Original Article

Lung Cancer Serum Spectra Using FTIR ART in Karbala, Iraq: A Comparative Study

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Abstract

Because lung cancer (L.C) still has a high incidence and mortality rate, it is essential to create an effective tool for early diagnosis. The serum can represent both pathologic and physiological impacts on a person, hence the FTIRATR approach was used to assess serum samples from both normal and (L.C) patients. The lipid, protein, and nucleic acid molecule concentrations on the serum of lung cancer patients were identified by the comparison of band spectral region. Cancer rates were increased compared to those who were in good health. Through this study, the spectra of samples of patients with an average age 58.49091±14.006444 and for healthy patients with an average age 58.56667±13.748187 and using independent test to found that the ratio R1(2959/1544) being effective to identifying the serum of (L.C) patients from the serum of normal. Additionally, the findings demonstrated that the (helix/sheet) ratios in (L.C) patients' serum were higher than those in normal serum. Additionally, it was shown that there is a statistically significant correlation between the patient and healthy group ratios that were looked at. Additionally, A2 and A9, as well as A4 and A6, had a high Pearson correlation that was statistically significant whether it was positive or negative. Accordingly, it can be argued that this study is the first of its sort for the Karbala Governorate, and its information can be regarded as crucial for subsequent research.

Keywords: Lung Cancer, The Karbala Governorate, The SPSS Software, and Serum.

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INTRODUCTION

lung cancer is a malignant tumour that is dangerous to human health because of its high incidence and mortality rate[1]. It is among the most prevalent cancers in the world, and both the incidence and death have risen over time. It will cause around (2.2) million new instances of cancer and (1.8) million deaths in 2020, making up 18% of all cancer-related mortality[2]. Early identification is crucial to the survival of patients with (L.C), whose survival probability is still expected to be less than (15%). Due to an increase in smokers, genetic predisposition, and deteriorating environmental conditions in recent years [3]. If lung cancer can be detected early and properly treated, the survival rate will significantly increase [4,5]. The three modalities of CT, chest X-ray, and bronchoscope [6] are currently routinely employed to evaluate (L.C) medically. Although these methods can be used to identify (L.C.), they are less effective at calculating (L.C.) at an early stage[7]. Therefore, it is crucial to create an effective strategy for the initial diagnosis of (L.C) [8]. All disease processes are accompanied by biochemical changes in diseased tissues, cells, and organs; these changes typically begin before cell morphological changes or the appearance of physical symptoms. Since FTIRATR spectra have the potential to reflect these changes at the molecular level, they can be used to make early diagnoses of disorders [9]. A crucial component of blood that readily separates from the human body is the serum. Cancer cells' metabolites cause the component changes of biomolecules in serum [10], , and using the FTIRATR approach to find these differences may offer a new tool for cancer diagnostics. Because they require few samples and are inexpensive, vibrational spectroscopic methods like Raman spectroscopy and (FTIRATR technique) have recently been used often in biological samples [11, 12]. Unlikely Raman spectroscopy has some certain drawbacks due to its large fluorescence background and low signal. However, these drawbacks are not related to FTIRATR technique [13]. Evaluated the rations of serum for (L.C) patients and normal by using FTIR spectroscopy by X Wang, et, al [14]. Kaznowska et al. used infrared spectroscopy to examine tissue samples from normal and (L.C) patients and discovered the related wavenumber variations of the functional groups in DNA, carbohydrates, lipids, phospholipids and proteins. [14]. also has been investigated by drying the s.erum on a BaF_2 window under vacuum and it was discovered that there were changes in the protein

secondary structure of serum between (L.C) patients and normal [15]. Given the importance of the lung as an essential organ in the human body, and based on what was mentioned, our study was for the purpose to use technique that may effectively contribute to identifying and detecting many variables related to lung cancer to detect it at an early stage. Therefore, serum samples were collected from the Imam Al-Hussein Center for Cancerous Tumors in Karbala Governorate, Iraq where the disease spreads significantly, and it is a study that is considered a basic pattern for later studies.

