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FTIR Spectral Investigation on Healthy and Cancerous Blood Samples-Acute Lymphocytic Leukemia (ALL) Coupled with Statistical Analysis

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ABSTRACT

The role of Fourier Transform Infra-Red (FTIR) spectroscopy in the clinical analysis has increased tremendously in the recent past, due to the development of sophisticated instruments and efficient data evaluation software. The application of FTIR spectroscopy in the analysis of blood is highly promising. This technique has many advantages over the regular clinical analysis like very minimum sample requirement, avoid of costly disposables, minimum manpower requirement etc. Blood is the preferred indicator of the pathophysiological condition of a living system. Cancer is a dreadful disease and continuous research is going on for the early detection, treatment and monitoring. Cancer in general is associated with the proliferation of the abnormal cells without control. Out of the many forms of cancer, leukemia is cancer of the blood or bone marrow characterized by an abnormal increase of white blood cells, which are also referred to as leukocytes. Leukemia is clinically and pathologically split into its acute and chronic form, and is categorized into four major types namely Acute Lymphocytic Leukemia (ALL), Acute Myelogenous Leukemia (AML), Chronic Lymphocytic Leukemia (CLL) and Chronic Myelogenous Leukemia (CML). ALL is a cancer of the white blood cells that normally fight infections. ALL accounts for the majority of the childhood leukemia that affects the young children between 3 and 12 years. ALL may also occur in adults. Studies indicate that leukemia is not inherited or contagious. Several factors are suspected, although scientists are unable to pinpoint the exact cause. No specific sets of preventions are available. The present study aims to characterize the blood samples of ALL patients with the healthy subjects using Fourier Transform Infra-Red (FTIR) spectroscopic technique. The FTIR spectra of ALL and healthy blood samples have been recorded. It is observed that the general shape of the spectra is similar but there is considerable change in the absorption of the characteristic peaks. As a measure to characterize the healthy and leukemia blood, the intensity ratio calculation have been carried out among some of the specific absorption peaks for both healthy and diseased samples. It is observed that the values are different in both the samples. Statistical analysis is performed to find whether the absorption ratios differ in the healthy and diseased groups employing Analysis of Variance (ANOVA) and independent t-test. Thus the role of FTIR spectroscopy in the clinical analysis of blood samples has been established both qualitatively and quantitatively.

Keywords: FTIR, Statistical analysis, ALL, ANOVA

INTRODUCTION

Leukemia is a cancer of blood and bone marrow and is characterized by an abnormal proliferation of white blood cells. Leukemia can be either acute or chronic. Acute leukemia usually grows quickly. Chronic leukemia usually grows slowly. Leukemia can also involve two different types of white blood cells. These are lymphoid cells and myeloid cells. For this reason, all of the different forms of leukemia are categorized into four major types namely Acute Lymphocytic Leukemia (ALL), Acute Myelogenous Leukemia (AML), Chronic Lymphocytic Leukemia (CLL) and Chronic Myelogenous Leukemia (CML).

ALL is the most common type of leukemia. It is common in young children but can also be seen in old people. CLL is usually seen in people over the age of 55. It almost never affects children. AML occurs more commonly in adults, than in children. CML is found in people between 20 to 80 years. It occurs more commonly in adults. ALL is the most common malignancy diagnosed in children, representing nearly one third of all pediatric cancers. The annual incidence rate for ALL is 30.9 cases per million populations. ALL is a major type of childhood leukemia with varying incidences in different countries from 0.9-4.7 per 100,000 children [1]. Radiation exposure, environmental agents, maternal alcohol consumption, paternal smoking, etc. are associated with increased risk of ALL in children [2]. ALL is a colonel hematological disorder arising due to genetic change in hemopoietic cells [3].

The role of Fourier Transform Infra-Red (FTIR) spectroscopy in the clinical analysis has increased tremendously in the recent past, due to the development of sophisticated instruments and efficient data evaluation software.

