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# A Comparative study between the spectra of prostate Cancer and healthy men using FTIR-ATR technique

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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 technique can be used not only for understanding the biological nature of the disease, but also for the diagnosis of the prostate 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 prostate cancer .we found the absorbance of patients with prostate cancer larger than the absorbance of health men .We found that some functional groups were lost in the spectrum of patients, in addition to the occurrence of a shift in the spectrum of patients from the spectrum of healthy people, which can be used in the early diagnosis of diseases. In this study, P-values were lower than 0.05 (P< 0.05) for each ratio $A_{1,}$ $A_{2,}$ $A_3$ showed 0.010,0.012,0.016 respectively, It was statistically significant, while the ratio A and $A_5$ It was not statistically significant , Therefore, it is possible to take into consideration the ratio values of each $A_{1,}$ $A_{2,}$ $A_3$ in the early diagnosis of prostate cancer, as well as in distinguishing between healthy and malignant tissues in the prostate gland in men.*

*Keywords: Fourier transform, Infrared spectroscopy, prostate cancer, spectra of serum men.*

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**Introduction:**

Cancer is a major public health problem worldwide and is a major cause of death , in 2019 by sex and cancer type, there will be approximately 1,762,450 cancer cases diagnosed, which is the equivalent of more than 4,800 new cases each day. 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 the prostate cancer about (174,650) case in 2019, prostate cancer is one of the most common males cancers and the second commonest 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 Iraqi Cancer Registry Annual Report , in in 2017 the number of prostate cancer cases (853)case , and the number of cases in Najaf (42) patients , 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 (61) cases[4]. The latest world health organization(WHO) data published in 2017 Prostate Cancer Deaths in Iraq reached 432 or 0.25% of total deaths The age adjusted Death Rate is 7.02 per 100,000 of population ranks Iraq 152 in the world [5]. 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[6]. usually means that disease is the unbalancing of these changes and this unbalancing can most readily be detected by chemical examinations of the body fluids or excreta[7] . The fast and reliable determination of concentration of blood, plasma or serum constituents is the major requirement of clinical chemistry and the use of multi-molecular biochemical analysis techniques such as Fourier transform infrared (FTIR) spectroscopy could support this purpose[8]. The diagnostic problems associated with the correct grading and staging of prostate cancer has led to an interest in the development of spectroscopic based diagnostic technique 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[9]. 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 [10]. also at a very early stage of the disease, due to the

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fact that any biochemical change in the tissue must precede any morphological manifestation of the disease itself[6]. The ability to diagnose the early onset of disease ,rapidly, unequivocally has multiple benefits[11]. The present work is an attempt to characterize the blood serum as normal or affected by prostate cancer with the aid of FTIR-ATR spectral technique[12]. 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[13]. The objective of the present work is to employ the spectroscopic technique to detect the changes 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.

**Materials and Methods**

Plastic syringe was used to collect 5 ml of venous blood , blood samples have been collected from 18 healthy volunteers and 29 prostate cancer patients which are divided by age groups (60-69),(70-79),(80-89), and (90-99) .Patient samples were collected from the middle Euphrates cancer center , and the healthy samples collected from the blood bank in Al-Najaf city, Iraq. The collected blood samples were centrifuged at 4000 rpm immediately and the serum was obtained., was used small bettri dishes, put in a volume of 0.1ml of serum , and it was mixed with 300 mg of Potassium Bromide (KBr) by the glass sticks. Take with cover to each sample, labeling it, The dishes which prepared to examined put into Vacuum dried for 3 minutes[14] .Dried samples were grinned by marble mortar Then examined by FTIR-ATR Instrument , 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 infrared spectra were baseline corrected and normalized to obtain accurate data. Also The function groups were analyzed as well as the specific influence of the relevant pathological characteristics of the cancer patients.

