محتوى الدهون و الكربوهيدرات مع زيادة درجة التسرطن (من **G1** إلى **G3**)، وفي المقابل فان البروتين والكولاجين والحمض النووي **(DNA)** قد بينت ارتفاع طفيف مع زيادة درجة التسرطن ، و هذا سيساعد في تصنيف ثلاث درجات تسرطن مختلفة . بذلك يتبين لنا ومن خلال دراستنا، أن مطياف **FT –IR** هو أداة جديدة وسريعة ودقيقة لتشخيص الأنسجة الغير سليمة.