Study The Differences of Sera between normal and malignant In human Breast using FTIR-ATR

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Abstract:

Blood being the chief circulatory medium in human body, participates in every functional activity by virtue of its circulation through every organ. Almost in all diseases the blood undergoes major changes in chemical and biochemical properties. The study of blood by spectroscopic techniques can be used not only for understanding the biological nature of the disease, but also for the diagnosis of the breast cancer .In the present work, Fourier Transform InfraRed (FTIR-ATR) technique is employed to study the spectral differences between a healthy serum and that affected with new breast cancer .we found the absorbance of patients with new breast cancer larger than the absorbance of health human.

Keywords: Fourier transforms, Infrared spectroscopy, breast cancer, spectra of serum human.

دراسة الفرق للمصل بين الطبيعي والخبيث في الثدي البشري باستخدام تقنية FTIR-ATR

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الخلاصة:

الدم هو الوسط الدائري الرئيسي في الجسم البشري ، يشارك في كل نشاط وظيفي استنادا الى توزيعه خلال كل عضو، في كل الامراض تقريبا يمر الدم بالتغيرات الرئيسية لخواصه الكيميائية الحيوية، دراسة الدم بالتقنيات الطيفية ممكن ان لا يستخدم فقط لفهم الطبيعة البايلوجية للمرض ، لكن ممكن لتشخيص سرطان الثدي . في العمل الحالي استخدمت تقنية تحويل فورير للأشعة تحت الحمراء لدراسة الاختلافات الطيفية بين المصل الطبيعي والمتأثر بالحالة الجديدة لسرطان الثدي . اذ وجدنا امتصاصية المصابين الجدد بسرطان الثدي اكبر من امتصاصية الانسان الطبيعي.

الكلمات المفتاحية: تحويلات فورير، مطيافية الأشعة تحت الحمراء، سرطان الثدي، أطياف المصل البشري.

Background: Most of the blood tests aiming for breast cancer screening rely on quantification of a single or few biomarkers. The objective of the present work is to employ the spectroscopic techniques to detect the changes in blood sera of breast cancer subjects and to evaluate the feasibility of detecting breast cancer by analyzing the total biochemical composition of sera using infrared spectroscopy rely on absorbance differences and the shift in wavenumbers between normal and the case of new breast cancer.

1. Introduction

Breast cancer is the most common malignancy in women in the Iraq and the second leading cause of death. New cases of breast cancer will be diagnosed in the Iraq in 2014 especially in Al- Muthana governorate formed 70% according tostatistical done in Middle Euphrates Center Cancer of Iraq for unknown of all cases ,early detection of breast is important cancer using total biochemical analysis a preliminary study annual screening mammograms starting aged 40. (Zelig, Barlev et al. 2015).Conventional mammography is known to have a sensitivity of about 66 % and specificity of about 92 %(Pisano, Gatsonis et al. 2005).In the last few decades, researchers have introduced the use of serum tumor markers for cancer screening .However, none of the markers tested has proved suitable for screening the entire population because of low specificity and sensitivity at the early stages of disease(Duffy 2006) To improve these results, attempts have been made to apply combinations of markers(Pisano, Gatsonis et al. 2005). Thus, multi molecular biochemical analysis could be useful for this purpose. Fourier transform infrared (FTIR-ATR) spectroscopy is a simple, rapid, reagents free biochemical tool that provides information on the total molecular composition of biological samples(Diem, Griffiths et al. 2008). Organic compounds absorb infrared light at an energy (wavenumber) corresponding to the nature of the bonds between its atoms, yielding a unique spectral "fingerprint". Thus, spectroscopy of a biological sample generates an absorption spectrum of the compounds in that sample, reflecting their molecular structure ,It is a powerful analytical biochemical and imaging method however, in a complex samples such as blood components .It has been found to be useful for the detection and characterization of a broad variety of cancer cells and tissues (Simonova and Karamancheva 2013).

