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Using a physical technique to compare the spectra of breast cancer patients, Najaf, Iraq

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Abstract Breast cancer is one of the most common malignancies in women. In this paper we employ UV-VISIBLE technique for diagnosing the breast cancer early **.**we have found fourth types of bands in the spectra of sera by this technique for female of breast cancer ,female who take chemotherapy and control (controls). First, second and third band regions (201-219,251-255,279) respectively cover the ultraviolet behavior of proteins but fourth band does not show absorbance of proteins, we noted appeared and disappeared some bands also increased the absorbance in patients which are represented an evidence for diagnosing breast cancer.

Keyword: Breast cancer, peak 279, Chemotherapy, UV-visible.

INTRODUCTION

The term “breast cancer” refers to a malignant tumor that has developed from cells in the breast. Usually breast cancer either begins in the cells of the lobules, which are the milk-producing glands, or the ducts, the passages that drain milk from the lobules to the nipple. Less commonly, breast cancer can begin in the stromal tissues, which include the fatty and fibrous connective tissues of the breast. This information is provided by breastcancer.org[1, 2].There are many ways to detect breast cancer, but there must be broader studies in this field, in the hope that we will reach early detection of this disease. Among the techniques that we will use for early detection is ultraviolet spectroscopy .We will study one of the most important and exciting advances in modern biochemistry has been the application of spectroscopic method, which measure the emission and absorption of energy of different frequencies by molecules and atoms[3]. Spectroscopic studies of proteins, nucleic acids and other biomolecules are providing many new insights into the structure and dynamic process in these molecules[4]. The application of UV absorption spectroscopic technique for the examination of the concentration of protein molecules has undergone significant change during the past couple of years[5].UV-Vis spectroscopy involves the absorption of UV and visible light by the molecule causing the promotion of an electron from ground electronic state to an excited electronic state of the transition [6, 7]. Absorption of this high energy light causes electronic excitation. The easily accessible part of the region is from 200 nm to 800 nm. This shows absorption only if conjugated π-electron systems are present[8]. Ultra violet radiation having wavelength less than 200 nm is more difficult to handle. The energies are sufficient to promote or excite a molecular electron to a higher energy orbital. Ultra-violet region is only a small part of the electromagnetic spectrum and it extends from 190 nm to 300 nm[9]. We are interested in the spectrum, which obtained when ultra-violet light passes through medium and not in the source spectrum. The interaction of ultraviolet radiations with matter may result either in the emission or in the absorption spectrum[10]. UV radiations excite the transparent substance from the lower to higher energy state via electronic transitions. The transitions induced by ultra violet are not common to all electronic structures; in fact these take place in conjugated systems. We are in a position to recognize the characteristic groups because the UV spectra are highly sensitive to environmental factors in biological macromolecules [9].As the energies involved in electronic spectra are large, these are associated with changes in rotational and vibrational states, which blur the observed spectrum, rendering it characterless in liquids[11]. However, even this highly characteristic of a particular molecular group lies both in its frequency and intensity[12]. In our research, we try to use serum spectra of patients who did not receive any new treatment and who received chemotherapy and compare the absorbance spectra of their serum with those of control controls using UV-visible spectroscopy technique for the purpose of laying the basis for future studies in this field.

Patients Characteristics

This study comprised 22 adult females patients with newly diagnosed of breast cancer for all types of breast cancer their ages ranges between 33 to 74 years. The group consisted only females, all the serum samples collected from people who lived in Iraq, Their medical records were examined. The control subjects were 25 control Iraqi people, the control group was used only for comparing serum levels.

