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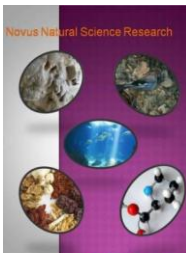

Investigation of the Spectroscopic Changes in the IR Spectra of Lymphocytic and Myeloid Leukemia

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ABSTRACT

The aim of this work was to use IR spectroscopy to investigate the spectroscopic changes in lymphocytic leukemia compared with normal blood. This can lead to suggest the IR spectroscopy as a diagnosis technique for leukemia. Four blood samples were collected from patients in Khartoum city diagnosed by histopathology as Acute lymphocytic leukemia (ALL), Chronic lymphocytic leukemia (CLL), Acute myeloid leukemia (AML) and Chronic myeloid leukemia (CML). The IR spectra of leukemia samples were compared with that of normal blood. Considerable numbers of peaks were recorded in the range between 400 and 4000 cm^{-1}. The IR spectra of the samples showed characteristic peaks in the spectral range between 900 and 3450 cm^{-1}. The locations of these peaks in the spectrum of normal blood sample and the spectra of leukemia blood samples are the same with respect to the positions while significant differences in their intensities were recorded. The comparison of these peaks showed significant differences in the intensities and in the intensity ratios between leukemia samples and normal blood. The results proved that IR spectroscopy can be used for diagnosis of lymphoid leukemia.

KEYWORDS: IR Spectroscopy; Spectroscopy in medical diagnosis; Diagnosis of leukemia, Vibrational spectroscopy.

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INTRODUCTION

A human body, on a simple level, is composed primarily of water, proteins, nucleic acids, lipids and carbohydrates. Changes in the body leading to diseases, such as cancer, are thought to be due to some biochemical changes in one or all of these components. If vibrational spectra are sensitive to the structure of these components, then they too must be changed with the diseased state. Since the pioneering work of Elliott and Ambrose1 in 1950 for proteins and Blout and Fields in 1949 for nucleic acids, infrared (IR) spectroscopy has in fact been shown to be very sensitive to the conformation of these biological building blocks [1].

Based on these facts, infrared 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) [2]. There are many types of leukocytes but the broadest categories are those arising from the lymphoid lineage and those from the myeloid

lineage. Leukemia 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 number of lymphocytes in the blood [3]. Leukemia is not a single disease. In fact, there are four major types: Acute lymphocytic leukemia (ALL), Chronic lymphocytic leukemia (CLL), Acute myeloid leukemia (AML) and Chronic myeloid leukemia (CML). This leukemia's differ in the age of onset, causes and risk factors, treatment, and the likelihood of survival [4,5].

Although all bio-molecules (proteins, nucleic acids, lipids and carbohydrates) are important, the nucleic acids RNA and DNA are especially important because they carry within their structure the hereditary information that determines the identity and structure of proteins. Each protein, unique in its structure and hence in its function, participates in the processes that characterize the individuality of the cell [1]. In the vibrational spectra, the amide bonds of proteins form so-called chromophores that give rise to nine strong characteristic bands that are named amide A, amide B and amides I–VII. Among these bands, amide I, which is due mostly to the C=O stretching vibrations of the peptide backbone, is by far the best characterized [6]. Lipids are critical to all biological media by forming the cell walls that keep biological media organized in their necessary compartments. Lipids of various kinds also help regulate the flow of needed biological molecules from one side of a lipid barrier to the other, often assisted by imbedded proteins that form the passages for these molecules called channels. The major absorption IR bands of a typical lipid are at 1738, 1465, 1255, 1168, 1095 and 1057, and 968 cm^{-1} [7]. For carbohydrates, the most common are sugars or saccharides. Sugars are present in biological media primarily as hexose sugars, such as glucose, where they are an immediate energy source. Pentose sugars are also present, mainly as the ribose component of the nucleic acid backbone of DNA and RNA, both as the component monomers and the much longer polymers, as well as in the energy-transducing oligomeric pieces. Polysaccharides in the body are found either in a free state or combined with proteins, in a complex known as glycoproteins. The only polysaccharide in the body that is not bound to a protein is glycogen [7].

The aim of this work was to investigate the changes in the structure of the most important chromophores of blood via the investigation of the spectroscopic changes in four types of leukemia using IR spectroscopy which may be, then, can be used as a diagnostic method for leukemia.

