Prostate Cancer Serum Spectra as Indicators of Early Diagnosis Using FTIR-ATR Fourier Transform Infrared Spectroscopy

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Abstract

Prostate cancer is the many typically diagnosed cancer in males. Prostate-specific antigen (PSA) testing allows early diagnosis of prostate cancer despite the poor sensitivity and specificity of the patient's blood, which requires the need for better tools to diagnose this disease. This study investigates the possibility of using Fourier transform infrared spectroscopy. (FTIR-ATR) for spectroscopic comparison between healthy and infected serum, where the highest absorbance was for prostate cancer patients in addition to the loss of some functional groups for the spectrum of patients and a shift in the spectrum of patients from the spectrum of healthy patients, which can be used in the accurate and early diagnosis of this type from cancer. This research revealed that P-values for each ratio I4, I6, and I7 were less than 0.05 (P 0.05). It was statistically significant, although the ratios I1, I2, I3, I5, I8, and I9 were not; hence, the ratio values of each I4, I6, I7 can be used in the early detection of prostate cancer and to distinguish between normal and malignant tissue in the prostate gland in men.

Keywords: FTIR Spectroscopy, Serum, Prostate Cancer, Prostate-specific Antigen (PSA).

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INTRODUCTION

Cancer is an enormous public health issue that affects individuals worldwide and is a significant cause of death; in 2019, by sex and cancer type, there will be approximately 1,762,450 cancer cases given a diagnosis, more than 4,800 new cases every day An estimated 9.6 million deaths in 2018 and 606,880 cancer deaths are projected to occur in the United States[1]. Estimated Number of New Cases for prostate cancer about (174,650) cases in 2019, prostate cancer the most widespread male cancer and the second most typical cause of cancer-related deaths of men whose prevalence increases with age[2]. The predominance of prostate cancer is different due to genetic and environmental factors[3]. According to the Iraqi Cancer Registry Annual Report, in in 2017 the number of prostate cancer cases (853) case, and the number of instances in Najaf (48) patients [4], while in 2018, The number of prostate cancer cases (1023) infection, The highest incidence was among the age group 70+ years, and the incidence increased with age, in the province of Najaf, the number of patients with prostate cancer (84) cases[5]. The latest world health organization (WHO) data published in 2017 Prostate Cancer Deaths in Iraq reached 432 or 0.25% of total deaths[6]. Iraq ranks 152 globally with an age-adjusted death rate of 7.02 per 100,000 people [7]. From a diagnostic and therapeutic point of view, it is fundamental to study the physical and chemical changes occurring in tissues and cells due to disease[8]. Usually, the disease is the unbalancing of these changes, and this unbalancing can most readily be detected by chemical examinations of the body fluids or excreta[9]. The rapid and responsible determination of the concentration of blood, plasma, or serum constituents is the primary requirement of clinical chemistry, and the use of multi-molecular biochemical analysis techniques such as Fourier transform infrared (FTIR) spectroscopy could support this purpose[10]. The diagnostic concerns associated with accurate prostate cancer grading and staging have directed interest in developing a spectroscopic-based diagnostic procedure. Spectroscopy has received quite a lot of attention for understanding the biological nature of the disease and the diagnosis of the disease in recent years[11]. FTIR spectroscopy technique is employed to study the spectral differences in the serum of healthy and affected by prostate cancer, Based on the differences in the spectral signatures[12]. Also, any biochemical change in the tissue must precede any morphological manifestation of the disease itself[13]. The ability to rapidly diagnose the disease's premature onset unequivocally has considerable advantages

[14]. The present work attempts to characterize the blood serum as normal or affected by prostate cancer with FTIR-ATR spectral technique, as shown in figure1. Fourier transform infrared spectroscopy can potentially improve clinical decision-making and patient outcomes by detecting biochemical changes in prostate cancer patients at the molecular level[15]. The purpose of the current assignment is to employ the spectroscopic process to detect the differences in blood sera of prostate cancer subjects and to evaluate the feasibility of detecting prostate cancer by analyzing the total biochemical composition of sera using infrared spectroscopy rely on differences absorbance and the shift in wavenumbers between normal and the case of prostate cancer[16].