Substances and Procedures

A total of 30 healthy adults and 55 people with lung cancer had blood samples obtained. Age groups (30-35), (35-40), (45-50), (50-55), (60-65), (65-70), (70-75), and (75-80) are used to categorize them (80-85). The Imam Hussain Cancer Center provided patient samples for collection. After drawing blood, the samples are put in a centrifuge, which rotates for five minutes to separate the serum from the blood cells and suspended particles. The blood serum is transferred to neutral glass tubes and stored in a refrigerator prepared for measurement. With the FTIR device Bruker IFS 66V spectrophotometer is a flexible research grade equipment that can measure in the visible, distant-IR, middle-IR, close-IR, and spectrum areas. Each spectral area necessitates a separate source, detector element arrangement and beam splitter, this article explains how to use the instrument for routine measurements in the middle-IR spectral range (4000 cm^{-1} to 400 cm^{-1}), as well as how to calibrate it. Where this device measures liquid substances, we will take a very small amount of 2-3 drops of serum using a micropipette and place it on the lens of the device, the signals were transmitted to a computer, and the data was analysed using a Windows based data software. [16].

Statistical Program

When obtaining data and information on scientific research procedures, specific instruments are needed to help with classification, analysis, and access to explanatory findings for the scientific researcher's study assumptions. Obtaining information from all of the community's vocabulary is extremely difficult, and requires large sums of money, in addition to the need to include a large number of participants in scientific research, so the sample method is the best solution to obtain the results in the shortest and with the least effort. As a result, we utilized to discover the mean± Std. Deviation, correlation and other statistics [17].

Results and Discussions

All FTIRATR spectrum were baseline adjusted and normalized to get accurate data. The study included two groups, the healthy group and the patients group, where the average age for each group was calculated as shown as shown in Table1. Also the function groups were analysed of the cancer patients

Table 1: Information for lung cancer patients and healthy people.

	Number	mean± Std. Deviation
healthy people	30	58.56667±13.748187
lung cancer patients	55	58.49091±14.006444

Serum's FTIRATR spectra reveals details about biomolecules such as functional groups, structure, bond types, and reactions. Biomarkers, or disease-related molecular alterations in bodily fluids and key tissues such as blood, are critical in aiding screening and diagnosis so that therapeutic treatments may begin as soon as feasible. The group frequency of the major elements of lung cancer indicated in Table2 is used to assign suitable vibrational bands to absorption bands of spectra. by using FTIR, we will first have characterized the spectral of normal case and lung cancer represented in figure 1, the FTIR absorption overlay spectra of normal and lung cancer were divided into 9 categories in figures 2((a), (b), (c), (d), (e), (f), (g), (h), (i)) by the following:

Table 2: Frequency assignment of FTIRATR vibrational bands in human serum samples

Sq.	Vibrational ban.d (cm^{-1}) Human serum	Vibrational band(cm^{-1}) lung cancer	Assignment.	Component group.
1.	3280	3293	H–O–H stretching The amide A band	Amino acid (amide A)
2.	2957	2958	CH_3 asymmetric and symmetric stretching	Fatty acid/ lipids
3.	2920	2931	Asymmetric CH_2 stretching	Fatty acid/ lipids
4.	1635	1650	The amide I and amide II bands (proteins)	amide II
5.	1453	1451	CH_2 deformation CH_2 scissoring	Amino acid
6.	1396	1399	CH_3 bending C=O stretch of COO–	Amide I
7.	1311	1313	The amide III band CH_2 twist	Cyclopropane
8.	1170	1169	C–O (H) stretching in proteins Ester C–O asymmetric stretch	Amide IV Amino acid