**Results and Discussions**

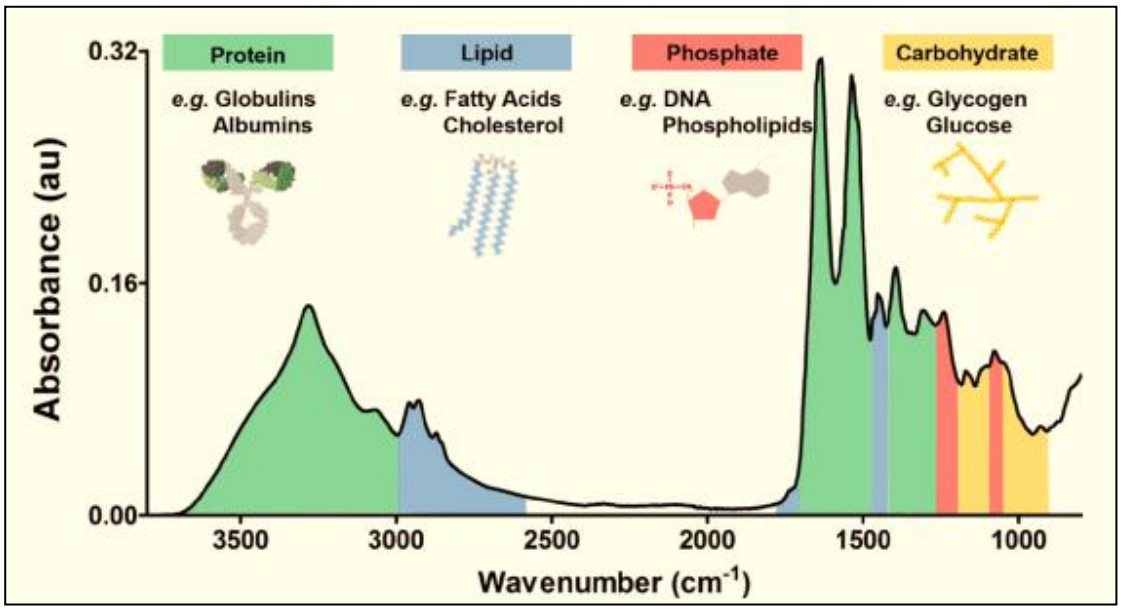
The infrared spectrum of serum provides useful information of biomolecules like structure, functional groups, types of bonds and its interactions. Biomarkers, which are defined as disease-related molecular changes in body fluids and vital tissues such as blood ,are essential in facilitating screening and diagnosis to allow clinical interventions to begin as soon as possible, A satisfactory vibrational band assignment of absorption bands of the spectra is done with the help of the group frequency of the various constituents of prostate cancer represented in table(1).

By using FTIR-ATR ,we will first characterized the spectral of normal case represented in figure (1) but differences among men with prostate cancer ,the representative normalized FTIR-ATR absorption overlay spectra of normal and prostate cancer after the ages were divided into 4 categories in figures((a),( b),(c),(d)) by the following :

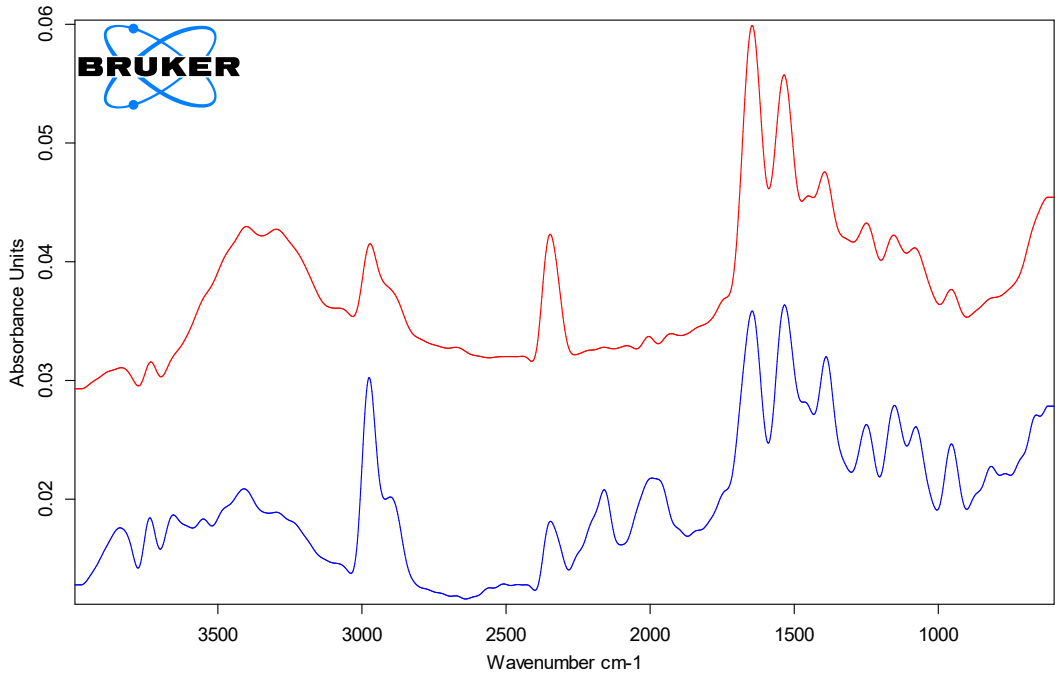
Table(1):Infrared vibrational band frequency assignment of human serum samples.