Mid Infrared spectroscopic method efficiently been used in the field of Medical-physical science for qualitative analysis of biological samples such as blood plasma and sera or tissues. Implementation of these techniques reduces time, resources and cuts cost(Berezin and Achilefu 2010). Spectroscopy is emerging as a potential diagnostic tool in the medical and pharmacological fields to provide information about the different chemical and morphological structures of healthy and patient .It reflects the physiological and pathological changes that take place in the tissues, which leads to the changes in the various sera and cellular constituents. Spectroscopy has received quite a lot of attention not only for understanding the biological nature of the disease, but also for the diagnosis of the disease in recent years. Almost in all diseases the blood undergoes major changes in chemical and biochemical properties. The application of spectroscopy for the study of biomedical compounds has increased tremendously in recent years since this gives the metabolic photography of the subject(Berezin and Achilefu 2010).

The advantage in the ever improving sensitivity of contemporary spectrometers with sophisticated computational techniques proved that FTIR-ATR spectroscopy could be exploited to explore the various biochemical alterations on the molecular and structural differences of the bio fluids of the human body(Hajiali, Heredia-Guerrero et al. 2015).

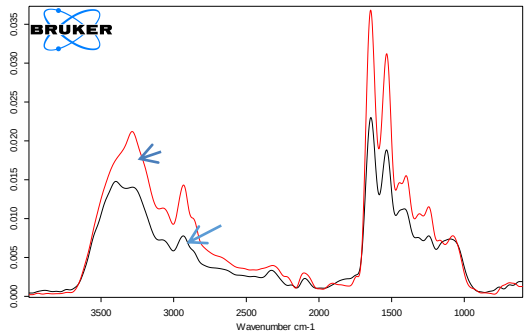
2. Materials and methods

Blood samples have been collected from 25 healthy volunteers and 22 breast cancer patients which are divided as a faction aged (25-30),(30-35),(35-40),(40-45),(45-50),(50-55),55-60),(60-65),(65-70)and(70-75). from the middle Euphrates center cancer , also collected samples control from Al Sader general hospital Al najaf statistical done in Middle Euphrates Center Cancer of Iraq for unknown of all cases ,early detection of breast is important cancer using total biochemical analysis a preliminary study annual screening mammograms starting aged 40. (Zelig, Barlev et al. 2015). Conventional mammography is known to have a sensitivity of about 66 % and specificity of about 92 % (Pisano, Gatsonis et al. 2005). In the last few decades, researchers have introduced the use of serum tumor markers for cancer screening .However, none of the markers tested has proved suitable for screening the entire population because of low specificity and sensitivity at the early stages of disease (Duffy 2006) To improve these results, attempts have been made to apply combinations of markers (Pisano, Gatsonis et al. 2005). Thus, multi molecular biochemical analysis could be useful for this purpose. Fourier transform infrared (FTIR-ATR) spectroscopy is a simple, rapid, reagents free biochemical tool that provides information on the total molecular composition of biological samples (Diem, Griffiths et al. 2008). Organic compounds absorb infrared light at an energy (wavenumber) corresponding to the nature of the bonds between its atoms, yielding a unique spectral "fingerprint". Thus, spectroscopy of a biological sample generates an absorption spectrum of the compounds in that sample, reflecting their molecular structure ,It is a powerful analytical biochemical and imaging method however, in a complex samples such as blood components .It has been found to be useful for the detection and characterization of a broad variety of cancer cells and tissues(Simonova and Karamancheva 2013).

