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Distinction of leukemia patients' and healthy persons' serum using FTIR spectroscopy

Daping Sheng [a,c], Xingcun Liu [c,*], Weizu Li [b], Yuchan Wang [b], Xianliang Chen [b], Xin Wang [b,*]

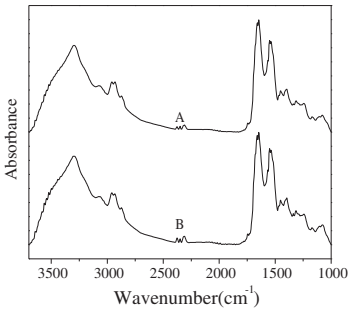
[a] National Synchrotron Radiation Laboratory, University of Science and Technology of China, Hefei, Anhui 230029, China
[b] School of Basic Medicine, Anhui Medical University, Hefei, Anhui 230032, China
[c] The First Affiliated Hospital, Anhui Medical University, Hefei, Anhui 230032, China

HIGHLIGHTS

- Leukemia patients' and healthy persons' serum was studied by FTIR spectroscopy.
- IR spectra of serum were investigated.
- H1075/H1542, H1045/H1467, H2959/H2931 ratios of the above samples were compared.
- Curve fitting was used to study differences of the above serum.
- FTIR spectroscopy can indentify leukemia patients' and healthy persons' serum.

GRAPHICAL ABSTRACT

FTIR spectroscopy was used to distinguish leukemia patients' serum (A) from healthy persons' serum (B).



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ABSTRACT

In this paper, FTIR spectroscopy was applied to compare the serum from leukemia patients with the serum from healthy persons. IR spectra of leukemia patients' serum were similar with IR spectra of healthy persons' serum, and they were all made up of proteins, lipids and nucleic acids, etc. In order to identify leukemia patients' serum and healthy persons' serum, the H1075/H1542, H1045/H1467, H2959/H2931 ratios were measured. The H2959/H2931 ratio had the highest significant difference among these ratios and might be a useful factor for identifying leukemia patients' serum and healthy persons' serum. Furthermore, from curve fitting, the RNA/DNA (A1115/A1028) ratios were observed to be lower in leukemia patients' serum than those in healthy persons' serum. The results indicated FTIR spectroscopic study of serum might be a useful tool in the field of leukemia research and diagnosis.

Introduction

Leukemia is a malignant tumor originating from malignant proliferation of hematopoietic stem or progenitor cells in peripheral blood, marrow, etc. Leukemia is the most commonly hematological malignancy, and its mortality in children and persons under 35 is highest among all tumors [1]. Therefore the early diagnosis and treatment are of great importance to increase the patients' survival rate.

There are several commonly used methods for leukemia diagnosis [2]: (1) The peripheral blood examination; (2) The bone marrow aspiration; (3) The French–American–British classification; (4) The routine cytochemical evaluation. But the above methods have several disadvantages such as depending largely on pathologists'

Abbreviations: FTIR, Fourier transform infrared; IR, infrared; AML, acute myelocytic leukemia; CML, chronic myelocytic leukemia; ALL, acute lymphoblastic leukemia; M, male; F, female.

* Corresponding authors. Tel.: +86 551 5161138; fax: +86 551 5161142 (X. Wang), tel.: +86 551 2923764; fax: +86 551 3633742 (X. Liu).
E-mail addresses: xcliu1969@tom.com (X. Liu), wxchem81@tom.com (X. Wang).

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judgments, complicated procedures and expensive instruments. Hence it is very urgent to develop a simple and rapid method for diagnosing leukemia at the early stage.

As FTIR spectroscopy can reveal biochemical changes in diseased tissues and cells [3,4], it has been used to study gastric cancer [3], cervical cancer [5], esophageal cancer [6], gallbladder cancer [7] and so on [8] in recent years. It has also been applied to leukemia research [9,10]. For example, white blood cells from an acute myeloid leukemia patient were investigated by FTIR spectroscopy and phosphate/lipid–protein ratio was found to decrease after the treatment [9]. FTIR spectroscopy was used to compare leukemia patients' lymphocytes with healthy persons' lymphocytes, and it could provide adequate information for differentiating the cells' state [11]. But the samples in these studies were mainly blood cells, and it was complicated to separate them from blood.

Compared with blood cells, serum is much easier to obtain and can reflect human beings' pathological changes [12,13]. FTIR spectroscopy was used to study human beings' serum, and the result showed that the contents of proteins, lipids and nucleic acids were different between the serum of healthy persons and cancer patients [14]. So FTIR spectroscopic examination of serum may be a potential tool for leukemia diagnosis. In this paper, FTIR spectroscopy was used to compare leukemia patients' serum with healthy persons' serum. The aim of this work is to investigate the possibility of distinguishing leukemia patients from healthy persons using serum's IR spectra.