| Sq. | Vibrational band($cm^{1}$) normal | Vibrational band($cm^{-1}$) Prostate cancer | Assignment | Component group |
|---|---|---|---|---|
| 1. | 3280-3309 | 3280-3298 | N-H asymmetric stretching of secondary amide of protein. N-H symmetric stretching | Amino acid (amide A) |
| 2. | 2925-2937 | 2925-2937 | CH2/CH stretching (Ethylene group). | Fatty acid/ lipids |
| 3. | 1700-1600 | 1648-1600 | C=O stretching (80% weakly coupled with C=N stretching (10%)and N-H deformation (10%) amide. | amide I |
| 4. | 1600-1500 | 1543-1500 | N=H deformation (60%)strongly coupled with C-N stretching (40%) amide.<br>N-H in plane bending vibration strongly coupled to C-N stretching vibration of protein. | amide II |
| 5. | 1400-1450 | 1400-1410 | CH3 asymmetric deformation COO- stretching of amino acids.( C=0 symmetric stretching of COO– ) | Amino acid |

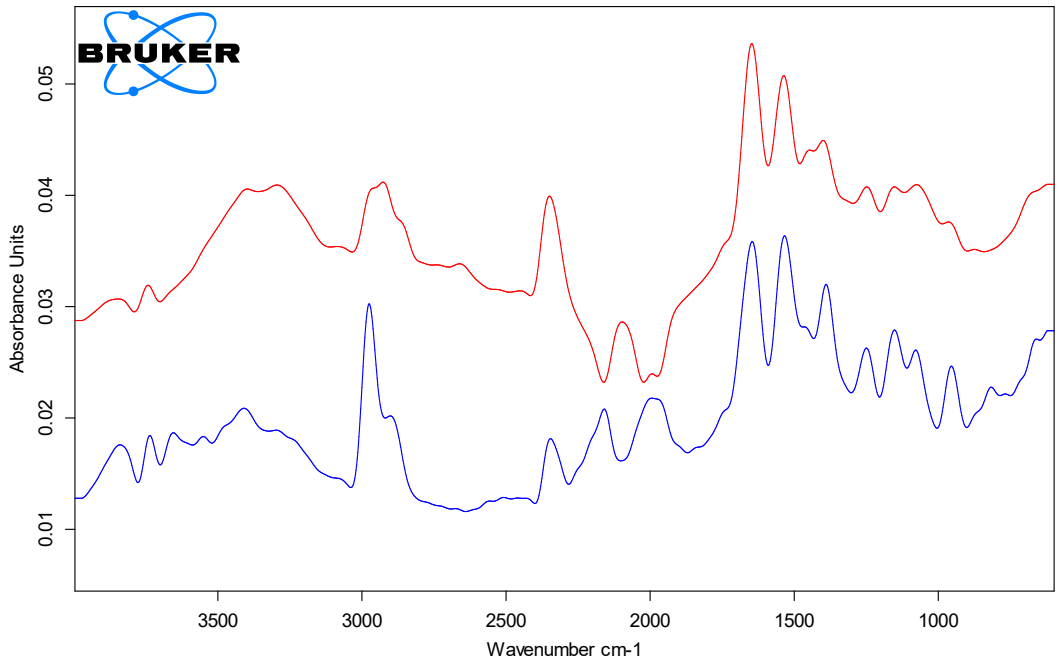
| | | | | |
|---|---|---|---|---|
| 6. | 1230-1330 | 1230-1249 | (N–H bend in plane and C–N stretch) | Amide III |
| 7. | 1045-1090 | 1068-1086 | C-O stretching (C-O symmetric stretching of glucose region) | Cyclo propane |
| 8. | 672-696 | | N-H asymmetric deformation coupled withCH2(methane)rocking amide V.<br>C-H out plane bending.(<br>NH2 wagging). | Amide IV<br>Amino acid |



