Identifying Differences Between Normal and Invasive Ductal Carcinoma grades for Breast tissues By FT-IR Spectroscopy

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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 were 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 G2 samples 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 cancer.

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

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 susceptible 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.

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>
<thead>
<tr>
<th>No.</th>
<th>Histology</th>
<th>Total number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>63</td>
</tr>
<tr>
<td>2</td>
<td>IDC G1</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>IDC G2</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>IDC G3</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Total samples number</td>
<td>98</td>
</tr>
</tbody>
</table>

G1 is grade1, G2 is grade2 and G3 is grade3

Results and Discussion:

Histological Examination Results

Upon removal during the operation, normal and malignant (IDCₙ) 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 (\(-\text{CH}_2\)) 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\(^a\)

<table>
<thead>
<tr>
<th>Peak position (cm(^{-1}))</th>
<th>Major assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3374</td>
<td>N-H stretching mode (protein)</td>
</tr>
<tr>
<td>3010</td>
<td>C-H stretching vibrations (lipid)</td>
</tr>
<tr>
<td>2925</td>
<td>CH(_2) antisymmetric stretching (lipid)</td>
</tr>
<tr>
<td>2854</td>
<td>CH(_2) symmetric stretching (lipid)</td>
</tr>
<tr>
<td>1743</td>
<td>C=O ester stretching mode (lipid)</td>
</tr>
<tr>
<td>1653</td>
<td>(C=O) carbonyl stretching, Amide I (protein)</td>
</tr>
<tr>
<td>1545</td>
<td>N-H bending mode, amide II (protein)</td>
</tr>
<tr>
<td>1450</td>
<td>CH(_3)CH(_2) deformation mode</td>
</tr>
<tr>
<td>1343</td>
<td>CH(_3)CH(_2) wagging mode</td>
</tr>
<tr>
<td>1236</td>
<td>(\nu_{as} (PO_2^-)) antisymmetric phosphate stretching (DNA)</td>
</tr>
<tr>
<td>1163</td>
<td>C-OH stretching vibration mode</td>
</tr>
<tr>
<td>1083</td>
<td>(\nu_{s} (PO_2^-)) symmetric phosphate stretching (DNA)</td>
</tr>
<tr>
<td>972</td>
<td>((\text{CH}_3)_2\text{N}^-) asymmetric stretching mode (lipid)</td>
</tr>
</tbody>
</table>

\(^{a}[3,5,11,18,19,20,21,22,23,24]\)

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 G1, G2 and G3 from N sample are +48%, +54.8% and +66.3% respectively.

Figures (3 and 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

With 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

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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.
تحديد الاختلافات بين نسيج الثدي السليم ودرجات سرطان الأقنية الغازية باستخدام مطياف FT-IR

إناس سليمان يوسف، نبيل إبراهيم فواز، بلال جاسر محمد

الخلاصة:
سرطان الثدي هو أحد أهم أشكال السرطانات الخبيثة التي تشكل تهديداً كبيراً لحياة النساء في العالم. لقد تم تسجيل طيف الأشعة تحت الحمراء لعدد 98 عينة من أنسجة الثدي التي تم تحديدها نسيجاً من قبل مختصين بعلم الأمراض بشكل مسبق إلى: 63 عينة سليمة و35 عينة سرطان الأقنية الغازية (IDC) والتي تم تصنيف الأخيرة إلى مختلف الدرجات وهي: 19 عينة درجة اولى (G1)، 7 عينات درجة ثانية (G2) و4 عينات درجة ثالثة (G3) في نطاق الترددات بين 0.44 سم⁻¹ و0.44 سم⁻¹. لقد تم الكشف عن العديد من الاختلافات الطيفية في مناطق التردد N–H والاهتزازات C–H، وحمض الأميدات ومنطقة 1400-950 سم⁻¹. لقد تم تحليل وتصنيف درجات مختلفة من سرطان الأقنية الغازية بهدف التقييم الطيفي أن يفرق بين هذه الدرجات، إن النتائج تكشف عن انخفاض كبير في محتوى الدهون والكربوهيدرات مع زيادة درجة السرطان (من G1 إلى G3)، وفي المقابل فان البروتين والكولاجين والحمض النووي (DNA) قد بنيت ارتفاع طيفي مع زيادة درجة السرطان. و هذا سيساعد في تصنيف ثلاث درجات سرطان مختلفة. بذلك يتيح لنا ومن خلال دراستنا، أن مطياف FT-IR هو أداة جديدة واعدة وسريعة ودقيقة للتشخيص الأنسجة الغير سليمة.