Mid Infrared spectroscopic method efficiently been used in the field of Medical-physical science for small bettri dishes with cover to each sample, labeling it, then weighted 300 mg of Potassium Bromid (KBr) by sensitive balance after blank it at the weight of the dishes .Measured 0.1ml of serum with change the yellow tip for each sample, then mixed it with KBr by glass sticks ,The dishes which were ground prepared to be examined put into Vacuum dried for 20-30 minutes ,Dried samples were grinned by marble mortar which is used with FTIR-ATR Instrument(Shaw, Kotowich et al. 1998). The pressed disc was put in the instrument after blanks it with the used KBr then recorded the spectrum to read concentration, after use the mortar and pistil should be cleaned with acetone and double distilled water, and put back on top of the oven for drying. The spectra were recorded in the region 4000-600 cm-1 using Bruker IFS 66V FTIR spectrophotometer. The collected signals were transferred to PC and data were processed by Windows based data program. All the spectra were baseline corrected and normalized to equal intensities and therefore compensated for the imprecision in the film preparation. The groups were analyzed as well as the specific influence of the relevant pathological characteristics of the cancer patients.

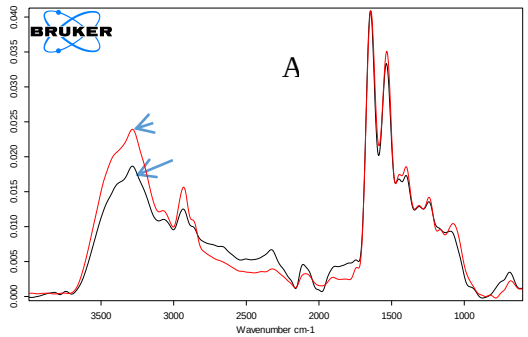
3. Results and discussions for fourier transform, infrared spectroscopy:

The infrared spectrum of serum provides useful information of biomolecules like structure, functional groups, types of bonds and their interactions. (Zelig, Barlev et al. 2015) Differences among women with malignant breast and benign breast ,the representative normalized FTIR-ATR absorption overlay spectra of normal and breast cancer in figures from(1 to 10)



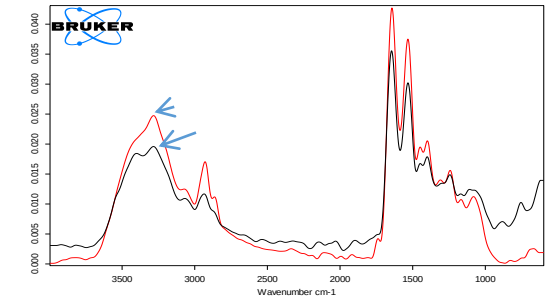
Figure(1):Comparison between new case of breast cancer(red)(A) and control(black)(B) for faction aged (25-30) years

(1)



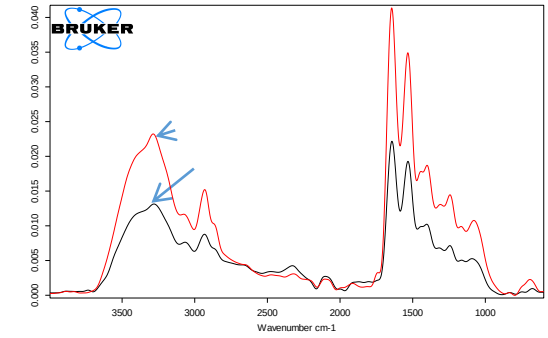
Figure(2):Comparison between new case of breast cancer(red)(A) and control(black)(B) for faction aged (30-35) years

(2)



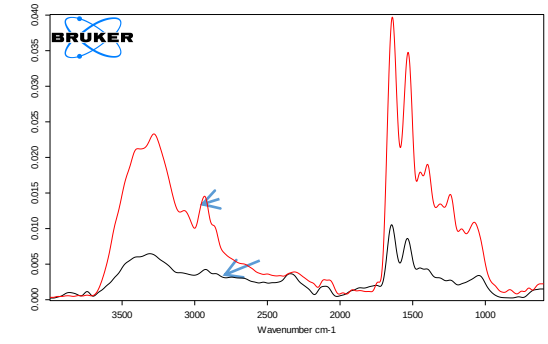
Figure(3):Comparison between new case of breast cancer(red)(A) and control(black)(B) for faction aged (35-40) years

(3)