Materials and methods

Materials

The blood samples of 30 leukemia patients (definite diagnosis and before treatment) were gained from the first affiliated hospital of Anhui Medical University, together with blood samples from 19 healthy volunteers (Table 1), they were centrifuged at 3000 r/min immediately and the serum was obtained. Moreover, the additional 10 serum samples (including six leukemia patients and four healthy persons) were used as blind test cases. All samples were stored at −80 °C and then examined by FTIR spectroscopy within 24 h. For each sample, about 5 μl serum was dropped onto a BaF_2 window homogeneously and dried under vacuum, and then a film was acquired for FTIR spectroscopic measurement.

FTIR spectroscopic measurements

FTIR spectroscopy was carried out using an IRAffinity-1 FTIR spectrometer with a DLATGS detector (SHIMADZU Corporation, Japan). For each spectrum, it was collected at 4000–800 cm^{-1} range with an 8 cm^{-1} resolution and 32 scans.

Data procession

All data were collected with IRsolution software and then stored using JCAMP format. Then these data were operated with OPUS5.5 software, they were baseline corrected in the 3700–1000 cm^{-1} region and then min–max normalized by scaling the entire spectrum to the absorbance of amide I (around 1646 cm^{-1}).

Table 1
The conditions of leukemia patients and healthy persons.

	Mean age ± SD	Sex		Diagnosis		
		M(n)	F(n)	AML(n)	CML(n)	ALL(n)
Leukemia patients	35.6 ± 16.21	16	14	22	4	4
Healthy persons	41 ± 13.22	9	10	–	–	–

Results and discussion

Analysis of the spectra

The average spectra of leukemia patients and healthy persons were gained and shown in Fig. 1. The main peaks and their corresponding assignments were listed in Table 2 [4,15–19]. From Table 2, it could be observed that there was no obvious peak shift between the spectra of leukemia patients and healthy persons.

Ratios of H1075/H1542, H1045/H1467, H2959/H2931

Human beings' serum is composed of proteins, lipids, nucleic acids and so on [20]. In order to distinguish leukemia patients' serum from healthy persons' serum, the peaks at 1079 cm^{-1} (baseline: 1140–1000 cm^{-1}), 1542 cm^{-1} (baseline: 1595–1479 cm^{-1}), 1045 cm^{-1} (baseline: 1140–1000 cm^{-1}), 1467 cm^{-1} (baseline: 1479–1426 cm^{-1}), 2959 cm^{-1} (baseline: 3002–2885 cm^{-1}), 2931 cm^{-1} (baseline: 3002–2885 cm^{-1}) were chosen and six corrected peak heights (H1045, H1079, H1467, H1542, H2959, H2931) were measured following Yano's method [16] (Fig. 2). Then the values of H1079/H1542, H1045/H1467 and H2959/H2931 were calculated (Table 3) and independent *t*-test was used to compare differences between leukemia patients' serum and healthy persons' serum.

The band around 1079 cm^{-1} is due to symmetric vibration of PO_2^- while the band around 1542 cm^{-1} is due to C–N stretching and N–H bending. The H1079/H1542 (DNA/proteins) ratio is a widely used factor for distinguishing healthy persons from cancer patients. In our study, the mean value of H1079/H1542 was 0.247 for leukemia patients while 0.284 for healthy persons. Independent *t*-test showed *P* value of H1079/H1542 was 0.2099 ($P > 0.05$), which indicated the ratios of leukemia patients and healthy persons were not significantly different. Moreover, the ratios of leukemia patients and healthy persons were all in the 0.024–0.485 range. So the H1079/H1542 ratio could not distinguish leukemia patients from healthy persons.

The band 1045 cm^{-1} owes to C–O stretching in carbohydrates and represents glycogen, the band 1467 cm^{-1} can represent cholesterol [16]. The H1045/H1467 ratio was applied to identifying

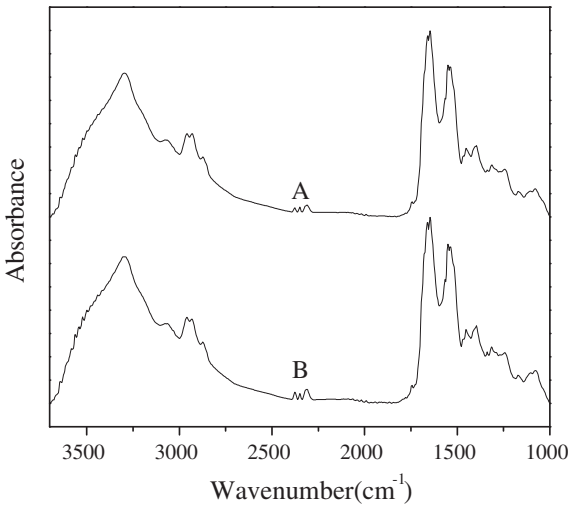

Fig. 1. Average IR spectra of serum from leukemia patients (A) and from healthy persons (B).