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Comparison between Blood Serum of Liver Cancer Patients and Healthy Controls Using UV–Visible Technology

Allawi Hamead Harjan[1*], Shihab Ahmed Jasim[2], Shaymaa A. Kadhim[1], Hussien Abid Ali Mraity[1], Azhar S. Alaboodi[1], Ali Abid Abojassim

[1]Department of Physics, Faculty of Science, University of Kufa, Iraq

[2]faculty of Agriculture, University of Kufa, Iraq

***Corresponding author:** Allawi Hamead Harjan**, Mobile: 009647831089442, E-mail:** allawi.alasadi@uokufa.edu.iq

ABSTRACT

Background: It is fundamental to investigate the physical and chemical changes occurring in tissues and cells due to certain diseases. **Objective:** Early diagnosis of liver cancer through spectral analysis of serum samples of patients and healthy subjects. **Patients and methods:** Fifty-two samples from liver cancer patient, and 45 from healthy, using UV-visible spectroscopy technology. By using the statistical program (version 20) and independent samples t-test, the averages of the blood serum for males with new case liver cancer were the highest compared to that of control group.

Results: Concerning the male patients with liver cancer who took chemotherapy and for all wavelengths except 225 nm, their blood serum average was higher compared to healthy subjects, and it was not statistically significant. Regarding the women who took chemotherapy, the situation of the absorption is seen to opposite to that the males who took chemotherapy too. **Conclusion:** The results showed that there are statistically significant differences between the spectrum of liver cancer patients and healthy people.

Keyword*:* Liver cancer, Statistical analysis, Spectra of serum, Uv-visible spectra of serum.

INTRODUCTION

Spectroscopy is concerned with the study of the interference between matter and electromagnetic radiation, which extends from high-energy gamma rays to very low-energy radio waves through X-rays, microwaves, ultraviolet, visible and infrared rays, where the interference diagram between matter and radiation is referred to as a spectrogram or spectrum [(1)]. Spectroscopy is also concerned with the study of the absorbed or emitted rays, which is an important technique for studying the arrangement of electrons in atoms.

UV radiation is a type of electromagnetic energy that can be used for many different purposes. It can treat skin conditions, disinfect water, and even cure some types of paint and diagnosis of liver cancer [(2)]. UV-Vis spectroscopy is a technique that uses the ultraviolet and visible portions of the electromagnetic spectrum to examine substances, the wavelength of a visible light is around 400-700 nanometers, while ultraviolet (UV) radiation spans from roughly 10-400 nanometers. UV radiation can cause sunburn, skin cancer, and other health problems. UV spectroscopy measures the intensity and wavelength of UV radiation. It can be used to detect harmful UV radiation and to identify unknown substances [(3)]. The easily accessible part of the region is extended from 200 nm to 800 nm. This shows the absorption only if conjugated π-electron systems are presented [(4)]. The ultraviolet region, extending from 190 nanometers to 300 nanometers, contains energies sufficient to excite a molecular electron to a higher energy orbital [(5)].

Research on ultra-violet light typically seeks to find out what spectrum is obtained from passing it through the medium rather than what spectrum is generated by the source. In the interaction of ultraviolet radiation with matter, emission, fusion, or absorption spectra may occur [(6)]. During the electronic transitions induced by ultraviolet radiation, the substance moves from a lower energy to a higher energy state. These transitions occur only within conjugated systems [(7)].

As high energies will cause rotational and vibrational changes in electronic spectra, this will blur the observed spectrum in liquids, rendering it essentially useless [(8)]. With the application of quantum optical spectroscopy to investigate breast tissues, a number of studies have examined the relationship between the spectra of different cancerous tumors. As seen by the UV-Visible spectral range, malignant breast tissues absorb and scatter more than normal breast tissues [(9)]. The aim of this study was to early diagnose liver cancer through spectral analysis of serum samples of patients and healthy subjects.

