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Laser Raman spectroscopy for spectroscopic characterization of chronic lymphocytic leukemia (CLL)

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ABSTRACT

In this work Laser Raman spectroscopy was used for spectroscopic characterization of chronic lymphocytic leukemia (CLL) blood samples. Eight samples collected from leukemia patient's type (CLL) were investigated using laser Raman spectrometer. The patients were diagnosed by histopathologies in Radiation and Isotopes Center Khartoum (RICK) and Alamal Hospital. The analysis of the Raman spectra was done for the peaks of proteins, lipids and nucleic acid. Significant differences in the spectra of CLL samples, compared with normal blood spectrum, were noticed. The results showed that Laser Raman spectroscopy can be used to diagnose efficiently the CLL via the spectral changes in the intensities of the spectral peaks and the changes in their Raman shifts.

1 – Introduction

Raman spectroscopy is an analytical, nondestructive technique that provides information about the molecular structure of the investigated sample. The Raman effect arises when an incident light excites molecules in the sample, which subsequently scatter the light. While most of this scattered light is at the same wavelength as the incident light, some is scattered at a different wavelength. This in-elastically scattered light is called Raman scatter (line) and results from the interaction of the incident light with the molecular motions or vibrations [1]. The spectral positions, intensities, and linewidths of the Raman lines, corresponding to vibrational energy levels, yield information on the composition, secondary structure and interaction of molecules.

Raman spectroscopy is an important technique for the elucidation of molecular structure, for locating functional groups or chemical bonds in the molecules [2]. A Raman spectrum of a given molecule consists of a series of peaks or “bands” each peak is shifted by one of the characteristic vibrational frequencies of that molecule [3]. Each molecule has its own characteristic spectrum, and, thus, a Raman spectrum can provide a “fingerprint” of a substance from which molecular composition can be determined. Further, the intensity of a band is proportional to the concentration of the molecules from which the band arises [4]. For example, proteins, nucleic acids, polysaccharides, and lipids have their own set of characteristic bands [5,6]. Based on these facts, Raman spectroscopy can be used to characterize any living tissue (include blood) and discover any biochemical change that can happen in the tissue cells, like that associated with leukemia. Leukemia cells originate from their own type of abnormal stem cell, called a leukemia stem cell (LSC) [7]. The change of normal tissue into a cancerous lesion is a slow process which involves alterations in the molecular level which, in later stages, alter the morphology and tissue architecture. At present, almost all cases of cancer are diagnosed by subjecting a tissue sample to histopathological examination (biopsy). However, histopatological examination is invasive and does not provide immediate feedback. The development of a technique that is less invasive and provides real time and quantitative information about tissue biochemistry is of great importance for cancer diagnosis. Many research groups have employed optical spectroscopy methods (i.e. Raman, Fourier Transform Infra Red (FTIR) and laser-induced fluorescence spectroscopy) to performance such diagnostic applications, aiming the early detection of cancer. The biochemical tumor markers such as proteins, enzymes and hormones, could be detected by analyzing the differences of Raman spectra taken from normal and pathologic tissues [8]. According to the fundamental theory of Raman spectroscopy and previous literatures, the molecular level information can be obtained directly from a micro-size (i.e. cell and bacteria) to even sub-nanosize (i.e. DNA or amino acids) specimen with real time analysis ability in a microliter of aqua sample. This great potential is based on several improvements in equipment, such as increased sensitivity and resolution in signal detectors, size-minimized and cost-reduced high-power laser, and improvement of other microelectronics and software [9].

The three major components in the blood cell population are erythrocytes, leukocytes, and platelets. There are many types of leukocytes but the broadest categories are those arising from the lymphoid lineage and those from the myeloid lineage. One of leukemia types is the Chronic lymphocytic leukemia (CLL) which results from an acquired (not present at birth) injury to the DNA of a single marrow cell that is destined to become a lymphocyte. Scientists do not yet understand what produces this change in the DNA. Once the marrow cell undergoes the leukemic change, it multiplies into many cells. These leukemic cells grow and survive better than normal cells; over time, they crowd out normal cells. The result is the uncontrolled growth of lymphocytic cells in the marrow, leading to an increase in the

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number of lymphocytes in the blood. The leukemic cells in CLL do not impede normal blood cell production as extensively as is the case with acute lymphocytic leukemia. This important distinction is the reason for the less severe early course of CLL [10]. Different techniques are used to diagnose this type of cancer.

This work aimed to characterize the spectroscopic changes in CLL patients, as a diagnostic procedure, using laser – Raman spectroscopy.

2 – The experimental part:

To record the Raman spectra of the samples a Laser Raman spectrometer model LIRA-300 (Australia) was used. The light source of this spectrometer is a diode pumped solid state laser with wavelength of 532 nm and output power of 40 mW. The data were collected and shown by the spectrometer as spectra.