Samples Collections

We used in this study (blood) samples collected from fastening women at each age (33-74) years. This work done at the middle Euphrates center cancer ,Al Najaf city, Iraq also collected samples of control from Al Sader general hospital al-Najaf al-Ashraf government, five milliliters freshly drawn blood from each patient was collected in clean and dry test tube without any anti-coagulant. The test tube was kept for 45 minutes at room temperature (22 ± 2℃) for the formation of clot. Sera of different patients were separated by centrifugation at 1500 r.p.m. up to 15 minutes and were collected in screw capped test tubes. We used Shimadzu (UV-Visible spectrophotometer) ,double-beam 190 - 1100 nm (UV 1800) featuring the highest resolution, the UV-1800 easily satisfies the standards of wavelength resolution, UV-1800 analyzed data on a computer by using UV Probe software.

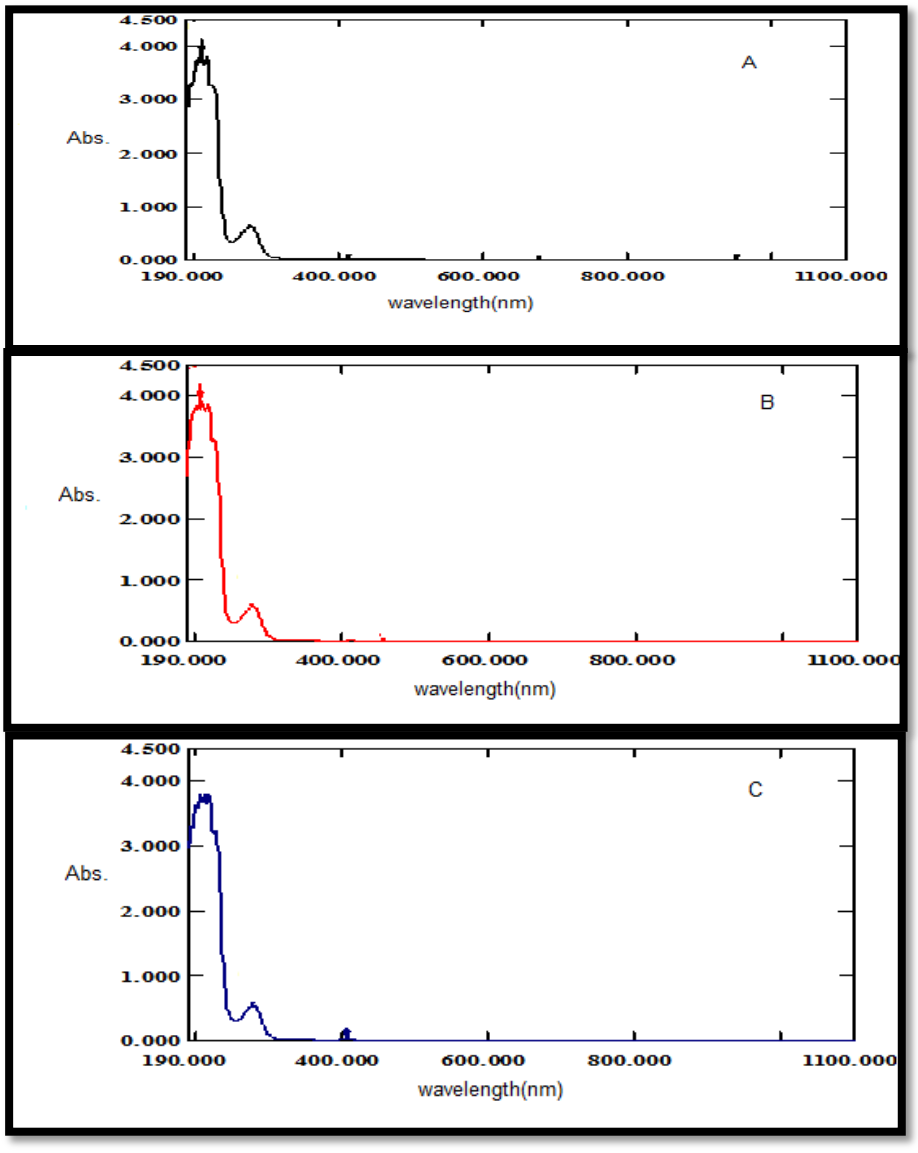
Sample Preparation

Take 25μl of kept serum by micropipette ,Put the measured serum at test tube then added 3ml of deionized water after take 25μl from it ,the tube shake circularly to mixed well, The instrument was blank by put deionizer water (diluting solution) at the tow cells which accessed with the instrument. The solution which wanted to measured put on one the tow quartz cell and The other remained, then the spectrum was recorded to read