MATERIALS AND METHODS

Samples collections and preparation:

Ninety-seven blood samples were collected from volunteers, of them 45 for healthy and 52 for liver cancer patients (male and female). Directly after the collection, each blood sample was centrifuged at 3000 rpm for 10 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 until dried and were collected in screw capped test tubes. Shimadzu (Uv-Visible spectrophotometer) was used, double-beam 190-1100 nm (UV 1800). Featuring the highest resolution, the UV-1800 easily satisfies the standards of wavelength resolution. The UV-1800

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analyzed data on a computer using UV Probe software [10]. Using micropipette, 25µl of serum was taken and put at a test tube then 2.75 ml of deionized water was added. The tube was shaken circularly to be mixed well. The instrument was blank by putting deionized water (diluting solution) at the two cells which was accessed with the instrument. The solution to be measured put on one of two quartz cells and the other remained, and then the spectrum was recorded to read concentration of serum.

Ethical approval: Informed consents from all patients were obtained. The Academic and Ethical Committee of the University of Kufa approved this study. The study was conducted in accordance with the World Medical Association Code of Ethics (Declaration of Helsinki) for human studies.

Statistical analysis

To calculate the difference between the two means, a T-test for independent samples was used (SPSS for Windows version 20, SPSS Inc., Chicago, IL, USA). In the absence of statistical significance, a P-value greater than 0.05 was considered insignificant, while a P-≤ 0.05 was considered significant [11].

RESULTS

In this study, we attempted to employ the diagnostic spectral differences between serum of healthy blood and serum of liver cancer patients, by strongly absorbing proteins present in serum at wavelength around 280 nm due to amino acids such as tyrosine and tryptophan.

The results in table (1) for males with newly diagnosed hepatocellular carcinoma, the absorbance of the blood spectrum of male patients was higher than that of healthy blood serum, but it was not statistically significant. Some values of absorbance for females of the newly diagnosed liver cancer were statistically significant (P-value less than 0.05) at wavelengths of 225 nm, 227 nm and 280 nm, whereby serum absorbance of female patients was higher than that of healthy females as shown in table (2).

Table (1): The group statistics and independent samples test for liver cancer patients of the new cases in males

Peaks	Cases	Mean ± St. Dev.	Levene's Test		t-test		
			F	Sig.	t	df	P-value
225 nm	Patient	2.97±0.66	14.989	0.001	0.919	19.862	0.369
	Health	2.57±1.53					
227 nm	Patient	2.98±0.43	17.864	0.001	1.175	17.541	0.256
	Health	2.51±1.45					
278 nm	Patient	0.99±0.22	17.914	0.001	1.846	20.464	0.079
	Health	0.72±0.51					
279 nm	Patient	0.97±0.23	16.800	0.001	1.669	20.844	0.110
	Health	0.72±0.51					
280 nm	Patient	0.93±0.24	15.642	0.001	1.505	21.193	0.147
	Health	0.71±0.50					
281 nm	Patient	0.90±0.24	14.472	0.001	1.329	21.546	0.198
	Health	0.70±0.50					

Table (2): The group statistics and independent samples test for liver cancer patients of the new cases in females

Peaks	Cases	Mean ± St. Dev.	Levene's Test		t-test		
			F	Sig.	t	df	P-value
225nm	Patient n=10	2.63±0.76	1.094	0.308	-2.594-	20	0.017
	Health n=12	3.33±0.49					
227nm	Patient n=10	2.33±0.97	7.182	0.014	-3.246-	20	0.004
	Health n=12	3.37±0.50					
278nm	Patient n=10	0.89±0.23	15.374	0.001	-1.432-	15.077	0.173
	Health n=12	1.00±0.09					
279nm	Patient n=10	0.86±0.25	19.189	0.00	-1.753-	14.538	0.101
	Health n=12	0.99±0.094					
280nm	Patient n=10	0.93±0.24	15.642	0.002	-1.612-	13.101	0.002
	Health n=12	0.71±0.50					
281nm	Patient n=10	0.78±0.29	25.032	0.001	-1.988-	20	0.061
	Health n=12	0.97±0.097					

Regarding male liver cancer patients who underwent chemotherapy, it was found that serum absorption in liver cancer patients was higher compared to healthy subjects. There was also a statistical significance for the value of absorbance at wavelength 225 nm as shown in table (3). From table (4), the case was different for females with liver cancer, as there was no statistical significance difference (P > 0.05) for the patients who received chemotherapy, and the absorbency of the blood serum of healthy females was the highest, so the relationship became close to normal.