Eight blood samples were collected from eight patients' diagnosed by histophathologiest as CLL patients. Each sample was put it in the sample cell of the spectrometer by injection of 1mL of blood in the cell and Raman spectrum was recorded in the region from 0 to 3599 cm^{-1}. The shift in wavenumber and the change in intensities of the scattered light in Raman spectra were compared with normal blood and analyzed.

3 - Results and discussion:

To determine the changes in the spectra of patient's blood samples, a comparison was done between the spectrum of normal blood and the collected samples. Raman spectrum for normal blood sample in the range from 0.0 to 3871.6 cm^{-1} is shown in figure 1 blow.

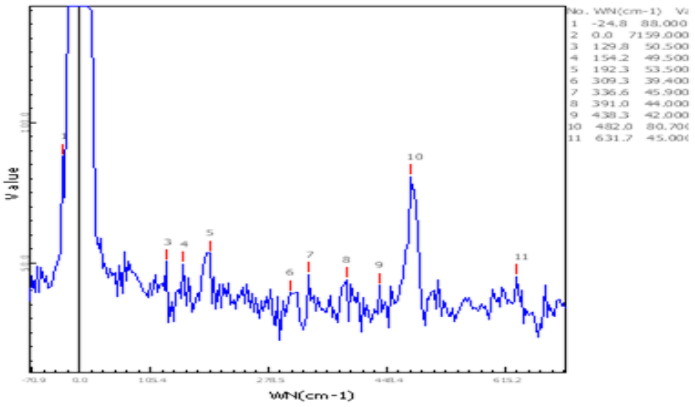

Figure (1a) Raman spectrum of normal blood in the range from 0.0 to 631.7 cm^{-1}

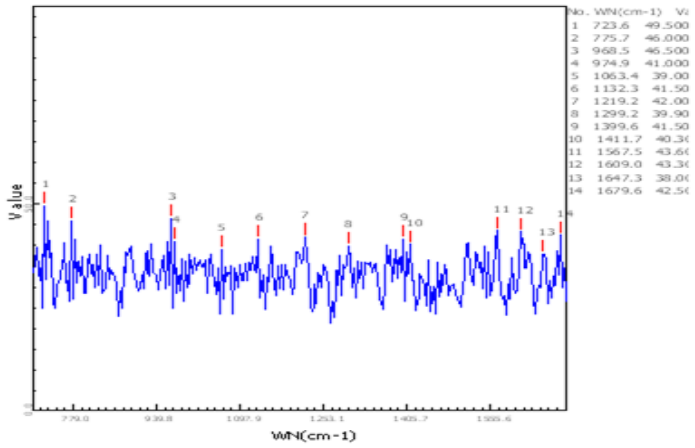

Figure (1b) Raman spectrum of normal blood in the range from 723.6 to 1679.6 cm^{-1}

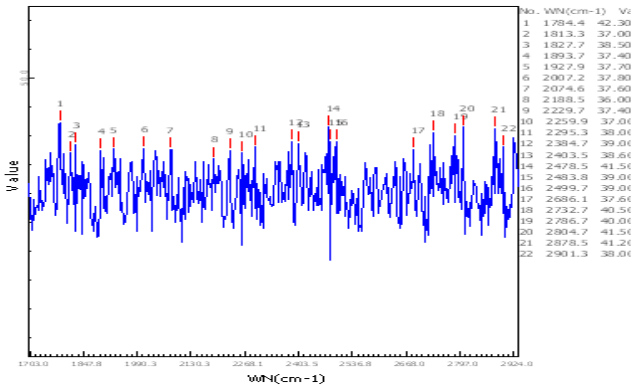

Figure (1c) Raman spectrum of normal blood in the range from 1784.4 to 2901.3cm^{-1}

Figures 2 to 4 show examples of the Raman spectra of the leukemia samples type (CLL) in the range from 0.0 to 3599 cm^{-1}. Table (1) lists the wavenumber for each peak, with its intensity, in the recorded spectra while table (2) illustrates the comparison with normal blood spectrum.

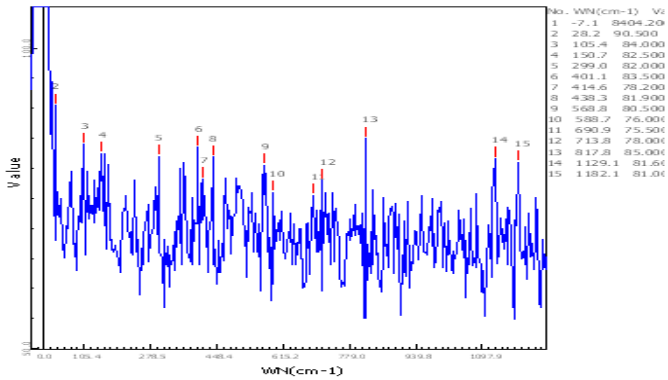

Figure (2a) Raman spectrum of chronic lymphocyte leukemia (CLL) sample no. (1) in the range from 0.0 to 1182.1cm^{-1}.