MATERIALS AND METHODS

The equipments

In this work, FTIR spectrometer model 8400S supplied from Shimadzu (Japan) was used to record the IR spectra of the samples**.**

The FTIR - 8400S has very smooth and precise moving mirror unit and dynamic alignment system to optimize and stabilize the interferometer unit. This system monitors the interferometer conditions using He-Ne laser and compares it with that of optimized conditions. Any detected discrepancies can be corrected by automatic alignment of the piezo actuators at the fixed mirror.

The samples

Four blood samples had been collected from leukemia patients, with different ages, diagnosed with CML, ALL, CLL and AML leukemia in histopathology lab of two hospitals; Alamal

hospital and Radiation and Isotopes Center - Khartoum (RICK). No sample preparation was required. Normal blood sample was used for comparison with the patients' samples.

The experimental procedure

Each blood sample was put it in the sample holder (KBr) of the FTIR spectrometer using automatic pepate of 100 ml. The spectra of the normal blood and the leukemia samples were recorded in the range between 400 and 4000 cm $^{-1}$ by computer connected with the spectrometer. The spectra were analyzed and compared.

RESULTS AND DISCUSSION

Figure (1) shows the IR spectrum of the normal blood sample, while the spectra of leukemia samples, obtained by the same spectrometer, are shown in figures (2) to (5). The wavenumbers and the intensities of the peaks in the spectra are listed in table (1).

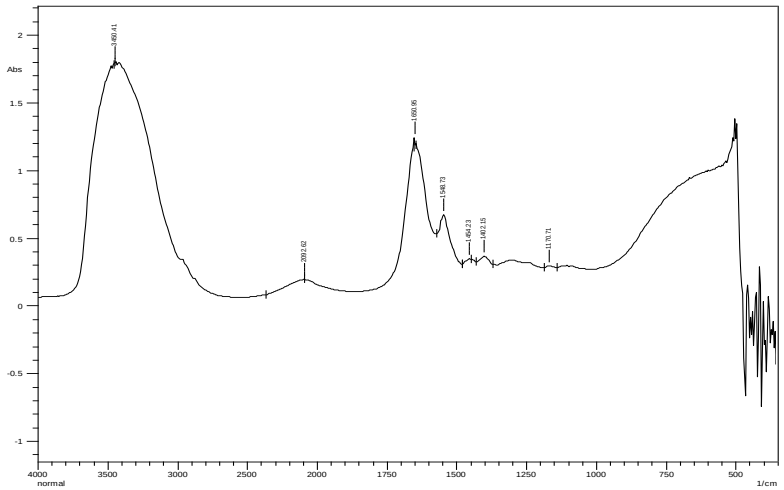

Figure: 1 IR spectrum of normal blood sample

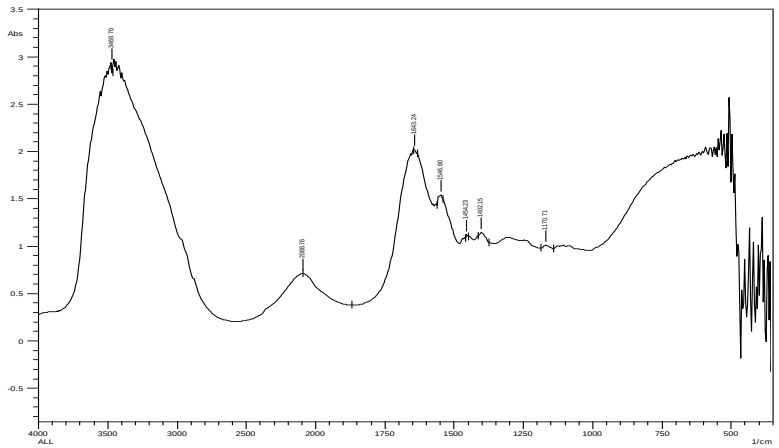

Figure: 2 IR spectrum of acute lymphocytic leukemia (ALL) blood sample

A vibration band assignment for the peaks in the spectra was done with the idea of the group frequencies present in the samples. The mid IR region (4000 - 400 cm^{-1}) can be further broken down into regions, the region 3700 - 3500 cm^{-1} represents hydroxyl groups, the region 3300 – 3200 cm^{-1} represents N-H in protein (Amide A). There are also other peaks of biological interest such as CH_2 and CH_3 stretching vibrations in fatty acids with (3050 - 2800 cm^{-1}), C = O, NH and C-N in proteins and peptides with (1750 - 1500 cm^{-1}) and C - C, C - O - C in polysaccharides with (1200 - 900 cm^{-1}) [8].