Table (3): The group statistics and independent samples test for liver cancer patients of the chemotherapy in males

Peaks	Cases	Mean ± St. Dev.	Levene's Test		t-test		
			F	Sig.	t	df	P-value
225nm	Patient	3.33±0.32	21.808	0.000	-2.119-	16.022	0.050
	Health	2.51±1.45					
227nm	Patient	3.11±0.47	17.576	0.000	-1.504-	17.921	0.150
	Health	2.49±1.48					
278nm	Patient	0.93±0.15	27.630	0.000	-1.522-	17.508	0.146
	Health	0.71±0.51					
279nm	Patient	0.89±0.17	25.548	0.000	-1.228-	18.090	0.235
	Health	0.72±0.51					
280nm	Patient	0.85±0.18	23.157	0.000	-0.971-	18.774	0.344
	Health	0.71±0.50					
281nm	Patient	0.80±0.19	20.816	0.000	-0.734-	19.442	0.472
	Health	0.70±0.50					

Table (4): The group statistics and independent samples test for liver cancer patients of the chemotherapy in females

Peaks	Cases	Mean ± SD.	Levene's Test		t-test		
			F	Sig.	t	df	P-value
225nm	Patient	3.28±0.35	8.135	0.009	0.134	14.112	0.895
	Health	3.31±0.53					
227nm	Patient	3.14±0.38	3.274	0.083	0.891	23	0.382
	Health	3.30±0.51					
278nm	Patient	0.99±0.16	3.240	0.085	0.057	23	0.955
	Health	1.00±0.094					
279nm	Patient	0.97±0.16	2.436	0.132	0.495	23	0.626
	Health	0.99±0.094					
280nm	Patient	093±0.17	2.201	0.151	0.884	23	0.386
	Health	0.98±0.095					
281nm	Patient	0.89±0.19	2.348	0.139	1.215	23	0.237
	Health	0.97±0.097					

The values of the absorbance of the spectrum of blood serum for patients with liver cancer for males were higher than the absorbance of the spectrum of healthy blood serum, as the highest average value of the absorbance was recorded at the wavelength of 227 nm and the lowest value was at the wavelength of 281 nm for the spectrum of patients, as well as the highest average value of the absorbance at the wavelength of 225 nm and its lowest value at the wavelength of 281nm for the spectrum of healthy as shown in figure (1). The values of the absorbance of the spectrum of blood serum for healthy blood serum for females were higher than the absorbance of the spectrum of patients with liver cancer, as the highest average value of the absorbance was recorded at the wavelength of 227 nm and the lowest value at the wavelength of 280 nm for the spectrum of healthy. Also, the highest average value of the absorbance at the wavelength of 225 nm and its lowest value at the wavelength of 281nm for the spectrum of patients as shown in figure (2). All the absorbance values of the blood serum spectra of patients receiving chemotherapy at the selected wavelengths were higher than the absorbance spectra of healthy male serum and this was illustrated by figure (3). All the absorbance values of the blood serum spectra of the healthy at the selected wavelengths were higher than the absorbance spectra of patients receiving chemotherapy females' serum and this was illustrated by figure (4).