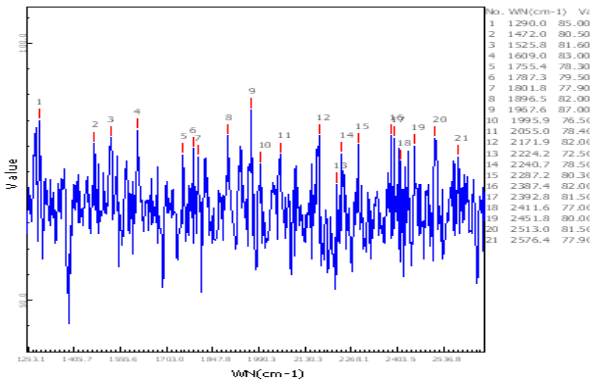

Figure (2b) Raman spectrum of chronic lymphocyte leukemia (CLL) sample no. (1) in the range from 1290 to 2576.4cm^{-1}.

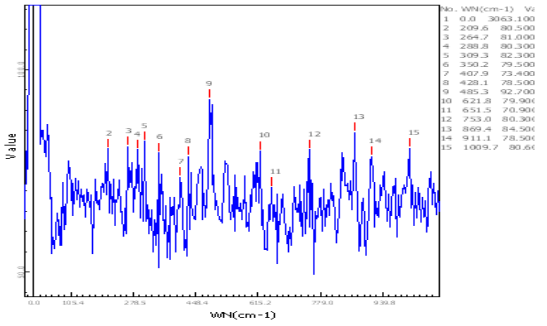

Figure (3a) Raman spectrums of chronic lymphocyte leukemia (CLL) sample no. (4) in the range from 0.0 to 1009.7cm^{-1}

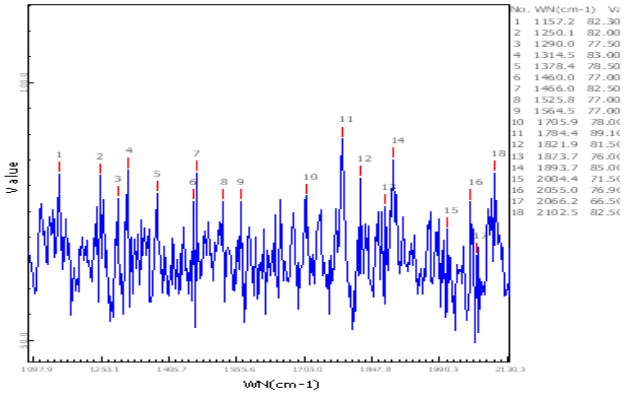

Figure (3b) Raman spectrum of chronic lymphocyte leukemia (CLL) sample no. (4) in the range from 1097.9 to 2130.5cm^{1}.

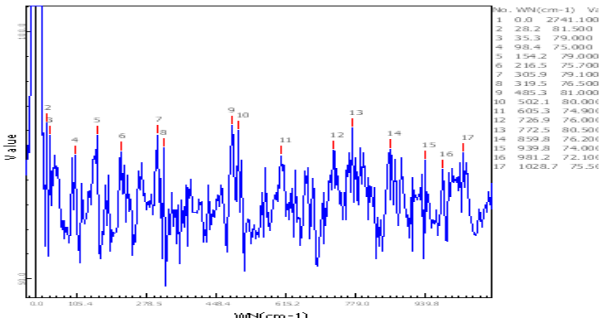

Figure (4a) Raman spectrum of chronic lymphocyte leukemia (CLL) sample no. (8) in the range from 0.0 to 1028.7cm^{1}.

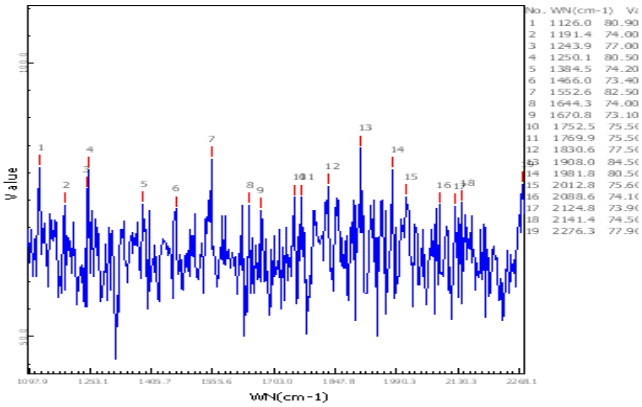

Figure (4b) Raman spectrum of chronic lymphocyte leukemia (CLL) sample no. (8) in the range from 1096 to 2276.3cm^{-1}

The characteristic vibrational peaks are mainly dominated by the protein constituents of the samples. A vibration band assignment was done with the idea of the group frequencies of the various chromophores present in the samples [11].