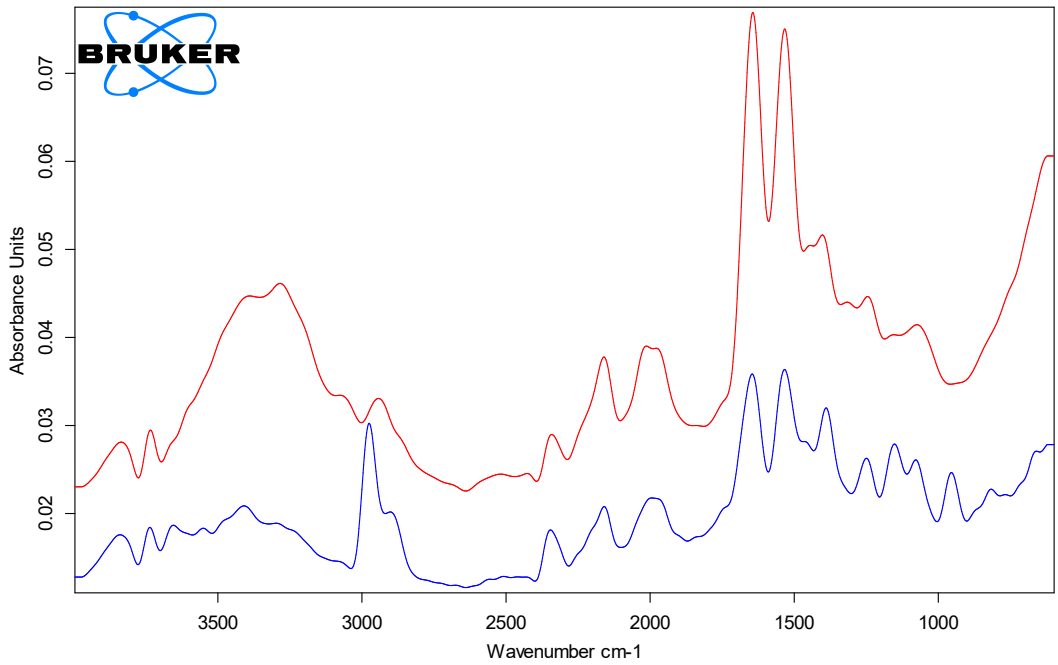

Figure(1):An unprocessed spectrum derived from human blood serum using attenuated total reflectance Fourier transform infrared (FTIR -ATR) spectroscopy. Spectral regions correspond to known bond vibrations and can therefore be associated with groups of biomolecules such as protein, lipid, phosphate and carbohydrates. Broad examples of blood serum constituents are listed [15, 16].