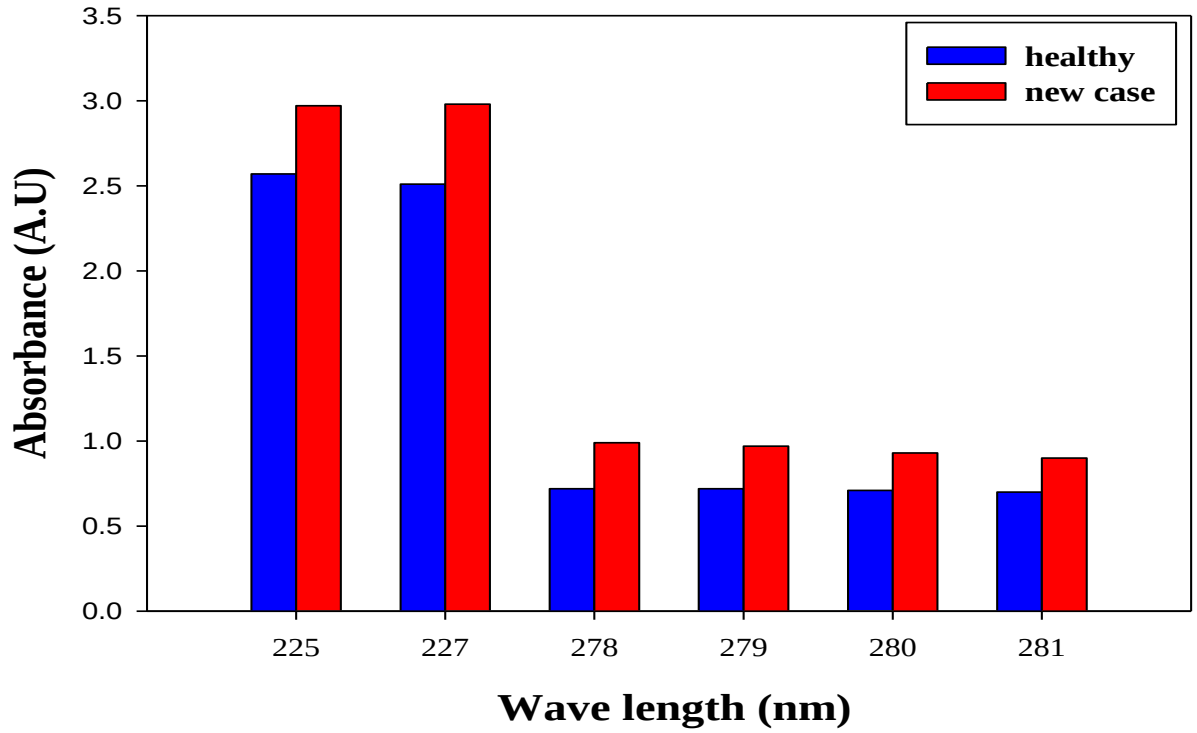

Figure (1): Comparing the spectrum of liver cancer patients (the new case) with the spectrum of healthy males.