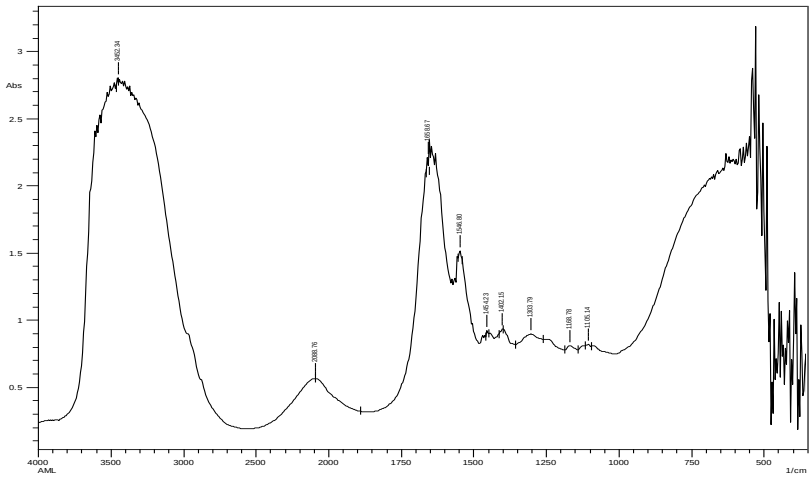

Figure: 3 IR spectrum of acute myeloid leukemia (AML) blood sample

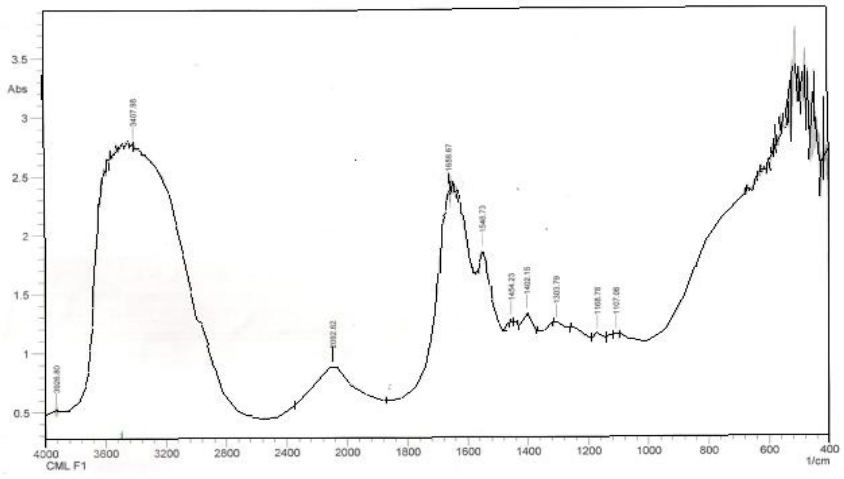

Figure: 4 IR spectrum of chronic myeloid leukemia (CML) blood sample

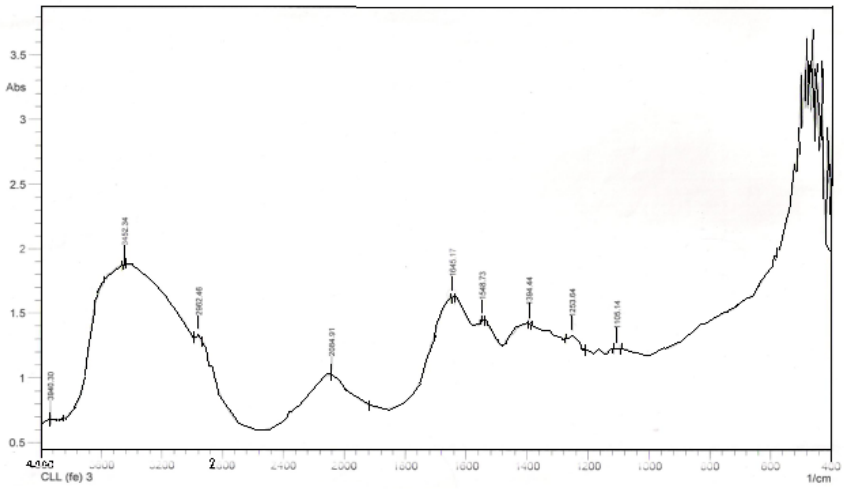

Figure: 5 IR spectrum of chronic lymphocytic leukemia (CLL) blood sample

The region at 1174-1000 cm^{-1} is assigned to strong stretching vibrations of the PO_2 and C – O groups of the phosphodiester deoxyribose (protein) structure, while 1499 - 1310 cm^{-1} is assigned to weak NH vibrations and CH in plane deformations of nucleic acids [9]. The spectral region 3600-3000 cm^{-1} comprises of C-H, O-H and N-H bond stretching vibrations of the

proteins. The prominent peak at 3200 cm^{-1} is due to the N-H stretching mode (Amide A) of proteins [10].