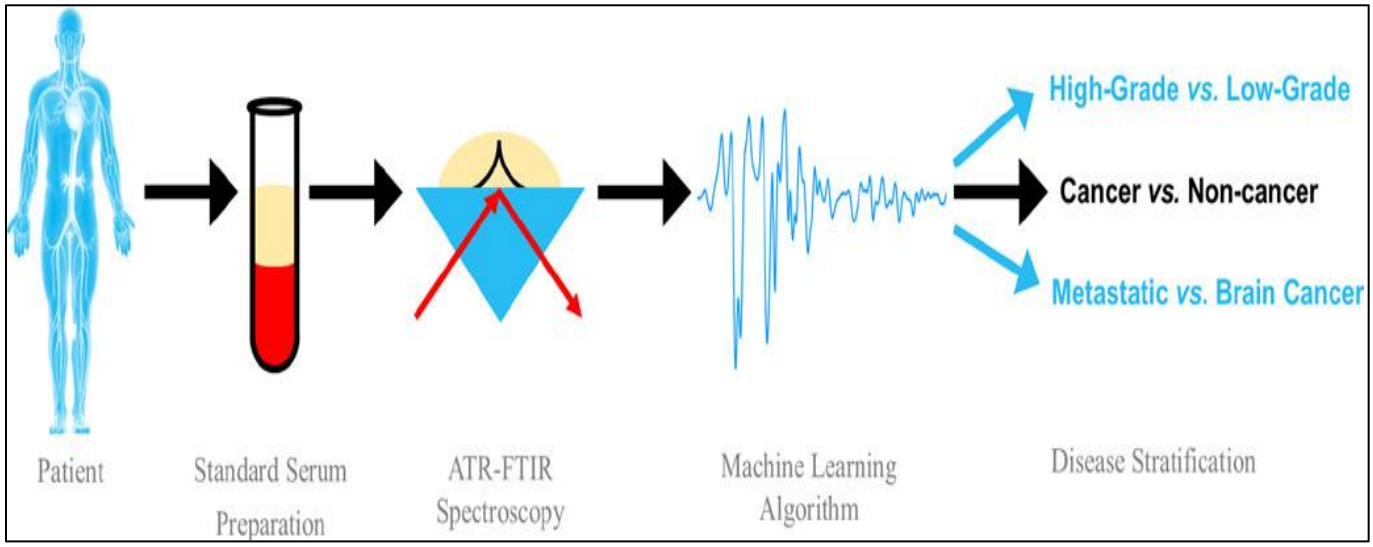

Figure 1. A Total Fourier Transform Diluted Reflectance (ATR-FTIR) Spectroscopy of Serum for Diagnosing and Stratifying Cancerous Tumors Using Machine Learning Algorithms[15].

Materials and Methods

About 100 blood specimens were taken from donors at the blood bank, and the Middle Euphrates Cancer Center and Main Blood Bank in Najaf Governorate, Iraq; half of the specimens were from healthy people, and the other half were from prostate cancer patients, using a plastic syringe with a volume of 5 ml for each specimen and centrifuged at 6000 rpm for 10 minutes to obtain blood serum; small bettri dishes with 0.1 ml of serum were used. It was mixed with 300 mg of Potassium Bromide (KBr) by the glass sticks. Take with cover to each sample, labeling it; the dishes prepared to examine put into Vacuum dried for 3 minutes[17]. Dried specimens were grinned by marble mortar, and these samples were divided into different age groups .All specimens were examined using a Bruker IFS 66V FTIR spectrophotometer, and the spectra were obtained in the region 4000-600 cm-1. All the infrared spectra were baseline corrected and normalized to obtain accurate data. Also, the function groups were analyzed and the specific influence of the relevant pathological characteristics of the cancer patients.

The data were analyzed using Statistical Package for Social Sciences (SPSS) ("SPSS\Statistics\20\stats.exe"). Independent Samples t-test was used to calculate the difference between the two means of absorbance for some wavenumbers in the spectrum of all samples.

Results and Discussions

The infrared spectrum of serum provides valuable information on biomolecules like structure, functional groups, types of bonds, and their interactions. Biomarkers, which are defined as disease-related molecular changes in body fluids and vital tissues such as blood, are critical in facilitating screening and diagnosis so that clinical interventions can proceed as soon as possible; an acceptable vibrational band assignment of absorption bands of the spectra is done using the category frequency of the diverse constituents of prostate cancer expressed in table 1.

Table 1. Human serum specimens' infrared vibrational band frequency assignment.