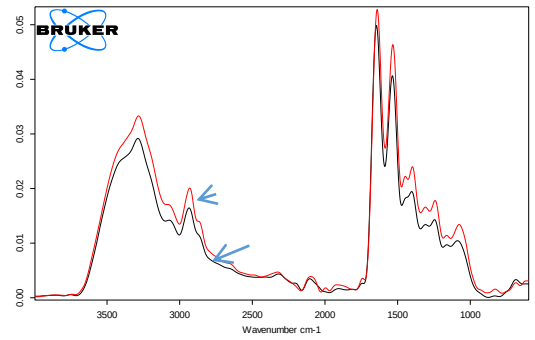
Figure(4):Comparison between new case of breast cancer(red)(A) and control(black)(B) for faction aged (40-45) years

(4)



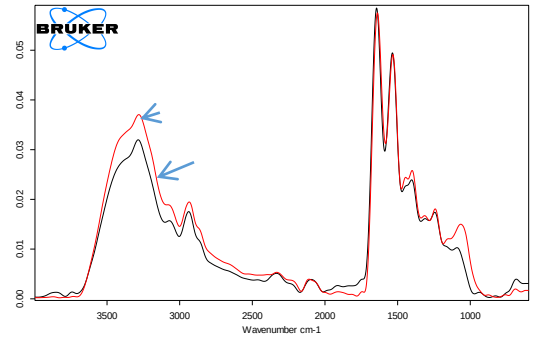
Figure(5):comparison between new case of breast cancer(red)(A) and control(black)(B) for faction aged (45-50) years

(5)



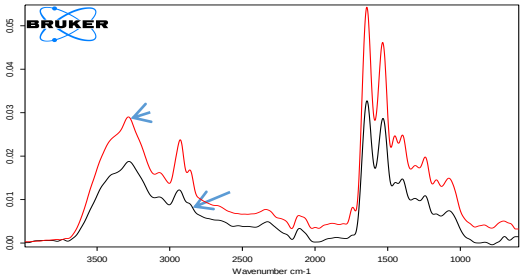
Figure(6): comparison between new case of breast cancer(red)(A) and control(black)(B) for faction aged (50-55) years

(6)



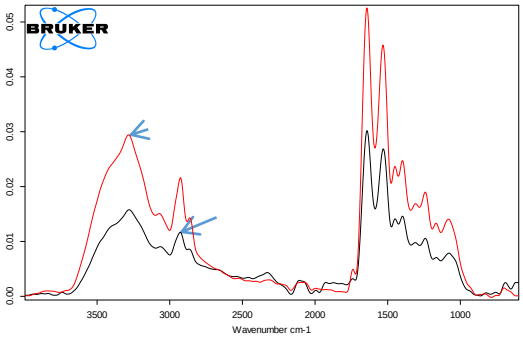
Figure(7):comparison between new case of breast cancer(red)(A) and control(black)(B) for faction aged (55-60) years

(7)



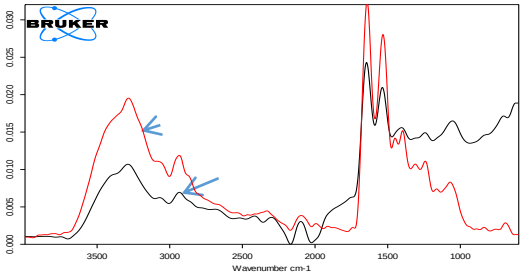
Figure(8):comparison between new case of breast cancer(red)(A) and control(black)(B) for faction aged (60-65) years

(8)



Figure(9):comparison between new case of breast cancer(red)(A) and control(black)(B) for faction aged (65-70) years

(9)



Figure(10):Comparison between new case of breast cancer(red)(A) and control(black)(B) for faction aged (70-75) years

(10)