Table: 1 The wave numbers and intensities of peaks in the IR spectra of all samples

Peak no.	Normal blood		CML sample		CLL sample		AML sample		ALL sample	
	WN (cm^{-1})	Intensity (a.u)	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	3450.41	1.80	3450.34	2.78	3452.34	1.88	3452.34	2.806	3469.70	2.93
2	2092.62	0.20	2092.62	0.86	2084.91	1.34	2088.76	0.565	2088.76	0.71
3	1650.95	1.25	1658.67	2.42	1645.17	1.05	1658.67	2.212	1643.24	2.03
4	1547.38	0.67	1548.73	1.88	1548.73	1.66	1546.80	1.515	1546.80	1.54
5	1454.23	0.35	1454.23	1.28	1454.23	1.48	1454.23	0.925	1454.23	1.13
6	1402.15	0.37	1402.15	1.33	1394.44	1.44	1402.15	0.938	1402.15	1.14
7	1303.79	0.25	1303.79	1.27	1303.64	1.41	1303.79	0.898	1303.79	1.09
8	1170.71	0.30	1168.78	1.19	1168.78	1.18	1168.78	0.811	1170.71	1.01
9	1105.14	0.19	1107.06	1.185	1105.14	1.37	1105.14	0.819	1105.14	0.925

In the FTIR spectra of the samples, it can be observed that the peak at 3450.41 cm^{-1}, referred to O-H stretching vibrations of hydroxyl group in the proteins, has an intensity of 1.80 in normal blood. This band is located at 3452.34, 3452.34 and 3469.70 cm^{-1} for AML, ALL and CLL samples with intensities of 1.88, 2.806 and 2.93, respectively. The intensities of these peaks in leukemia samples are increased 50% or more than that obtained for normal blood sample.

The peak at 2092 cm^{-1} is attributed to C – H asymmetric stretching vibrations bond of CH_2 methylene in fatty acids [11]. For normal blood sample this peak is located at 2092.62 cm^{-1} with intensity of 0.20, and can be observed at 2092.62 and 2092.64 cm^{-1} for CML and CLL samples with intensities of 0.86 and 1.34, respectively, while for ALL and AML samples this band is observed at 2088.76 cm^{-1} with intensities of 0.71 and 0.565.

The peak at 1650 cm^{-1} represents amide I of α – helical structure of proteins [12]. For normal blood this peak is located at 1650.95 cm^{-1} with intensity of 1.25 while for leukemia samples this peak is located at 1658.67 cm^{-1} for CML and AML samples with intensities of 2.42 and 2.212. This peak is located at 1645.1 and 1643.24 cm^{-1} in CLL and ALL spectra with intensities of 1.05 and 2.03, respectively.

The band at 1548 cm^{-1} is referred to the vibration of C – N bond in Amide II [13]. For normal blood this band is located at 1547.38 cm^{-1} with intensity of 0.67, and for CML and CLL samples this peak is observed at 1548.73 cm^{-1} with intensities of 1.88 and 1.66, while for AML and ALL sample it is observed at 1456.80 cm^{-1} with intensities of 1.515 and 1.54, respectively. The increment in the intensity was very significant.

A peak at 1450.23 cm^{-1} with intensity of 0.35 is observed in normal blood spectrum. For CML, ALL, and AML samples this peak is located at 1454.23 cm^{-1} with intensities of 1.33, 0.938 and 1.14, while for CLL sample it is located at 1394.44 cm^{-1} with intensity of 1.44. This peak represents the asymmetric bending vibration of CH_3 in lipids [12].

The bands at wavenumbers 1310-1240 cm^{-1} are referred to amide III bond of protein [11]. In the spectra of the samples, it can be noticed that there is a peak at 1303.79 cm^{-1} in the spectrum

of normal blood with intensity of 0.25, while for CML, ALL, AML samples the intensities are 1.27, 0.898 and 1.09, respectively.