Absorption Band for healthy and injured (cm^{-1})	Assignment	Component group
3281.78 - 3298.10	N-H asymmetric stretching of the secondary Amide of protein. N-H symmetric stretching	Amino acid(amide A)
2926.66 - 2938.90	CH2/CH stretching (Ethylene group).	Fatty acid/ lipids
1600.07 -1700.07	C=O stretching (80% weakly coupled with C=N stretching (10%)and N-H deformation (10%) Amide.	Amide I
1500.07 -1600.07	N=H deformation (60%)strongly coupled with CNstretching (40%) Amide. N-H in-plane bending vibration strongly coupled to C-N stretching vibration of protein.	Amide II
1400.06 -1451.08	CH3 asymmetric deformation COO- stretching of amino acids. (C=0 symmetric stretching of COO)	Amino acid
1230.67 -1249.03	(N–H bend in-plane and C–N stretch)	Amide III
1046.98 -1091.88	C-O stretching (C-O symmetric extension of glucose region)	Cyclo propane
673.50 - 695.94	N-H asymmetric deformation coupled withCH2(methane)rocking amide V. C-H out plane bending. (NH2 wagging).	Amide IVAmino acid

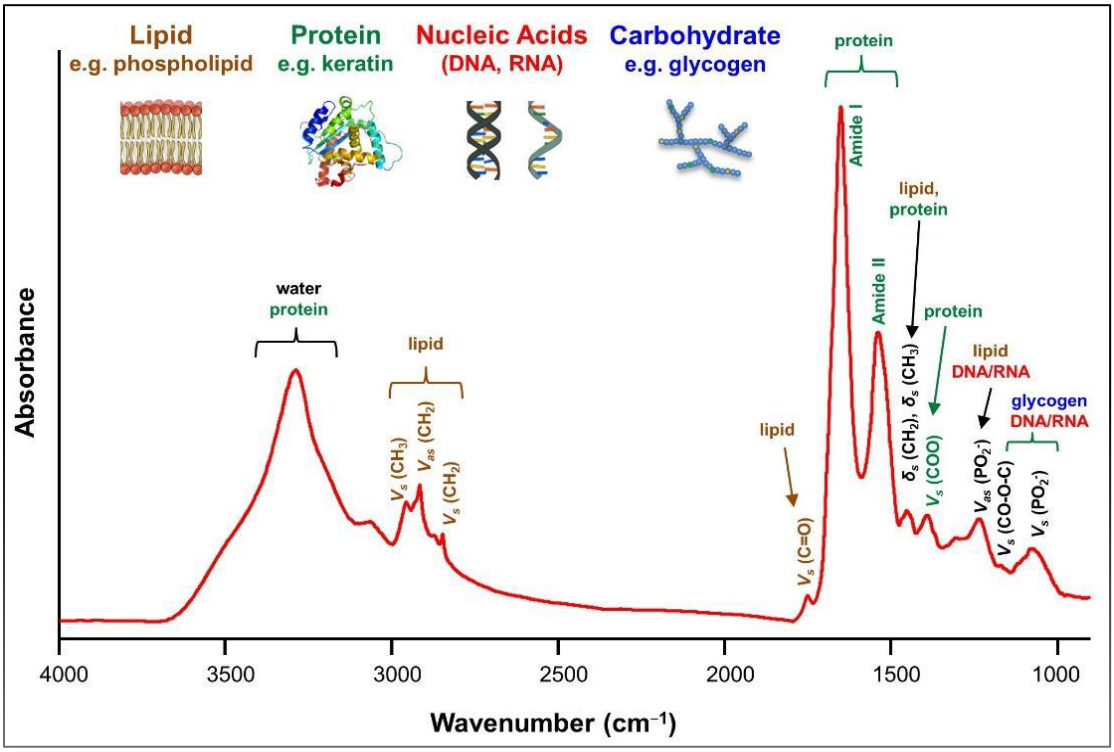

Figure 2. Common Fourier transforms infrared (FTIR) bands for biomolecules in prostate cancer for unprocessed spectrum derived from human blood serum. Spectral regions correspond to known bond Vibrations can be associated with groups of biomolecules such as protein, lipid, phosphate, and carbohydrates. Broad examples of blood serum constituents are listed [14, 15].