FTIR spectroscopy is a non-invasive, reagent free, diagnostic tool and it possesses several advantages over the regular clinical methods, like very small amounts of sample requirement, high sensitivity, produces instantaneous, accurate and precise results, reliability of its measurement and avoidance of costly disposables and minimum manpower requirement. FTIR spectroscopy has been used by scientists as a powerful tool in studying the molecular structure of compounds [4]. It has been applied in biology for studying the structure and conformation of molecules like proteins, nucleic acids and lipids [5-7]. The mid infrared region has been shown to be useful in the identification of disease patterns of human sera [8]. Precise quantification of serum components, such as glucose, total protein, cholesterol, urea etc., has been achieved using mid IR spectroscopy [9]. The main aim of this study is to determine the spectral variation of the cancer affected blood samples with healthy human blood samples. The FTIR spectrum of a blood sample exhibits characteristic absorption peaks due to the specific functional groups which are present in the sample. It is observed that the positions of the peaks are the same in both the samples but the amount of absorptions are different thus giving qualitative analysis. The spectral variations are analyzed quantitatively by finding the intensity ratios among some of the specific absorption peaks. It is observed that the values are significantly different in the normal and the cancer samples. The results are further validated with statistical analysis by applying the independent t-test and ANOVA calculations, which indicate that the spectral variations are statistically significant. Thus, the veracity of FTIR spectroscopy in the clinical analysis of blood could be established.

MATERIALS AND METHODS

The present study aims at the FTIR spectral investigation on healthy and cancer affected blood samples. In the present work, blood samples from 20 children affected with ALL and healthy children are employed for the spectral analysis. 2 ml of blood of each individual has been collected. The blood sample is centrifuged and the serum is separated and is used for spectral recordings. Healthy blood samples from children below age 12 and ALL samples between age 3 and 12 years are collected.

RESULTS AND DISCUSSION

The FTIR spectra of the samples had been recorded at Sophisticated Analytical Instrumentation Facility (SAIF), Indian Institute of Technology (IIT), Chennai-36, using Spectrum-One Perkin- Elmer FTIR Spectrophotometer. The spectra are recorded in the mid infrared region of 4000-400 cm^{-1} in the absorption mode. The FTIR spectra are obtained by spreading a small volume of serum on a thallium bromide cell (IR transparent material) and allowed to dry for few minutes to remove the water content [10]. The dried serum forms a thin uniform film on the cell [11]. Infrared transparent thallium bromide cell without the sample has been scanned as background for each spectrum and 16 scans were co-added at a spectra resolution of ± 1 cm^{-1}. As the spectra are baseline corrected and they are normalized to acquire identical area under the curves.
A satisfactory vibrational band assignment of the absorption bands of the spectra has been done with the idea of the group frequency of the various constituents of the blood samples [12]. Table 1 presents a satisfactory vibrational band assignment of blood samples.

The vibration band at 3296 cm^{-1} is due to the N-H stretching vibration of the secondary amides of protein. The asymmetric and symmetric stretching vibrations of the methyl group of proteins and lipids are found to be present at 2960 and 2874 cm^{-1} respectively. The other two vibration bands in C-H stretching region are found to be present near 2934 and 2851 cm^{-1}, which are due to an asymmetric and symmetric stretching vibration of the methylene group. The strong absorption and present at 1660 cm^{-1} is attributed to C=O stretching of amide I of the proteins [13]. In the same way the presence of the band at 1554 cm^{-1} is due to the amide II NH bending vibration that are strongly coupled to the CN stretching vibrations of the protein amide groups. The peaks at 1457 and 1315 cm^{-1} are considered to be due to the asymmetric and symmetric deformations of the methyl group of proteins. The peak at 1398 cm^{-1} may also considered due to COO^- stretching of ionized amino acid chains, suggesting an increased contribution from carboxalate. The lipid phosphate band due to the asymmetric PO_2 stretching vibration is found to occur at 1240 cm^{-1}. The spectral region 1169-1081 cm^{-1} is predominantly occupied by the C-O stretching vibrations of glucose. The absorption peaks present at 1169, 1153, 1107, 1079 and 1035 cm^{-1} are considered to be due to the different C-O stretching vibrations of C-O-H and C-O-C bonds. The weak absorption band at 955 cm^{-1} is considered to be due to PO_2 symmetric stretching of the phosphate bond of proteins. The medium strong vibration bond present at 625 cm^{-1} is assigned as N-H out-of-plane bending with the contribution of C-N torsional vibrations.