concentration of serum. Blood was collected into stainless steel injection vein without any additives. Directly after collection, each blood sample was centrifuged at 3000 rpm for 5 minutes in order to separate blood cells and suspended particles from blood serum. Sera were transferred into neutral glass vials and stored in a freezer at (-20 C°).

Results and discussions

Ultraviolet absorption spectra of new breast cancer patients(B.C .case)** were recorded and compared with control group (N. case)* also with patients whom take dose of chemotherapy group (T.CT)***as shown in figures1 for different ages variance between (33years to 74 years).

Figure1. Ultra Violet spectrum of control females(A) ,new case of breast cancer(B) and females that take chemotherapy



N .case =control females*
B.C.case= new state of breast cancer**
T.CT = pateints whom taked dose of chemotherapy***

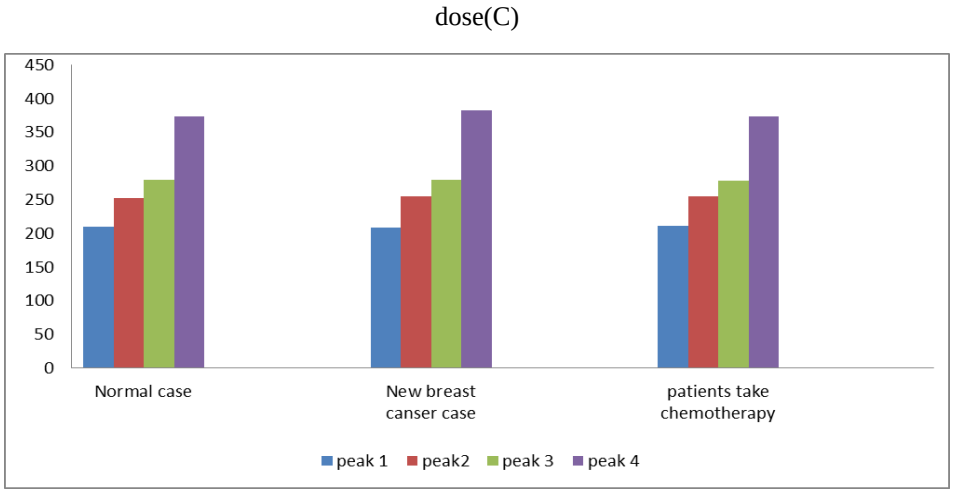


Figure2. Comparative among the mean of each peak for three groups.

The figure 1 represented the spectra of Ultra Violet spectrum of control females(A) ,new case of breast cancer (B) and females that take chemotherapy dose(C) with aged (33 – 74) years ,from these spectra we observed some important bands such as (201-219,251-255, 279 and 316-396) respectively.

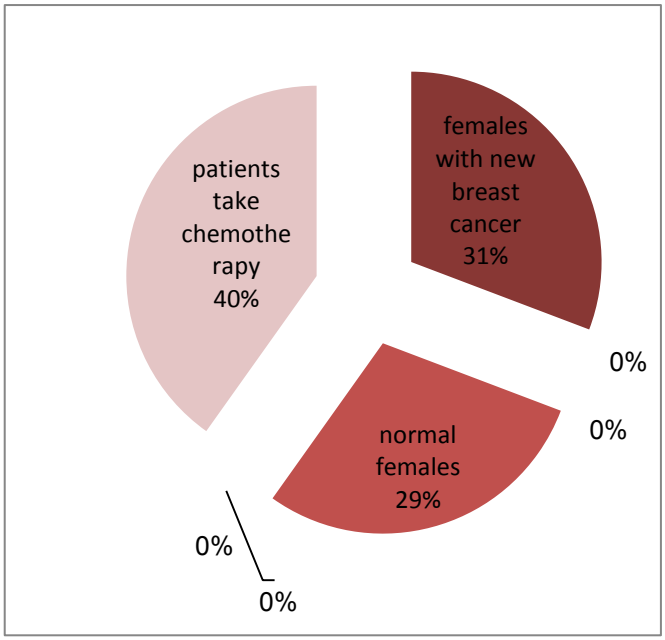


Figure3. Percentage absorbance of peak 279nm for three groups.