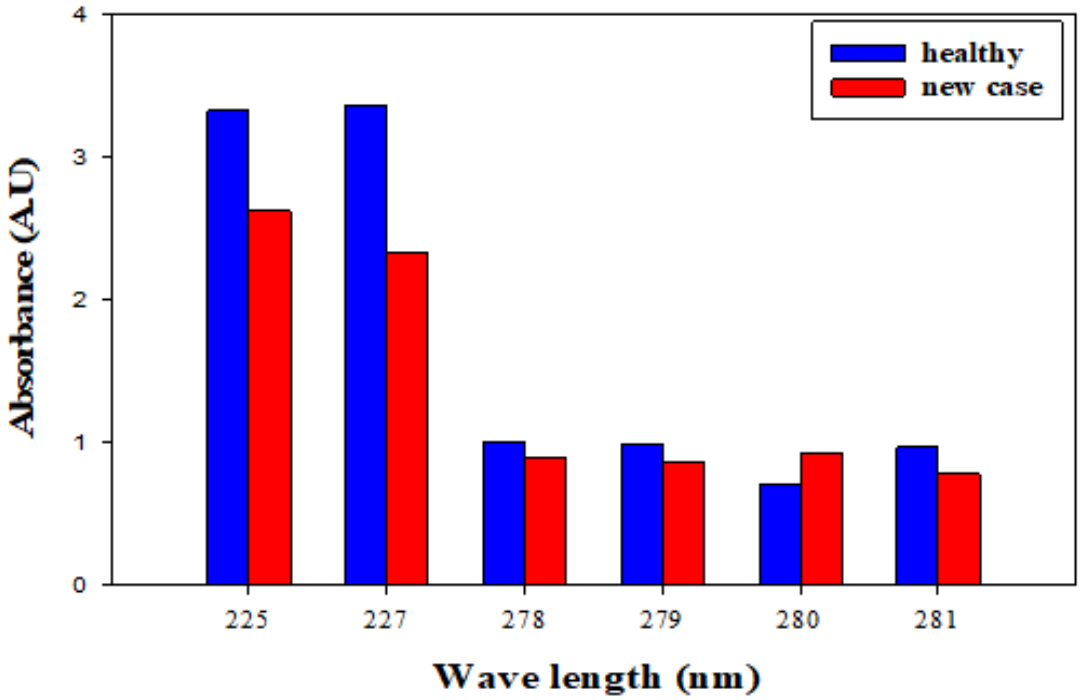

Figure (2): Comparing the spectrum of liver cancer patients (the new case) with the spectrum of healthy females.

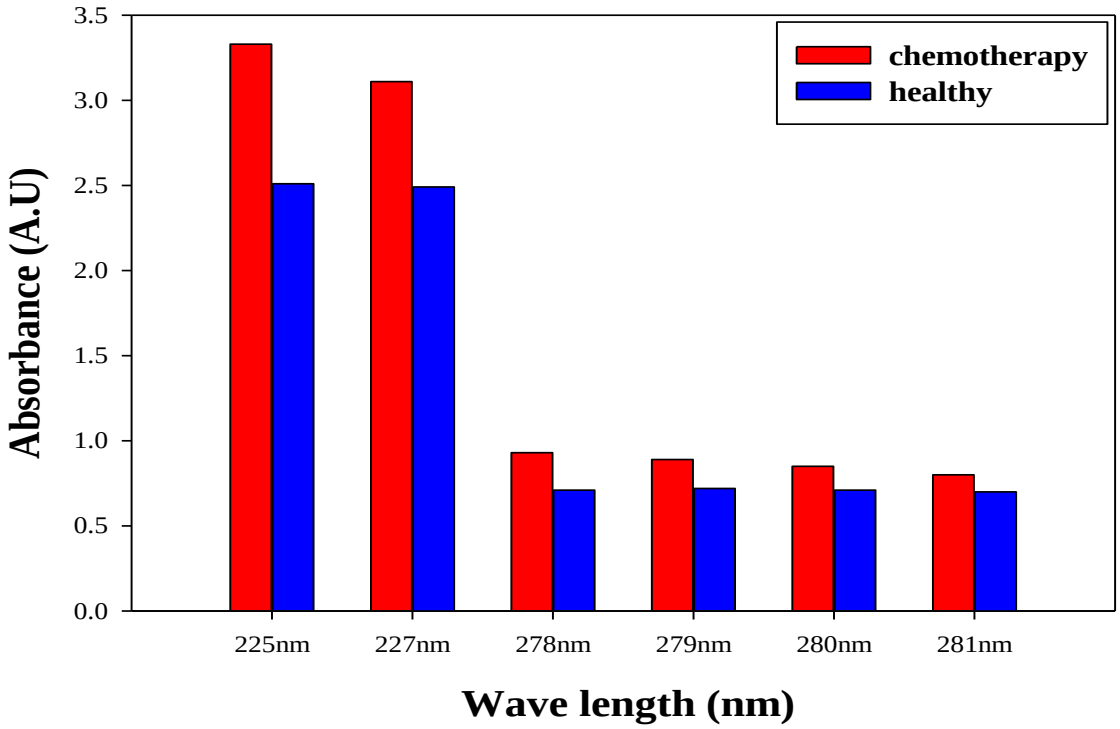

Figure (3): Comparing the spectrum of liver cancer patients (chemotherapy) with the spectrum of healthy males.