Table 1: Infrared vibrational band assignment of blood sample

Wavenumber (cm^{-1})	Vibrational band assignment
3295	N-H stretching of secondary amides of protein: Amide A
2960	CH_3 asymmetric stretching of proteins and lipids
2934	CH_2/CH stretching
2874	CH_3 symmetric stretching of proteins and lipids
2851	CH_2/CH stretching
1660	C=O stretching coupled with C-N stretching and NH deformation-amide I
1554	NH deformation strongly coupled with C-N stretching amide II
1457	CH_3 asymmetric deformation
1398	CH_3 asymmetric deformation COO^- stretching of amino acids
1315	CH_3 symmetric deformation
1240	PO_2 asymmetric stretching of lipid phosphates
1169	C-O stretching
1128	C-O stretching
1081	C-O stretching
955	PO_2 symmetric stretching of lipid phosphates
699	NH asymmetric deformation coupled with CH_2 rocking amide V
625	O=C-N deformation coupled with other ring deformation amide IV

The infrared spectrum thus provides various useful information of a bio molecule like structure, functional groups, types of bonds and its interactions. Figure 1 represents the FTIR spectrum of a healthy blood sample. The FTIR spectra of ALL blood samples are recorded in the same way (Figure 2). It is observed that the FTIR spectrum exhibits vibrational bands characteristics of the various group frequencies. Represents the average FTIR spectrum of healthy and ALL blood samples superimposed on each other. The spectrum of a healthy blood sample and the diseased blood samples are the same with respect to the positions of the peaks but different in terms of the absorption levels of the peaks. The amount of absorption is decreased in ALL blood samples than that of healthy ones. The difference in the absorption of the healthy and ALL samples characterize the samples qualitatively.

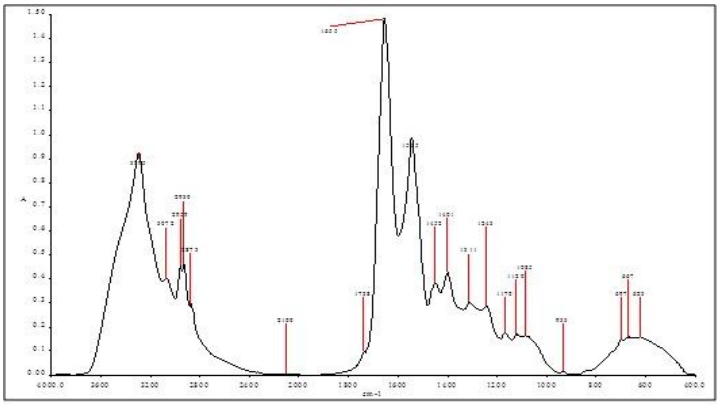

Figure 1: FTIR spectrum of normal blood sample

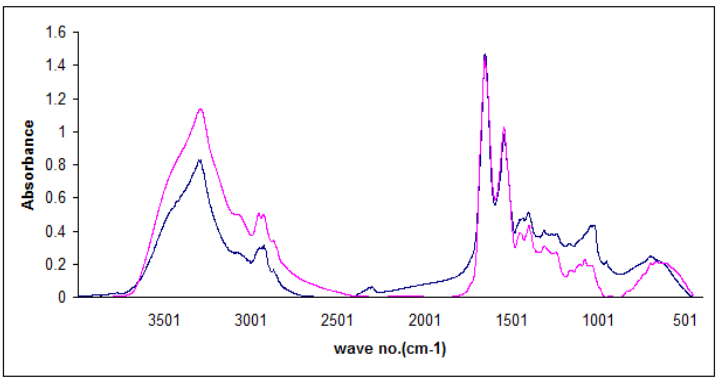

Figure 2: FTIR spectrum of healthy children and ALL blood samples
Healthy sample; ALL sample

In order to quantify the results further, intensity ratio calculation among some of the specific absorption peaks of the sample is employed [14]. Seven intensity ratio parameters i.e., $A_{3295}/_{2960}$, $A_{2960}/_{1655}$, $A_{1655}/_{1546}$, $A_{1546}/_{1452}$, $A_{1452}/_{1403}$, $A_{1317}/_{1081}$ and $A_{1081}/_{700}$ are calculated respectively among the prominent absorption peaks. It is observed that these values are decreased in ALL samples than that of the healthy ones. Tables 2 and 3 shows the intensity ratio calculations of health Children and ALL blood samples. Thus it is observed that both individual spectral absorption of the peaks and also intensity ratios are decreased in ALL samples compared to that of healthy ones.