Table(1): Comparative among important commonly function groups of wavelengths

Groups accordance for wavenumber		Mean	Std. Deviation	Std. Error Mean	Levene's Test (F)	Sig.	P-value	T-test
(698.6-667.8 cm^{-1})	Normal	687.2	5.221	2.131	3.700	.087	.727	.360
	New case	685.3	11.825	5.288				
(1246.1- 1241.4cm^{-1})	Normal	1243.6	1.407	.469	1.345	.262	.015	2.721
	New case	1242.2	.937	.296				
(1536.4-1530.3cm^{-1})	Normal	1534.2	2.281	.721	21.563	.000	.135	1.565
	New case	1533.0	.785	.248				
(1646.2-1639.0 cm^{-1})	Normal	1644.2	1.564	.494	.049	.828	.015	2.698
	New case	1642.2	1.777	.562				
(2937.8-2925.5 cm^{-1})	Normal	2933.2	3.699	1.169	.827	.375	.113	1.668
	New case	2930.9	2.554	.807				
(3297.3-3277.0cm^{-1})	Normal	3297.3	36.465	11.531	5.884	.026	.235	1.229

The figures from (1) to (10) represented the spectra of FTIR-ATR of normal samples and new case for the ages ranged from (25-30) to (70-75) years ,where observed from these figures for all ages the absorbance of new case is larger than normal case for each wavenumber consequently ,we can consider these results indicator to use the FTIR-ATR as tool diagnosis of breast cancer ,and this result agreement with the world publishers studies.

Table(2): Comparative between absorbance of normal case and new breast cancer for important commonly function

Groups accordance for absorbance		Mean	Std. Deviation	Std. Error Mean	Levene's Test (F)	Sig.	P-value	T-test
(698.6-667.8 cm^{-1})	Normal	.0025	.0010	.0004	3.989	.077	.568	.592
	New case	.0022	.0004	.0002				
(1246.1-1241.4cm^{-1})	Normal	.0120	.0032	.0010	.803	.383	.112	-1.677-
	New case	.0149	.0042	.0013				
(1536.4-1530.3cm^{-1})	Normal	.0279	.0120	.0038	.075	.788	.079	-1.863-
	New case	.0371	.0099	.0031				
(1646.2-1639.0 cm^{-1})	Normal	.0329	.0140	.0044	.092	.766	.091	-1.788-
	New case	.0432	.0115	.0036				
(2937.8-2925.5 cm^{-1})	Normal	.0107	.0053	.0017	.057	.814	.016	-2.653-
	New case	.0167	.0047	.0014				
(3297.3-3277.0cm^{-1})	Normal	.0176	.0088	.0027	.082	.778	.055	-2.056-
	New case	.0251	.0074	.0023				

Table(3): Correlations between mean wavelength and mean absorbance for patients

		mean wavelength	mean absorbance
mean wavelength	Pearson Correlation	1	.214
	Sig. (2-tailed)		.684
mean absorbance	Pearson Correlation	.214	1
	Sig. (2-tailed)	.684	

4. Statistical analysis

Statistical analysis was based on comparing the values of wavenumbers to each control group as compared to the cancerous group. There was a statistical analysis of the entire data with the help of the present SPSS statistical package Version 20. This data was further presented as mean , Standard Deviation of Means (S.D.M).There was also a comparison exercise done between the wavelength for two groups that was carried out for each function group with the help of t-test and p- value .From table(1) was

found high statistically significant for (1246.1-1241.4cm^{-1}),(1536.4-1530.3cm^{-1}), (1646.2-1639.0 cm^{-1}) and (3297.3-3277.0cm^{-1}) where was p-value < 0.05 while (698.6-667.8 cm^{-1}) and (2937.8-2925.5 cm^{-1}) was non-significant statically p-value was larger than 0.05.

From table(2) when comparative absorbance of selected functional group we found (2937.8-2925.5 cm^{-1}).

and (3297.3-3277.0cm^{-1}) was significant while (1646.2-1639.0 cm^{-1}) , (1536.4-1530.3cm^{-1}) and (698.6-667.8 cm^{-1}) was less significant while (1246.1- 1241.4cm^{-1}) non-significant statically.