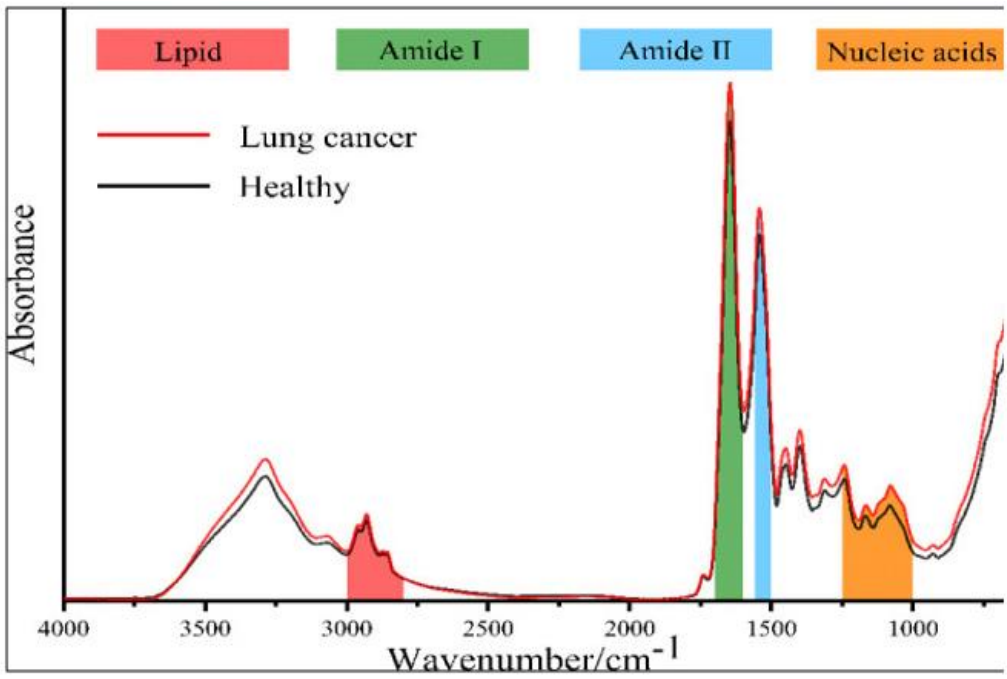
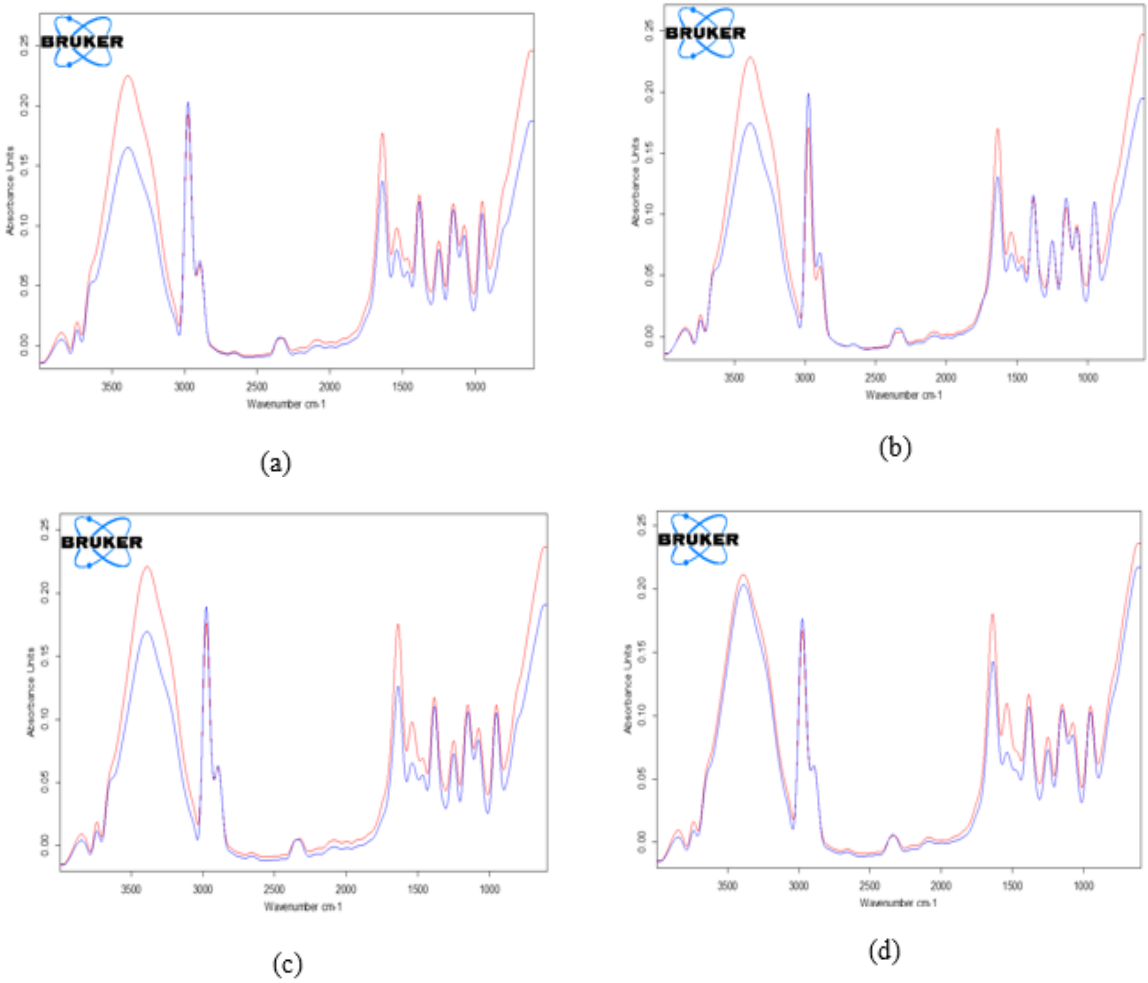


Figure 1: The mean FTIRATR spectrum of serum from lung cancer patients and normal [15].