Table 2: Intensity ratio calculations of the specific modes of vibration of healthy children blood sample

Sample	$A_{3295}/_{2960}$	$A_{2960}/_{1655}$	$A_{1655}/_{1546}$	$A_{1546}/_{1452}$	$A_{1452}/_{1403}$	$A_{1317}/_{1081}$	$A_{1081}/_{700}$
1	2.7998	0.2475	1.5955	3.2002	0.759	1.6017	0.8539
2	2.8147	0.2476	1.5753	3.2928	0.7732	1.6927	0.7483
3	2.8825	0.2657	1.5695	3.1511	0.7772	1.6696	0.7015
4	2.5762	0.2849	1.5642	3.1512	0.7486	1.6901	0.7019
5	2.7291	0.2417	1.5735	3.1797	0.7771	1.6481	0.8413
6	2.3689	0.2027	1.5049	3.1501	0.7897	1.6184	0.7912
7	2.7831	0.2901	1.5093	3.0217	0.7754	1.6014	0.7071
8	2.6528	0.2463	1.5201	3.0102	0.7041	1.6409	0.8473
9	2.6456	0.2456	1.5043	3.0142	0.7564	1.6404	0.7018
10	2.4455	0.2356	1.5302	3.0145	0.7546	1.6489	0.7145
11	2.6574	0.2456	1.5467	3.1543	0.7345	1.6245	0.7476
12	2.5643	0.2797	1.5345	3.2001	0.7543	1.6785	0.7123
13	2.4655	0.2346	1.5666	3.1256	0.7523	1.6678	0.8785
14	2.4622	0.2567	1.5876	3.1876	0.6945	1.6986	0.7348
15	2.6111	0.2668	1.5342	3.1678	0.7342	1.6245	0.7541
16	2.6456	0.2567	1.5423	3.2546	0.7456	1.6456	0.8545
17	2.7011	0.2685	1.5564	3.1785	0.7123	1.6023	0.7413
18	2.5432	0.2435	1.5575	3.1645	0.7894	1.6543	0.7015
19	2.6781	0.2634	1.5432	3.1456	0.7342	1.6416	0.7076
20	2.4673	0.2533	1.5546	3.1345	0.7456	1.6461	0.8136
Average	2.4637	0.2581	1.5574	2.6362	0.9400	1.5645	1.2633

Table 3: Intensity ratio calculations of the specific modes of vibration of ALL blood sample

Sample	$A_{3295}/_{2960}$	$A_{2960}/_{1655}$	$A_{1655}/_{1546}$	$A_{1546}/_{1452}$	$A_{1452}/_{1403}$	$A_{1317}/_{1081}$	$A_{1081}/_{700}$
1	2.0142	0.3167	1.4461	1.5489	1.5408	1.6869	1.5566
2	2.1048	0.3407	1.4209	1.6309	1.6809	1.0643	1.6346
3	2.1532	0.3513	1.4675	1.899	1.6982	3.9426	1.6362
4	2.2377	0.2715	1.4106	1.9115	1.2829	2.9426	1.7663
5	2.0366	0.2337	1.4141	2.4408	1.4541	1.9173	1.5378
6	2.2489	0.2781	1.5776	2.2482	1.4654	2.2898	1.6904
7	2.0304	0.2751	1.4249	2.284	1.4579	2.014	1.6057
8	2.0012	0.2964	1.4324	2.2282	1.4776	2.001	1.5202
9	2.2599	0.3421	1.5464	1.5517	1.2775	1.3041	1.5429
10	2.4366	0.2327	1.4541	2.4408	1.4536	1.9173	1.5368
11	2.1345	0.2435	1.4567	1.8245	1.4082	1.6869	1.6566
12	2.1208	0.2567	1.4204	1.6309	1.3609	1.0643	1.5946
13	2.1578	0.2398	1.4677	1.8956	1.4782	3.9426	1.6062
14	2.2001	0.2467	1.4113	1.9267	1.4329	2.9426	1.6163
15	2.0956	0.2411	1.4134	2.1456	1.4548	1.9173	1.6378
16	2.2078	0.2556	1.4655	2.4567	1.4654	2.2898	1.6678
17	2.0603	0.2457	1.4134	2.3456	1.4829	2.2898	1.6004
18	2.0897	0.2333	1.4123	2.2841	1.4641	2.0141	1.6057
19	2.2512	0.2378	1.5011	2.2282	1.4654	2.0013	1.6202
20	2.1687	0.2751	1.4249	1.5517	1.4779	1.3041	1.5599
Average	2.1505	0.2406	1.4490	2.0236	1.4639	2.1266	1.6096

Statistics is the study of the collection, analysis, interpretation, presentation, and organization of data. The two statistical methods employed in this study are Analysis of Variance (ANOVA) and independent t-test. In statistics, ANOVA is a collection of statistical models used in order to analyze the differences among group means and their associated procedures and the independent sample (or two-sample) t-test is used to compare the means of two independent samples [15]. The statistical method used for the validation of the FTIR spectroscopic analysis is the Independent Sample t- test by using the SPSS software package. It is an inferential statistical test that determines whether there is a statistically significant difference between the means in two unrelated variables. The Independent sample t- test is first employed between the healthy and diseased samples. Table 4 describes the summary of the statistics (minimum, median, maximum, average, above average and below average) describing the amount of variation present in the sample.