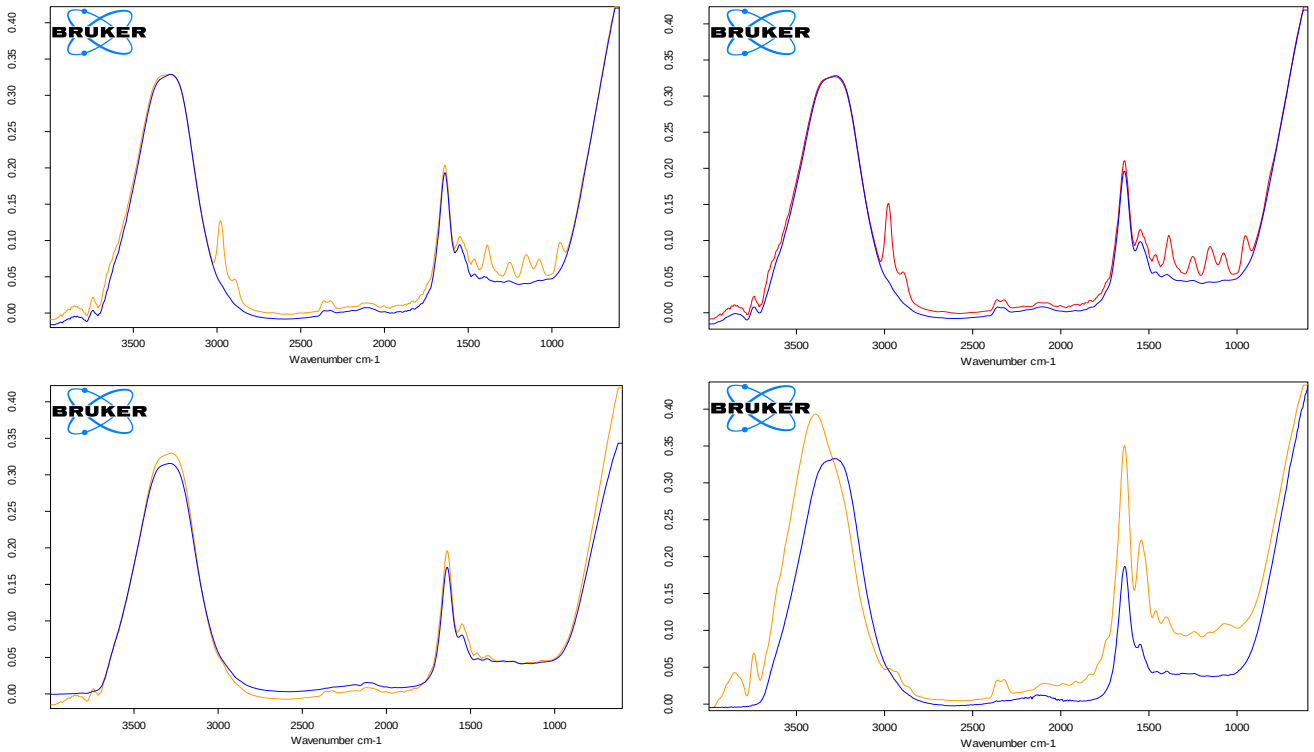

Fig. 3. Shows overlay of FTIR spectrum of healthy(blue) and patient blood serum(red) samples with prostate cancer for different age groups

The study focused on the spectra analysis using FTIR-ATR, tapping into the fundamental understanding of the spectra absorption principle. We will first characterize the spectral of normal case represented in figure1. However, differences among men with prostate cancer, the representative normalized FTIR-ATR absorption overlay spectra of normal and prostate cancer after dividing the ages into different categories in figure 3. by the following.

The Amide, I, and Amide II regions,(1700–1500 cm^{-1}) of the absorption spectrum give us information about protein content and secondary structure, where Amide I is the most intense absorption band in proteins stretching vibrations of the C=O (70-85%) and C-N groups are primarily responsible for this (10-20 %). Between 1600 and 1700 cm^{-1}, its frequency is detected. Also, Amide II is found in the 1510 and 1580 cm^{-1} region, and it is more complex than Amide I. Amide II derives mainly from in-plane N-H bending (40-60% of the potential energy). The rest of the potential energy arises from the C-N (18-40%) and the C-C (about10%)stretching vibrations [16]. The 1300–800 cm^{-1} region is due to vibrations of functional groups such as PO2 −, CO, and CC present in proteins, nucleic acids, and carbohydrates where the phospholipids band due to the asymmetric P-O stretching of PO2 occurs at 1240 cm^{-1} [17].