The bands seen at 1200-900 cm^{-1} are referred to C – O – C, C - O dominated by the ring vibration of polysaccharide (carbohydrates) [14]. For normal blood there is a peak located at 1170.7 cm^{-1} with intensity of 0.30. This peak is located at 1168.78 cm^{-1} for CML, CLL and AML samples with intensities of 1.19, 1.18 and 0.811, respectively, while for ALL sample it is located at 1170.71 cm^{-1} with intensity of 1.01.

The comparison of peaks intensities in normal blood sample and CML, CLL, AML and ALL samples is shown in figure (6) below.

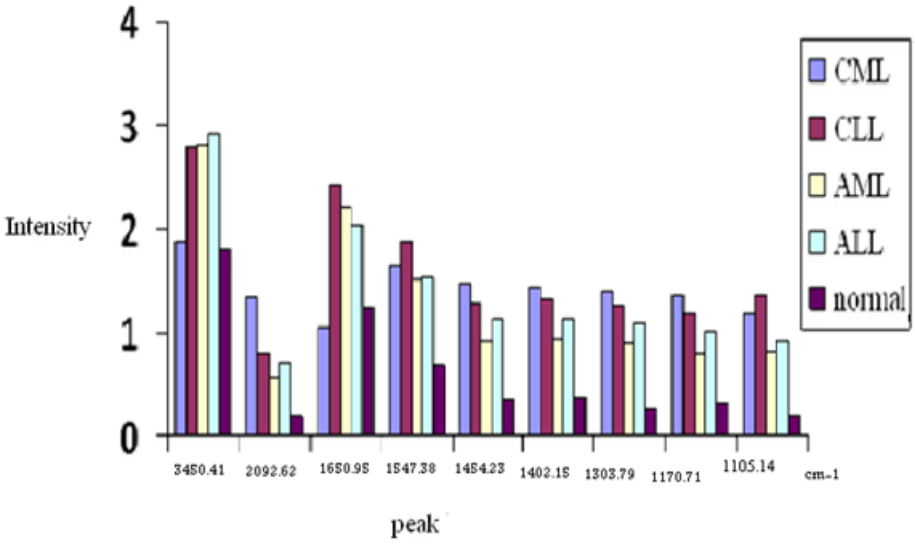

Figure: 6 A comparison of intensities between normal blood sample and leukemia samples

The intensities of the peaks related to leukemia samples are increased significantly compared with that of normal blood sample. The increment of intensities for AML, ALL, CML and CLL samples is referred to the increase in the amount of immature cell of white blood cell (blasts).

In order to compare the results further, four intensity ratios: R_1 (I_{2092}/I_{3450}) for lipids, R_2 (I_{1550}/I_{1650}) for protein, R_3 (I_{1402}/I_{1454}) and R_4 (I_{1170}/I_{1303}) for nucleic acids are calculated and compared with that of normal blood sample. The result of the comparison is listed in table (2).

Table: 2 A comparison between normal blood sample and the four leukemia samples using intensity ratios

Ratio	Normal blood	CML	CLL	AML	ALL
$R_1(I_{2091}/I_{3450})$	0.11	0.309	0.712	0.2	0.24
$R_2(I_{1548}/I_{1650})$	0.536	0.776	0.684	0.746	0.758
$R_3(I_{1402}/I_{1454})$	1.057	1.039	0.97	1.014	1.008
$R_4(I_{1170}/I_{1303})$	1.2	0.937	0.836	0.903	0.926

The (I_{2091}/I_{3450}) for lipids and (I_{1548}/I_{1650}) for proteins ratios were increased significantly for CML, AML, ALL and CLL compared with that of normal sample, while the ratios (I_{1402}/I_{1454}) and (I_{1170}/I_{1303}) for nucleic acids, were decreased in leukemia samples compared with normal sample. The increment in intensity ratios in the leukemia spectra indicates a local higher protein concentration in the leukemia cells [15].

One of the more prominent changes that occur with cancer is the increment in cellular nucleic content [16].

CONCLUSION

The spectra recorded by FTIR spectrometer showed characteristic peaks at 900 -1200, 1240-1310, 1400 - 1455, 1548, 1650, 2091 and 3450 cm^{-1}.

These peaks are the characteristics of various group frequencies. The locations of these peaks in the spectrum of normal blood sample and the spectra of leukemia blood samples are the same with respect to the positions while significant differences in their intensities were recorded.

The intensity increasing means deformation of some groups of vibration modes because the molecular structure of blood is changed duo to the increment of white blood cells.

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