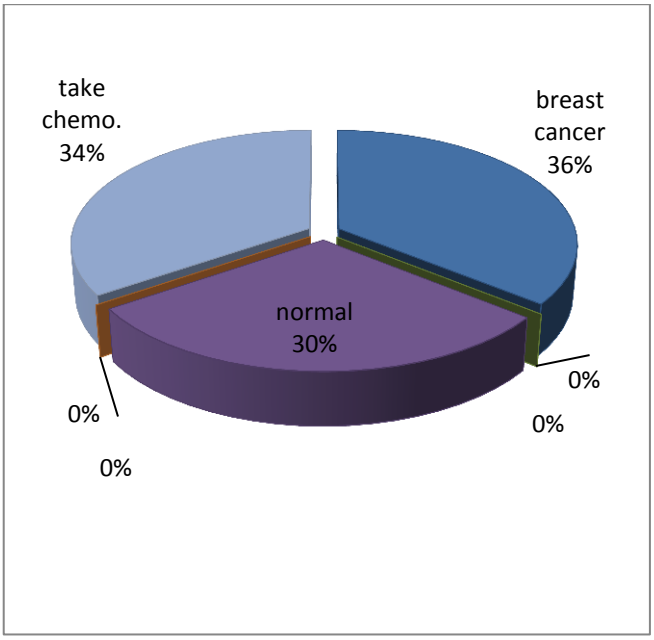


Figure4. Percentage absorbance of peak (251-255)nm for three groups.

From figure 3, we found the percentage absorbance of patient whom take chemotherapy was highest when compared with the new breast cancer and control case which may be resulting from treatment by the chemo drug. From figure 4, we found the percentage absorbance of peak(251-255) nm for the case new breast cancer was highest when compared with the case whom take chemotherapy and control case which may be this peak is rather more effective by the breast cancer from another peaks. We have found four important types of bands in the spectra of serum human for three groups , controls patients with new case and patients who take chemotherapy dose. First , second and three band regions cover the ultraviolet behavior of proteins but four band does not show absorbance of proteins because the absorbance intensity greater than 310 nm.

Table1: Statistical analysis for control females ,new breast cancer and the females whom take chemotherapy for absorbance to bands (251-255)nm and (279nm) nm

	Groups of peak(251-255nm)absorbance				
Groups	Levine's Test	Sig.	P-value	Sig.	T_ test
control & New case(breast)	3.440	0.071	0.231	NS	1.215
control & Take chemotherapy	6.235	0.017	0.039	Sig.	-2.128-
New case(breast) & Take chemotherapy	2.812	0.101	0.338	NS	.969
	Groups of peak(279nm)absorbance				
Groups	Levine's Test	Sig.	P-value	Sig.	T _test
control & New case(breast)	.022	0.882	0.161	NS	1.426
control & Take chemotherapy	16.045	0.000	0.057	NS	- 1.959-
New case(breast) & Take chemotherapy	16.134	0.000	0.073	NS	-1.841-

Table2: Statistical comparison for control females ,new breast cancer and the females whom take chemotherapy for absorbance to bands (**251- 255**)nm

Groups(251- 255)nm	Mean± SD	Std. Error mean	Median	Upper limit	Lower limit
Control absorbance	0.297±.037	0.0079	0.293	0.377	0.22
Breast cancer absorbance	0.488±.696	0. 148	0.343	0.701	0.270
Chemotherapy absorbance	0.344±.066	0.014	0.320	0.472	0.238
Age(years)	50±12.5	2.673	48.50	74	33