The pearson correlation coefficient (*r*) measures the strength and direction of linear relationships between two or more random variables and ranges from −1 to 1 (Howarth and Sinding-Larsen 1983, Paine 1998, Bluman 2003). In the present study *r* is used to describe the interrelationships between the wave length and absorbance for patients of breast cancer at a significance level (p).

The calculation of *r* revealed that there is weak correlation clarity. From table(3) we found positive linear relationship between mean wavelength and mean absorbance where the pearson correlation was 0.214 with non-significant p-value > 0.05.

This results was shown in table (1) for commonly function groups where the new case of breast cancer are became larger than the normal case and the shift in all function groups of breast cancer was to left either to right.

The 1800–1500 cm^{-1} (amide I and amide II) region contains mostly information on protein content and secondary structure. The 1300–800 cm^{-1} region is due to vibrations of functional groups such as PO_2 −, CO and CC present in proteins, lipids, nucleic acids, and carbohydrates where the lipid phosphate band due to the asymmetric P-O stretching of PO2 occurs at 1240 cm^{-1} (Movasaghi, Rehman et al. 2008).

The sample constituents namely lipid, protein and carbohydrates form the characteristic bands on the infrared spectrum in the frequency range between 3100 – 2800 cm^{-1},1800-1400 cm^{-1} and 1400-900 cm^{-1} respectively .Vibration band assignment has been carried out on the infrared spectrum of sera on comparing the position relative intensity and shape of the bands with the bands of related molecules(Subramanian, Sundaraganesan et al. 2010). There are two very strong prominent amide absorptions one at 1642 cm^{-1} due to C=O symmetric stretching and corresponds to Amide I band and another at 1536 cm^{-1} due to strong N-H in plane bending and termed as an Amide II band. The strong characteristic band at 3285cm^{-1} (Calin, Dumitru et al. 2002) due to N-H symmetric stretching confirmed the existence of amino acid group.

We did not found the medium band at 2873cm^{-1}due to C-H asymmetric and symmetric stretching of CH3 group established the presence

of lipids and the medium bands at 2854 cm^{-1} due to C-H symmetric stretching of CH2 group established the presence of lipids / fatty acids for normal and breast cancer(Gunasekaran and Uthra 2008). The medium bands at 1400 cm^{-1} , 1449 cm^{-1} signified the presence of amino acid due to C=O symmetric stretching of COO-- and asymmetric C-H scissoring of –CH3 group. The medium weak bands at 1313 cm^{-1}, 698 cm^{-1} represent the presence of amide III & amide IV due to C-H / N-H deformation(Romeo, Dukor et al. 2008) of C-H out plane bending. The weak bands at 1065-1091 cm^{-1} gives rise to the existence of glucose due to C-O symmetric stretching, C-C symmetric stretching, C-O symmetric stretching respectively and methyne gives rises to weak band at 667-696 cm^{-1} due to NH2 wagging corresponding to amino acid group.

5. Conclusions

We proved that FTIR-ATR spectroscopy of serum human is apotentially feasible and efficient tool for the early detection of breast neoplasms. An important application of our study is the distinction between benign lesions (considered as part of the non-cancer group) and malignant tumors thus reducing false positive results at screening. Furthermore, the correlation of specific spectral changes with clinical parameters of cancer patients indicates for possible contribution to diagnosis and prognosis.

Analysis of the blood sera spectrum for different samples under FTIR spectroscopic technique showed that there are some differences between each and every spectrum. Analysis of the blood Serum is a useful tool for determination of diseases in human body. Since FTIR spectrum gives the molecular finger print out of the sample,as compared to the clinic test, It plays a vital role in bio medical field for diagnostic purpose. it has been demonstrated that the study of IR spectra of serum samples may be used to differentiate between the healthy and breast cancer subjects.

Some remarkable differences are elucidated in terms of FTIR-ATR spectral profiles ,absorption bands, wave numbers and the intensity ratio parameters and satisfactory analysis has been made. It can be observed that in the case of breast cancer samples, the absorbance for the various fundamental modes of vibrations of fourth vital regions is larger than that for the normal samples.

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