Table 4: Statistics of Healthy Children (HC) and ALL samples

Five number Summary	HC-R1	ALL-R1	HC-R2	ALL-R2	HC-R3	ALL-R3	HC-R4	ALL-R4	HC-R5	ALL-R5	HCR-6	ALL-R6	HCR-7	ALL-R7
Minimum	2.3689	2.0012	0.2027	0.2327	1.5043	1.4106	3.0102	1.5489	0.6945	1.2775	1.6014	1.0643	0.702	1.5202
Median	2.6456	2.14385	0.2505	0.2562	1.5507	1.4287	3.1528	2.0362	0.7533	1.4648	1.6459	2.0012	0.744	1.606
Maximum	2.8825	2.4366	0.2901	0.3513	1.5955	1.5776	3.2928	2.4567	0.7897	1.6982	1.6986	3.9426	0.879	1.7663
Average	2.6247	2.1505	0.2538	0.2707	1.5485	1.4491	3.1449	2.0237	0.7506	1.464	1.6468	2.1266	0.763	1.6097
Above Average	11	11	9	9	10	8	14	10	11	11	9	7	7	9
Below Average	9	9	11	11	10	12	6	10	9	9	11	13	13	11

From Table 4, from the above and below average category, it is observed that in ratios HC-R1, HC-R5 and ALL-R1, ALL-R5 of the healthy and ALL samples, the observations are 55% and 45% respectively, similarly it is 45% and 55% for HC-R2 and ALL-R2 samples. The maximum of observations in above average is 70% in HC-R4 and 55% in ALL-R1 and ALL-R5, similarly the maximum of observations below average is 65% in HC-R7 and ALL-R6. In the healthy group, the minimum values range from 0.2027 (HC-R2) to 3.0102 (HC-R4) to 0.2327 (ALL-R2) and 2.0012 (ALL-R1) in ALL groups. One the other end the maximum values ranges from 0.2901 (HC-R2) to 3.2928 (HC-R4) and 0.3513 (ALL-R2) to 3.9426 (ALL-R6) for healthy and ALL groups. Table 5 represents the independent t-test for normal and ALL blood samples. It also represents whether significant difference exist between means of healthy and diseased groups at 7 different levels of intensity ratios. It can be concluded that between healthy and ALL sample, except in R2, there exist a mean level difference in all other groups.

Table 5: Independent t-test

Variables	R1		R2		R 3		R 4		R 5		R 6		R 7	
	N	t-statistics (p-value)	N	t-statistics (p-value)	N	t-statistics (p-value)	N	t-statistics (p-value)	N	t-statistics (p-value)	N	t-statistics (p-value)	N	t-statistics (p-value)
HC	20	12.12 (0.00)	20	-1.73 (0.09)	20	8.33 (0.00)	20	15.05 (0.00)	20	-30.74 (0.00)	20	-2.68 (0.01)	20	-43.81 (0.00)

CONCLUSION

FTIR spectroscopy has been employed as a means to distinguish ALL with the healthy samples. In the present work, spectral changes that occurred in the WBC of an ALL patient and their possible utilization for monitoring biochemistry of WBC are investigated. Characteristic band alterations are identified in both healthy and diseased samples. Similarly Intensity ratio calculations among specific bands are calculated. There are significant fluctuations in the ratios under investigation which can be attributed to the changes in the bio molecular structure between healthy and leukemic samples. These parameters may be used as possible markers to indicate and suggest that FTIR-spectroscopy may provide a rapid optical method for continuous monitoring or evaluation of WBC population. The spectral results are validated by applying statistical calculations, namely the independent t-test. The independent t- test is able to significant ratio level for the healthy and diseased samples for a given spectral peak in provide a better the entire frequency region of 4000-400 cm^{-1}. Thus, in the present work, it has been demonstrated that the independent t- test combined with FTIR spectroscopy can be efficiently applied for the analysis of healthy and leukemic samples.

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