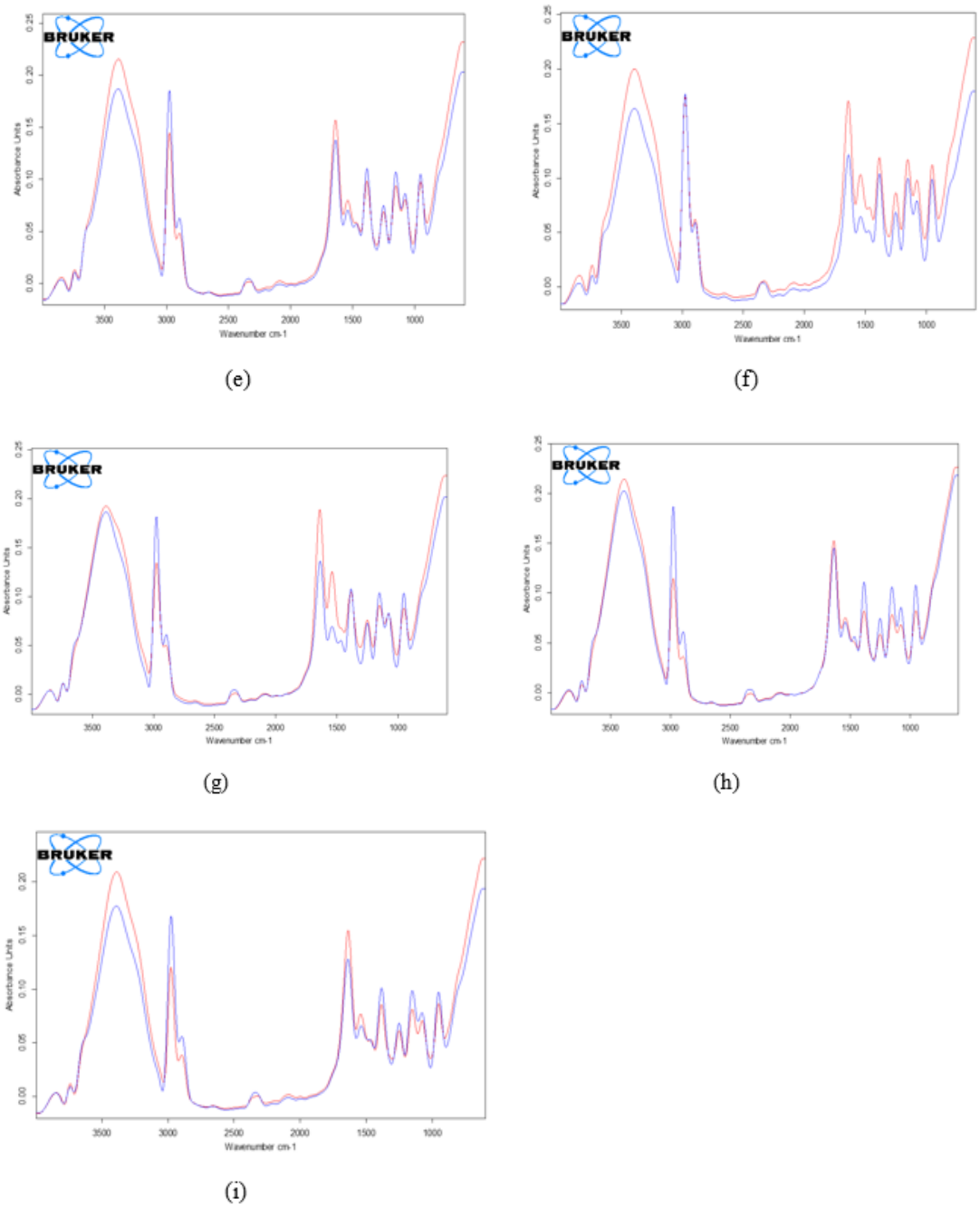


Figure 2: comparative between lung cancer patient (red) and healthy people (blue) by age groups (30-35), (35-40), (45-50), (50-55), (60-65), (65-70), (70-75), (75-80) and (80-85) respectively.