The specimen components, i.e., carbohydrates, protein, phosphate, and lipid, constitute the characteristic bands on the infrared spectrum in 1400-900 cm^{-1},1800-1400 cm^{-1}, and 3200 – 2800 cm^{-1}, respectively. On the infrared spectra of sera, vibration bands were assigned by comparing the bands' position, relative intensity, and shape with the bands of related molecules [18]. And considerable differences in infrared absorbance of these biomolecules have been observed in the present investigation of different normal sera. 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 as an Amide II band. The characteristic solid band at 3285cm^{-1} [19] due to N-H symmetric stretching confirmed the existence of the Amino acid group. 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. We found (2925-2937)$cm^{-1,}$ which is represented lipid (fatty acid and cholesterol) in most spectra of the patients due to CH2/CH stretching (Ethylene group). The medium-weak bands at 1230-1330 cm^{-1} represent Amide III, while the band 672-696 cm^{-1} in the spectrum of prostate cancer was missed, which is defined (Amide IV Amino acid) that due to N-H asymmetric deformation coupled with CH2 (methane) rocking Amide V [20]. The weak bands at 1065-1091 cm^{-1} give rise to glucose's existence due to C-O symmetric stretching, C-C symmetric stretching, and C-O symmetric stretching.

As well, we have noticed that the region of the wavenumbers 3280-3309cm-1, which represent(Amide A), is not found in

all spectrum of prostate cancer patients, and the region 1400-1450, Cm^{-1}which means Amino acid did not appear in some spectrum of patients in the (70-79) age group and appeared in other age groups.

Table 2. A comparison of intensity ratio parameters of sera from healthy subjects and Prostate cancer patients by T-test results.

Intensity ratio parameter	Case	Mean	Std.Dev	Std.Err.	Levene's Test		T-test		
					F	Sig.	t	df	P-value
I_1 (3304.22/3067.48)	*H.G.	2.976	0.285	0.040	56.04	0.000	-4.509-	64.98	0.000
	**PCa	3.456	0.697	0.097					
I_2 (3255.24/2906.25)	H.G.	16.89	4.552	0.644	0.227	0.635	5.137	98.00	0.000
	PCa	12.37	4.250	.6010					
I_3 (3255.24/1706.19)	H.G.	37.26	13.69	1.936	120.5	0.000	16.83	49.56	0.000
	PCa	4.575	1.031	0.146					
I_4 (2906.25/1106.17)	H.G.	0.493	0.211	0.030	0.039	0.844	0.219	98.00	0.827
	PCa	0.485	0.172	0.024					
I_5 (2873.59/2930.74)	H.G.	0.461	0.120	0.017	0.048	0.827	-2.470-	98.00	0.015
	PCa	0.519	0.115	0.016					
I_6 (1706.19/1506.18)	H.G.	0.833	0.063	0.009	8.637	0.004	3.149	88.59	0.002
	PCa	0.785	0.089	0.013					
I_7 (1606.19/1057.18)	H.G.	2.725	0.305	0.043	2.040	0.156	6.137	98.00	0.000
	PCa	2.376	0.262	0.037					
I_8 (1079.63/1544.96)	H.G.	0.485	0.081	0.011	4.038	0.047	-3.321-	96.88	0.001
	PCa	0.542	0.090	0.013					
I_9 (1400.06 /1451.08)	H.G.	1.026	0.229	0.032	0.004	0.947	-0.560-	98.00	0.577
	PCa	1.046	0.090	0.013					
I_{10} (1314.34/ 1404.14)	H.G.	0.911	0.268	0.038	0.455	0.501	3.061	98.00	0.003
	PCa	0.789	0.085	0.012					
I_{11} (1240.87/1053.10)	H.G.	1.004	0.044	0.006	4.569	0.035	-1.003-	73.19	0.319
	PCa	1.017	0.086	0.012					
I_{12} (1257.19/1006.16)	H.G.	0.960	0.047	0.007	61.68	0.000	-5.045-	58.96	0.000
	PCa	1.070	0.145	0.021					
I_{13} (1030.65/1051.06)	H.G.	1.018	0.021	0.003	41.77	0.000	7.015	64.78	0.000
	PCa	0.963	0.051	0.007					
I_{14} (1106.17/1057.18)	H.G.	0.962	0.015	0.002	0.000	0.998	0.933	92.00	0.353
	PCa	0.957	0.027	0.004					
I_{15} (1079.63/1242.91)	H.G.	0.998	0.038	0.005	1.775	0.186	-4.025-	98.00	0.000
	PCa	1.040	0.063	0.009					

*H.G. = Healthy groups.