The intensities of the vibration bands of amid I, amid II, amid A and amid VI in leukemia samples were increased significantly compared with that of normal sample. The intensity of the band at wavenumber 2800 cm^{-1}, represents CH_2 and CH_3 stretching vibration from fatty acids (lipids), was increased for leukemia samples compared with normal blood. Also CH_2 groups in phase mode of vibration at wavenumber 724 cm^{-1} was more intense for normal blood sample than that in leukemia samples. The wavenumber at 407 cm^{-1}, referred to carbohydrate, was increased in intensity in leukemia samples.

Table (1) The wavenumber and intensities of peaks in the Raman spectra of the normal and three CLL blood samples obtained by Raman spectrometer

Peak No.	Normal blood		CLL Sample No. (1)		CLL Sample No. (4)		CLL Sample No. (8)	
	WN (cm^{-1})	Intensity (a.u)	WN (cm^{-1})	Intensity (a.u)	WN (cm^{-1})	Intensity (a.u)	WN (cm^{-1})	Intensity (a.u)
1	309.3	39.4	305.9	82	309.3	82.3	305.9	79.1
2	391	44	407.9	83.5	407.9	73.4	407.9	76.5
3	482	80	-	-	485.3	92.7	468.5	90.6
4	631.7	45	654.8	89.8	651.5	70.9	-	-
5	723.9	49	720.3	78	753	80.3	739.9	79.1
6	1132.3	41.5	1129.1	81.6	-	-	1126	80.9
7	1460	-	1460	77	1450	82.3	-	-
8	1567.5	43.6	1525.8	81.6	1528.8	81.1	1531.8	81.6
9	1893.7	37.4	1893.7	75.5	1908	75	1902.2	82
10	2007.2	37.8	1993.1	85	2004.4	71.5	2001.6	76.5
11	-	-	2518	81.5	2528.9	74	2531.5	78
12	3164.7	37.5	-	-	3193.9	77.9	3181.8	74.9

Table (2): analysis of Raman spectra for normal and (CLL) blood samples

Peak position (cm^{-1})	Major assignments	Raman shift for normal blood (cm^{-1})	Intensity for normal blood	Raman shift for CLL samples (cm^{-1})	Intensity for CLL samples
407	carbohydrate	-	-	407.9	83.5, 73.4 ,76
628	Amid VI	631.7	45	654.8, 651.5	89.8 , 79.9
720	C-H rocking of › CH_2 – methylene group in lipids	723.6	49	720.3, 753, 739.9	78, 79.1, 80.3
1120	strong C – N vibration bond of Ribose	1132.3	41.5	1129.1, 1126	81.6 , 80
1548	vibration of C-N bond of proteins (Amide II).	1567	43.6	1525.8, 1528.8, 1531.8	81.6, 81.1, 81.6
3200	N - H vibration bond of proteins (Amide A)	3230.4	36.6	3193.9, 3181.8	77.9 , 74.9

The intensity of the band at wavenumber 1140 cm^{-1}, which represents the stretching vibration of C-O bond in amino acid [12], was decreased in all leukemia samples.

Besides that, one can observe a considerable intensity differences in the major Raman signals of leukemia cancer samples, such as proteins band Amid I (1667cm^{-1}) represent the β – turns, (1655 cm^{-1}) α-helical, amid II (1548 cm^{-1}) , amid III (1310-1240 cm^{-1}), N-H amid A (3200cm^{-1}) , amid VI (628 cm^{-1}) , 1002 cm^{-1} attributed to phenyl ring breathing mode, (3700-3500 cm^{-1}) attributed to hydroxyl groups, (835cm^{-1}) CH_2 vibration bond and 1120 cm^{-1} strong C-N vibration mode of ribose characteristic of proteins [13], the lipids – scissoring vibration (1445 cm^{-1}), the C-H stretching vibration of fatty acids at 2921cm^{-1} and C-H rocking of › CH_2 - methylene groups in lipids, the nucleic acids (1499-1310 cm^{-1}) and 779 cm^{-1} CH_2 group [14].

4 – Conclusions

Raman technique provides precise information about the changes in the molecular structure of the blood due to leukemia type CLL. Significant differences in the peaks intensities and peaks shift in the spectra of normal blood and CLL samples were noticed. Raman spectroscopy can be utilized to investigate any molecular changes in the blood constituents so as this technique can be used for diagnosis of CLL.

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