Table 3: FTIR spectral results of intensity ratio parameters of healthy and lung cancer

Ratios cm^{-1}	lung cancer patient (mean± Std. Deviation)	Healthy person (mean± Std. Deviation)	(P value)
R_1(2959/1544)	0.778118±.3696046	1.833911±.2546010	0.000
R_2(1651/1544)	2.014701±0.3083259	1.887982 ±0.2326435	0.036
R_3(1079/1544)	0.858051±0.1607398	1.315249±0.0711373	0.000
R_4(1079/1242)	1.157117±0.1241302	1.210843±0.0637250	0.010
R_5(1079/1169)	1.040050 ±0.0908171	0.989983±0.0321859	0.000
R_6(1106/1057)	0.915929±0.0853596	0.876065±0.0316094	0.003
R_7(1257/1006)	1.533909±0.6165074	2.202381±0.2245023	0.000
R_8(1506/1057)	1.245322±0.2761236	1.068229±0.1003311	0.000
R_9(α-helix/ β-sheet)	1.854418±0.1701405	1.663741±0.1280934	0.000

Table 4: The correlation factor between the studied ratios

		R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9
R_1(2959/1544)	P. C	1	.361-**	.655**	.354**	-.187-	-.477-**	.522**	.025	-.351-**
	(p-value)		.007	.000	.008	.172	.000	.000	.855	.009
R_2(1651/1544)	P. C		1	.039	-.006-	.232	.198	-.551-**	-.658-**	.733**
	(p-value)			.778	.965	.089	.148	.000	.000	.000
R_3(1079/1544)	P. C			1	-.113-	-.304-*	.223	.251	-.559-**	-.268-*
	(p-value)				.411	.024	.102	.065	.000	.048
R_4(1079/1242)	P. C				1	.703**	-.897-**	.132	.287*	.144
	(p-value)					.000	.000	.338	.034	.294
R_5(1079/1169)	P. C					1	-.456-**	-.127-	.016	.488**
	(p-value)						.000	.356	.910	.000
R_6(1106/1057)	P. C						1	-.218-	-.485-**	-.041-
	(p-value)							.109	.000	.768
R_7(1257/1006)	P. C							1	.259	-.447-**
	(p-value)								.056	.001
R_8(1506/1057)	P. C								1	-.515-**
	(p-value)									.000
R_9(α-helix/ β-sheet)	P. C									1
	(p-value)									
** Corre.lation is signi.ficant at the .01 level (2-.tailed).										
* Corr.elation is signi.ficant at the .05 level (2-.tailed).										
*** Pe.arson Corr.elation=P.C										

From Table 3, these results obtained by using the independent sampling test are documented, p- value, mean ± std. deviation, show that R_1(lipids/proteins) mean ± std. deviation was.778118±.3696046 for lung cancer patients while 1.833911±.2546010 for healthy groups, The P-value of (R_1=.000 P less than .05) showing that the ratios were much larger for healthy persons than (L.C) patients. R_2(proteins /proteins) may represent changes variation in the structure and composition of proteins. the band frequency of about 1651 cm^{-1} is the amide I. (proteins) [18], where the mean ± std. deviation was 2.014701±.3083259 for lung cancer patients while 1.887982 ±.2326435 for healthy groups, also indicating the composition. of N–.H. bending also .C–.N .stretching increased in comparison to the content of ..carbonyl stretching. in the proteins of (L.C) patients serum. The P.-value of (R_2=.000 P less than .05), showing that the ratios were much larger for (L.C) patients than normal. The band frequency of about 1079 cm^{-1} is PsO^{-}_2 Symmetric. stretching of nucleic acids, R_3(nucleic acids /proteins) can evaluate the DNA levels[19], the mean ± std. deviation was.858051±.1607398 for lung cancer patients while 1.315249±.0711373 for healthy groups indicating the DsNA. Content. decreased in (L.C) patients sserum. The drop in DsNA. content might be linked to (L.C) cell necrosis and apoptosis, as well as DNA produced by (L.C) cells. [20], This was not in agreement with Wang's findings. [21].The Ps-value of (R_3=.000, P less than.05) showing that the ratios were much larger for healthy persons than (L.C) patients. The band frequency of about 1242 cm^{-1} is PO^{-}_2 symmetric stretching of nucleic acids and R_4(nucleic acids / nucleic acids) can indicate structural changes of nucleic acids[18], the mean ±. std. deviation was 1.157117±.1241302 for lung cancer patients while 1.210843±.0637250 for healthy groups. The P-value of (R_4=.010 P less than 05) showing that the ratios were much larger for healthy persons than (L.C) patients. The band frequency about 1169 cm^{-1} receives major contributions from C–O (H) groups of threonine, tyrosine and serine residues in proteins [23], R_5(nucleic acids /proteins) may be used to determine the relative amount of nucleic acids in a sample [18] , the mean ± std. deviation was 1.040050 ±.0908171for (L.C) patients while .989983±.0321859 for normal groups, indicating the content of nucleic acids increased in lung cancer patients serum. The P-value of (R_5=.000 P less than .05) showing that the ratios were much larger for (L.C) patients than normal. We know 1106 is due to the different C–O. stretching vibrations of C.–O–H and C–O..–C bonds. The peaks at 1057 cm^{-1} wavenumbers correspond to C-O stretching (C-O symmetric stretching of glucose region)[22], in (carbohydrates). The mean ± std. deviation of R_6(nucleic acids / nucleic acids) was (.915929±.0853596) for lung cancer patient while (.876065±.0316094) for healthy groups. The P-value of (R_6= .003 P less than 05), showing that the ratios were much larger for (L.C) patients than normal. Then 1257 cm^{-1} is due to $PO^{-}_{2.}$ Antisymmetric[23].The peaks at 1006 cm^{-1} represented the glucose absorption features (carbohydrates.). The mean ± std. deviation of R_7(nucleic acids / nucleic acids) was 1.533909±.6165074 for lung cancer patient while 2.202381±.2245023 for healthy groups. 1506 cm^{-1} corresponding N..-H in plane bending vibration strongly