**PCa =Prostate cancer.

The results were analyzed using statistical program Statistical Package for Social Sciences (SPSS). Statistical processes were performed which included descriptive ratio statistics, means and independent samples test which contain Levene's test for equality of variances and t-test for equality of means and shown in Table 4.16. I was used to calculate the difference between the two means of absorbance for some wave numbers in the spectrum of all samples. The mean and std. deviation of the intensities I_1(3304.22/3067.48), I2 (3255.24/2906.25), I_3(3255.24/1706.19), I_5(2873.59/2930.74), I_6(1706.19/1506.18), I_7 (1606.19/1057.18), I_8(1079.63/1544.96), I_{10}(1314.34 /1404.14), I_{12}(1257.19/1006.16), I_{13} (1030.65/1051.06) and I_{15}(1079.63/1242.91) respectively were (3.456±0.697),

(12.37±4.250), (4.575±1.031), (0.519±0.115), (0.785±0.089), (2.376±0.262), (0.542±0.090), (0.789±0.085), (1.070±0.145), (0.963±0.051) and (1.040±0.063) respectively for prostate cancer patient while (2.976±0.285), (16.89±4.552), (37.26±13.69), (0.461±0.120), (0.833±0.063), (2.725±0.305), (0.485±0.081), (0.911±0.268), (0.960±0.047), (1.018±0.021) and (0.998±0.038) respectively for healthy persons, making it clear that the ratio was significantly higher for prostate cancer patients serum than this for healthy persons serum

Also for the ratio I_1,I_2,I_3,I_5,I_6,I_7,I_8,I_{10},I_{12},I_{13} and I_{15} ($P<0.05$), was statistically significant for all P-values less than 0.05 as shown in Table 4.16, so obviously, there are differences between the means of values through which the disease can be diagnosed.

The mean and std.deviation of the intensities I_4(2906.25/1106.17), I_9(1400.06 /1451.08), I_{11}(1240.87/1053.10) and I_{14}(1106.17/1057.18) respectively were (0.485±0.172), (1.046±0.090), (1.017±0.086) and (0.957±0.027) respectively for prostate cancer patients while (0.493±0.211), (1.026±0.229), (1.004±0.044) and (0.962±0.015) respectively for healthy persons, were not statistically significantly where the P values were greater than 0.05, as shown in Table 4.16.

The current study of various normal and cancerous seras has revealed significant differences in infrared absorption of these vital ingredients. The current work is an attempt to characterize the blood serum of prostate cancer patients and healthy men and understand in this study, and we observed that the absorbance of all patients that appeared in the spectra of FTIR-ATR for all age groups is more significant than that for the normal samples. Finally, the results of the spectroscopic study can be considered as evidence of reliance on this method.

Conclusion

The consequences obtained in this research did not support the $1030cm^{-1}/1080cm^{-1}$ peak ratio as the key discriminating factor between benign and malignant pathologies. This may have been because the sample areas in the study were more diverse, or the peak ratio difference may be accounted for by differences in cell cycle position at the time of analysis. The peak ratio $1400cm^{-1}$ / $1450cm^{-1}$ differed between pathologies, with the ratio closer to 1.0 in malignant tissue instead of approximately 0.6 in benign tissue. This region corresponds to proteins and lipids (cholesterol) and may represent higher protein concentrations in cancerous cells with prominent nuclei. It has also been suggested that the amount of cholesterol in malignant tissues is lower.

Also, the intensity ratio was calculated; it was found that some of the proportions were statistically significant for I1(protein/protein), I2(keratin/keratin), I3(phospholipid/ cholesterol), I4(Glucose/ Phspholipid) (I5 (Carbohydrates/carbohydrates), I6 (protein/carbohydrates) and I7 (protein/carbohydrates) which can be taken into consideration for the early diagnosis of prostate cancer. In contrast, the ratio was statistically non-significant for I8 (carbohydrates/carbohydrates) and I9 (protein/carbohydrates). As a result, we've consistently demonstrated that the FTIR-ATR spectroscopy of human serum is a potentially suitable and effective tool for the early detection of prostate cancer.

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