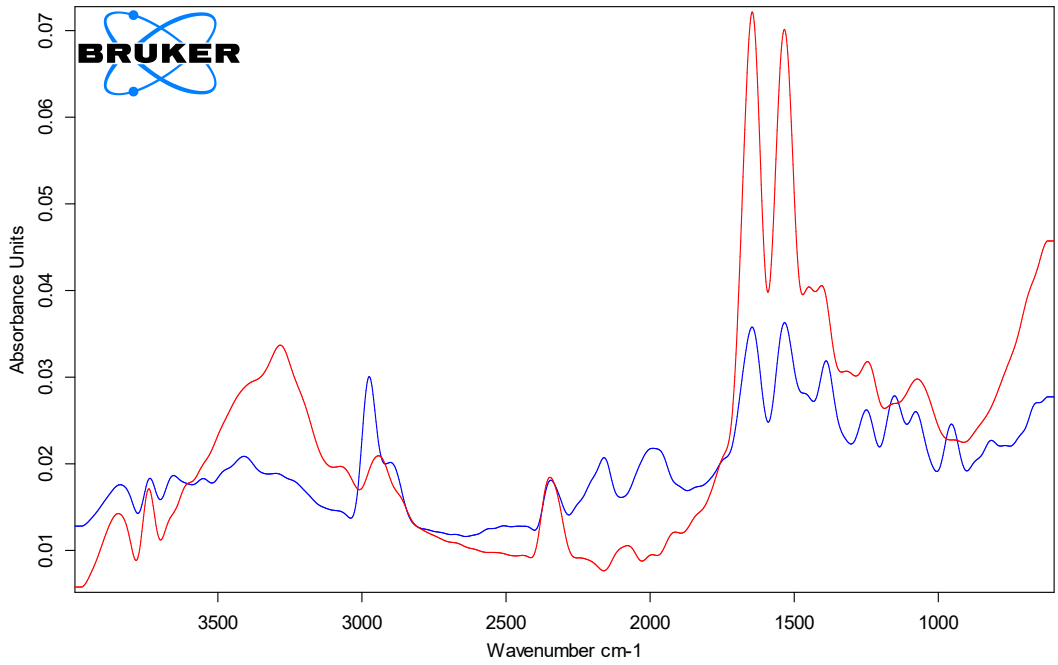

Figure(2 -a):comparative between prostate cancer patient (red) and health man (blue) for faction aged (60-69) years.




Figure(2 -b):Comparative between Prostate cancer patient (red) and health man (blue) for faction aged (70-79) years.




Figure(2-c):comparative between prostate cancer patient (red) and health man (blue) for faction aged (80-89) years.




Figure(2-d):comparative between prostate cancer patient (red) and health man (blue) for faction aged (90-99) years.

The 1700–1500 $cm^{-1}$ (amide I and amide II) region contains mostly information on protein content and secondary structure , where **Amide I** is the most intense absorption band in proteins. It is primary governed by the stretching vibrations of the C=O (70-85%) and C-N groups (10-20%). Its frequency is found in the range between 1600 and 1700 $cm^{-1}$. 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 [17] The 1300–800 $cm^{-1}$ region is due to

vibrations of functional groups such as $PO_2$ −, 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 $cm^{-1}$ [18].

The sample constituents namely lipid, protein, phosphate, and carbohydrates form the characteristic bands on the infrared spectrum in the frequency ranges 3200 – 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[19]. And considerable differences in infrared absorbance of these bio molecules 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 and termed as an Amide II band. The strong characteristic band at 3285$cm^{-1}$ [20] due to N-H symmetric stretching confirmed the existence of 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 which due to CH2/CH stretching (Ethylene group).The medium weak bands at 1230-1330 $cm^{-1}$ that represents Amide III ,while the band 672-696 $cm^{-1}$ in the spectrum of prostate cancer was missed which is represented (Amide IV Amino acid) that due to N-H asymmetric deformation coupled withCH2(methane)rocking amide V [21]. 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 .

As well, we have noticed that the region of the wavenumbers 3280-3309$cm^{-1}$ which represent( Amide A) is not found in all spectrum of prostate cancer patients and the region 1400-1450 $Cm^{-1}$which represents 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 health subjects and Prostate cancer patients by T-test results.

| Ratios | prostate cancer patient(mean± Std. Deviation) | Healthy person (mean±Std. Deviation) | Independent t-test(P value) |
|---|---|---|---|
| A1(1106/1057 $cm^{-1}$) | .995± .012 | 1.130±.081 | .010 |
| A2(1257/1006$cm^{-1}$) | 1.138±.060 | 1.068±.056 | .012 |
| A3(1606/1057$cm^{-1}$) | 1.295±.163 | 1.121±.062 | .016 |
| A4(1057/1006 $cm^{-1}$) | 1.087±.038 | 1.130±.081 | .259 |
| A5(1506/1057 cm-1) | 1.614±.383 | 1.359±.233 | .130 |

We know the 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 and std. deviation of A1(1106/1057) was ( .995± .012) for prostate cancer patient while(1.130±.081) for healthy persons .The P value of A1(1106/1057) was .010 (P<0.05),making it clear the ratio was significantly lower for prostate cancer patients' serum than this for healthy persons' serum.