Table3: Statistical comparison for control females ,new breast cancer and the females whom take chemotherapy for absorbance to bands (279)nm

Groups(279nm)	Mean± SD	Std. Error Mean	Median	Upper limit	Lower limit
control absorbance	0.068±.578	0.0145	0.574	0.739	0.448
Breast cancer absorbance	0.611±.067	0.0144	0.617	0.757	0.453
Chemotherapy absorbance	1.070±1.168	0.249	0.645	0.958	0.434
Age(years)	50±12.5	2.673	48.50	74	33

It was found through the use of the ANOVA test between the three studied groups for the two studied bands as well that it was not statistically significant where for three groups of band(251-255)nm(p value larger than 0.05), while it was statistically significant for three groups of band 279nm) where p value less than 0.05, Which can be used as an indicator to indicate the presence or predict the presence of various diseases according to the study.

Statistical Analysis

We take three groups (control, new breast case and the state whom take chemotherapy),each groups consist of 22 females .Simple descriptive statistics as mean, standard deviation (SD), 5% trimmed mean, median, range and percentiles were applied to the analytical data obtained for three groups, also the upper and lower limits were recorded for each group in this study, and all results were given as the mean± standard deviation (SD) value , the data analysis were performed by SPSS 20.0 statistical program among groups . If P- value was less than 0.05, it was considered statistically significant but if larger than 0.05 the value of any parameter in non-significant statically[13].The table 1,2 and 3 represented the comparative between band (251-255)nm and band 279 nm were the comparative groups was (control & new case) ,(control & take chemotherapy) and (new case & take chemotherapy) we found the value of Levine's test for three comparative groups 3.440,6.235 and 2.812 respectively where was high significant for second group also p-value was significant for band (251-255)nm, while for the band 279nm we found the value of Levine's test for three comparative groups .022,16.045 and 16.134 respectively where was high significant for second and third group also p-value was nearly significant in second group but non-significant to first and third group for band (251-255)nm. Data of absorbance for 279 nm band and (251-255)nm were also studied and the result of all parameters for two band represented in table 4 and 5 also analysis of variance (ANOVA) was evaluated by statistical program(spss20)which was higher statistically significant for 279nm band and non. statistically significant for (251-255)nm bands.

The ultraviolet spectra of proteins have been made the subject of study. It has been well established that the spectra of amino acids show proteins have a high intensity absorption band in the neighborhood area of 190 nm. A similar band is found in simple peptides, with absorptivity increasing with increasing chain length in oligopeptides. The band at 190 nm is not available with aliphatic amino acids. It has been seen in several proteins the molar absorptivity per peptide bond at 205 nm falls in the range 260 nm to 310 nm .The absorption spectrum of proteins is of great interest and made easy to study [14].

CONCLUSION

The UV absorbance spectrum for a biomolecule is sum of the spectra of component parts. The UV absorbance for nucleic acids is found from 190 nm to 394 nm. This spectrum is due to transitions of the purine and pyrimidine bases compared by another research[15, 16].We found from our study the absorbance in band (251-255)nm , control < patient take chemotherapy < new breast cancer, while in band 279nm control < new breast cancer < patient take chemotherapy ,the band 279nm appeared in all

groups ,the range of bands(201-219)nm variance among groups also the third band and fourth band, we observed absorbance the statically significances among three groups in band 279nm and band (251-255)nm. This spectroscopy confirms that the absorbance by proteins above 300 nm is not possible and no protein absorb at this wavelength. We have found the absorbance peaks above this wavelength. Other spectroscopic techniques may help in the study of this absorbance when compared by global publishes. Tryptophan (Amino acids, including tryptophan, are used as building blocks in protein biosynthesis, and proteins are required to sustain life) , have most intense transitions. Many proteins have few tryptophan compared with the other aromatic groups. These transitions are not dominated in the near ultraviolet regions.

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