coupled to C.-N stretching vibration of protein[24] . The peaks at 1057 cm^{-1} wavenumbers correspond to C.-O stretching (C-O. symmetric stretching. of glucose region) in (carbohydrates). The (P-value) of (R_7=.000 P less than.05) showing that the ratios were much larger for healthy persons than (L.C) patients. The mean of R_8(proteins / nucleic acids) was 1.245322±.2761236 for lung cancer patients while 1.068229±.1003311for healthy groups. The (P-value) of (R_8=.000 P <.05), showing that the ratios were much larger for (L.C) patients than normal. The band frequency about 1655 cm^{-1} (α-helix) and 1685 cm^{-1} (β-sheet) is the amide I (proteins), moreover, the relative content of α-helix high in lung cancer patients serum. and R_9(α-helix / β-sheet) can reflect the changes of proteins structures and components[25], the mean ± std. deviation was 1.854418±.1701405 for lung cancer patients while 1.663741±.1280934 for normal groups, recommending the content of N.–H bending and C.–N stretching greater in comparison to the content of carbonyl stretching in the proteins of (L.C) patients sersum. The (P-value) of (R_9=.000, P less than 05), showing that the ratios were much larger for (L.C) patients than normal.

From the table 4, the results of the Pearson factor were documented to find out the strength of the relationship among the studied ratios of the two groups of lung cancer and healthy people, Pearson correlation was high, whether positive or negative, and statistically significant between R_2 and R_9, Also R_4 and R_6. for Pearson's correlation was moderate, whether positive or negative, and statistically significant between R_1 and (R_3, R_7), R_4 and (R_5), R_2 and (R_7,R_8), R_3 and (R_8) , R_8 and (R_9). But Pearson's correlation was low whether positive or negative, and statistically significant between R_1 and R_4), R_5 and (R_9), R_1 and (R_9), R_5 and (R_6) , R_7 and (R_9). finally Pearson's correlation was negligible whether positive or negative, was not statistically significant between R_1 and R_5,R_8), likewise R_2 and (R_3,R_4,R_5,R_6), R_3 and (R_4,R_6,R_7), R_4 and (R_7,R_9) , R_5 and (R_7,R_8) R_6 and (R_7,R_9), R_7 and (R_8), and statistically significant between R_3 and (R_9),also R_4 and (R_8).

Conclusions

FTIRATR spectroscopy was used to compare serum samples from (L.C) patients and normal. The protein, lipid, and nucleic acid absorption bands were all present in the IR spectra of the serum above. The R_1(lipids/proteins) ratio can be an effective in identifying the serum of (L.C) patients from that of normal. Furthermore, the secondary architectures of proteins in malignant and normal serum were different. When (L.C) struck, the relative level of α-helix was likely to be high. Some ratios can be used as markers to predict (L.C) occurrence. As a result of the aforesaid findings, serum IR spectram may be effective in identifying (L.C).

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