Then the 1257 $cm^{-1}$ is due to $PO^{-}_2$ Antisymmetric str. DNA/RNA [23]. The peaks at 1006 $cm^{-1}$ represented The glucose absorption features(carbohydrates). The mean and std. deviation of A2(1257/1006) was (1.138±.060) for prostate cancer patient while(1.068±.056) for healthy persons .The P value of A2(1257/1006)) was .012 (P<0.05),making it clear the ratio was significantly higher for prostate cancer patients' serum than this for healthy persons' serum.

The 1606 $cm^{-1}$corresponds to C=O stretching vibrations, is the most sensitive part of the protein when determining the secondary structure[24], while 1057 $cm^{-1}$ due to the different C–O stretching vibrations of C–O–H and C–O–C bonds[25]. The peaks at 1057 $cm^{-1}$ wavenumbers correspond to C-O stretching (C-O symmetric stretching of glucose region) in (carbohydrates). The mean of A3(1606/1057)was 1.295±.163 for prostate cancer patients while 1.121±.062 for healthy persons The P value of 1606/1057 was .016 (P<0.05),making it clear the 1606/1057 ratio was significantly higher for prostate cancer patients' serum than this for healthy persons' serum.

The peaks at 1057 $cm^{-1}$ wavenumbers correspond to C-O stretching (C-O symmetric stretching of glucose region) in (carbohydrates). The peaks at 1006 $cm^{-1}$ represented The glucose absorption features(carbohydrates) , The mean of A4(1057/1006) was 1.087±.038 for prostate cancer patients while 1.130±.081 for healthy persons. The P value of

1057/1006 was .259 (P>0.05),making it clear the 1606/1057 ratio was non significantly lower for prostate cancer patients' serum than this for healthy persons' serum.
1506 $cm^{-1}$ corresponding N-H in plane bending vibration strongly coupled to C-N stretching vibration of protein[26]. The peaks at 1057 $cm^{-1}$ wavenumbers correspond to C-O stretching (C-O symmetric stretching of glucose region) in (carbohydrates). The mean of A5(1506/1057) was 1.614±.383 for prostate cancer patients while 1.359±.233 for healthy persons. The P value of 1506/1057 was .130 (P>0.05),making it clear the 1506/1057 ratio was non significantly higher for prostate cancer patients' serum than this for healthy persons' serum.

**Statistical Analysis**

The results were analyzed using Statistical Package for Social Sciences (SPSS) ("SPSS\Statistics\20\stats.exe" )Independent Samples t-test was used to calculate difference between the two means of absorbance for some wavenumbers in the spectrum of all samples . The p-value <0.05 was considered as significant. the p-value is (.010 , .012 and .016) for (A1(1106/1057$cm^{-1}$) ,A2(1257/1006$cm^{-1}$) and (1606/1057$cm^{-1}$)) ,and, there is difference between the two means is statistically significantly where was the P values less than 0.05 while the p value (.259 and .130) for the ratio A4(1057/1006 $cm^{-1}$) and A5(1506/1057 cm-1) was not statistically significantly where was the P values greater than 0.05.

**Conclusion**

Infrared spectroscopy has long been used to characterize chemical compounds Hence, the pathophysiological conditions of the body with the aid of FTIR-ATR spectral techniques, also a robust tool with great potential for clinical application which extends beyond screening, diagnosis, Analysis of the blood sera spectrum for different samples under FTIR-ATR spectroscopic technique showed that there are some differences between each and every spectrum in terms of spectral profiles, absorption bands, wave numbers and satisfactory analysis has been made, Analysis of the blood Serum is a useful tool for determination of diseases in human body.
The current work is an attempt to characterize the blood serum of prostate cancer patients and healthy men and understand in this study ,we observed that the absorbance of all patients that appeared in the spectra of FTIR-ATR for all age groups is greater than that for the normal samples. Also the intensity ratio were calculated, It was found that some of the ratio were statistically significant for $A_1$(carbohydrates / carbohydrates), $A_2$(protein / carbohydrates) and $A_3$(protein / carbohydrates) which can be taken into consideration for the early diagnosis of prostate cancer while the ratio were statistically non-significant for A4(carbohydrates / carbohydrates) and $A_5$(protein / carbohydrates) Thus, we have demonstrated that FTIR-ATR spectroscopy of human serum is a potentially feasible and efficient tool for the early detection of prostate cancer.

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