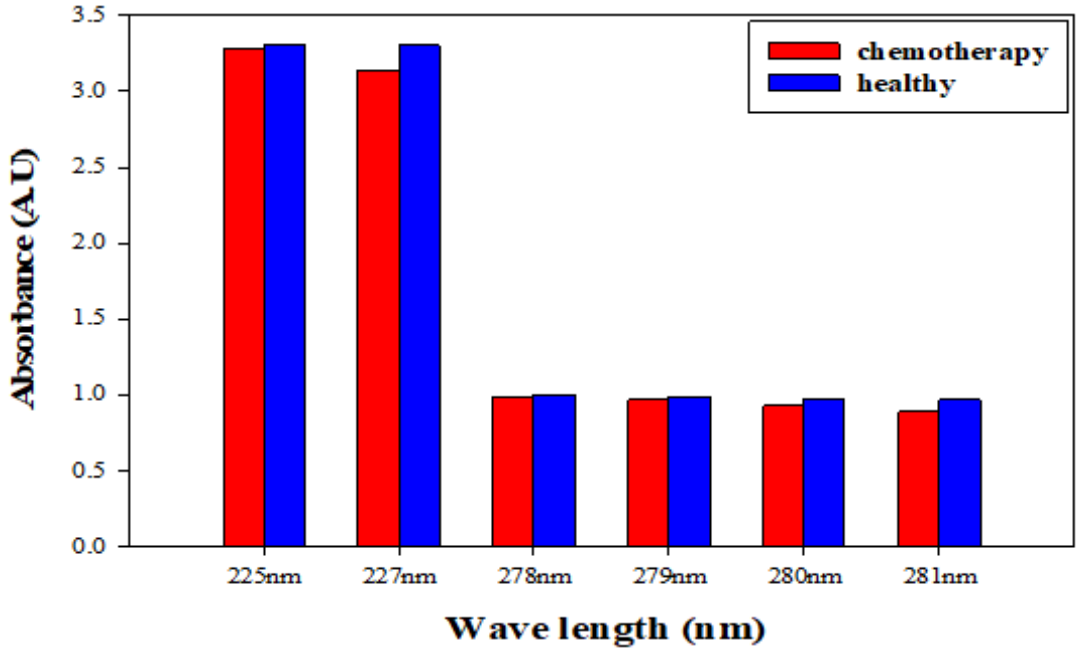

Figure (4): Comparing the spectrum of liver cancer patients (chemotherapy) with the spectrum of healthy females.

DISCUSSION

There is a new type of blood serum that uses ultraviolet (UV)–visible technology to fight liver cancer. This new test can identify liver cancer with a very high degree of accuracy, making it an important tool for early diagnosis. UV radiation has been shown to be effective in the treatment of liver cancer [12]. Photodynamic therapy (PDT) is a form of UV radiation that uses photosensitizing agents, such as hematoporphyrin derivative (HPD) or methyl aminolevulinate (MAL), to trigger a chemical reaction in cancer cells that causes cell death [13].

In PDT, the photosensitizer is administered intravenously and is taken up by the cancer cells. Liver cancer is the most common form of cancer in men and women combined [14].

The American Cancer Society estimates that about 1 in 7 men and 1 in 3 women will develop liver cancer at some point in their lifetime [15]. The five-year survival rate for people diagnosed with liver cancer is about 80%, but that number can be increased significantly through early detection. The symptoms of liver cancer often don't appear until the cancer has already progressed. As a result, many people with liver cancer aren't diagnosed until their cancer is advanced [16]. UVC–Ultra violet chlorine is a type of radiation that has been shown to be effective in the diagnosis of liver cancer. This is because it can pass through the outer layer of the skin and into the tissue beneath [17].

Once in the tissue, the UVC radiation can react with the chemicals in cells and produce a photochemical effect. UV –visible technology is a type of imaging technology that uses ultraviolet light to see inside the body. It is much less invasive than current testing methods, so patients don't have to have surgery [18].

The inside of the body is lined with blood vessels that carry blood throughout the body, white blood cells move through the blood vessels and perform different functions, when something is wrong with the blood vessels or organs, the white blood cells may not function properly [19]. The microscope uses ultraviolet light to light up the blood vessels in the body. When excited by the UV light, certain molecules in the blood vessels glow bright green [20].

CONCLUSIONS

Based on the results, it can be concluded that for the newly diagnosed males with liver cancer, there was a significant difference in the absorbance of blood serum measured by the technique UV-visible spectroscopy for all wavelength and that the statistical significance difference was noticed between the mean of the studied groups and vice versa for the mean absorbance of blood serum.

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