APPLICATION OF FTIR MICROSCOPIC SPECTROSCOPY FOR THE FOLLOW-UP OF CHILDHOOD LEUKEMIA CHEMOTHERAPY

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ABSTRACT

Acute Lymphoblastic Leukemia (ALL) accounts for majority of the childhood leukemia. Outcome of children with ALL treatment has improved dramatically. Sensitive techniques are available today for detection of minimal residual disease in children with ALL, which provide insight into the effective cytotoxic treatment. Here, we present a case study, where lymphocytes isolated from two children before and after the treatment were characterized using microscopic Fourier Transform Infrared spectroscopy. Significant changes in the absorbance and spectral pattern in the wavenumber region between 800-1800 cm⁻¹ were found after the treatment. Preliminary analysis of the spectra revealed that the protein content decreased in the T-type ALL patient before the treatment in comparison to the age matched controls. The chemotherapy treatment resulted in decreased nucleic acids, total carbohydrates and cholesterol contents to a remarkable extent in both B and T-type ALL patients.

Keywords: Acute lymphoblastic leukemia, childhood, chemotherapy, FTIR microspectroscopy.

1. INTRODUCTION

Among children, leukemia accounts for one third of all childhood cancers. Acute lymphoblastic leukemia (ALL) is a major type of childhood leukemia with varying incidence in different countries from 0.9 to 4.7 per 100,000 children (1). Radiation, environmental agents, maternal alcohol consumption and paternal smoking are associated with increased risk of ALL in children (2). ALL is a clonal hematological disorder arising due to genetic changes in hemopoietic cells (3). Mainly, the genetic alterations in transcription factor oncogenes are implicated in the process of leukemogenesis (4). Treatment of ALL using combination therapy has improved the survival rate drastically in the case of children (5). The treatment protocol has different phases during which cocktail of drugs are administered to the patient. The drugs, which have vital role in therapy, are Prednisone, Adriyamycin, L-Asparginase, MTX and ARA-c (6, 7). In the clinics, highly sensitive PCR techniques are applied to detect the minimal residual disease (8). This is in addition to the blast count performed by conventional methods (9).

In the past few years, FTIR has been used in the diagnosis of cancer. Gao.,T et.al (10) has carried out FTIR study of human breast, normal and carcinomal tissues. They reported that their method of analysis results in nearly 100% diagnostic accuracy of carcinoimal tissues from normal ones. The chronic lymphocyctic leukemia could be well characterized by FTIR based on lipid and DNA content and the overall spectral characters (11). Also the diagnosis of lung cancer was done using FTIR by measuring the ratio of the peak intensities of the 1030 cm⁻¹ and 1080 cm⁻¹ bands (originated mainly in glycogen and phosphodiester groups of nucleic acids) which differs greatly between normal and lung cancer samples (12). The grading of lymphoid tumors could be achieved by FTIR microscopy (FTIR-MC) (13). The examples mentioned above clearly suggest that FTIR can be a powerful tool in the diagnosis of cancer. The main advantages of this technique are simple, quick and economically viable. Our group has already obtained good success in the diagnosis of colon cancer using modern FTIR microspectroscopy (14, 15, 16).

In this report, we present the application of FTIR-MC in the follow-up of chemotherapy treatment of two children who had B and T-cell type ALL. It is interesting to monitor the effect of various drugs at the molecular level in the first phase of the
treatment. This is the first report of this kind showing the potential of FTIR-MC for the follow-up of leukemia chemotherapy treatment in children.

2. MATERIALS AND METHODS

2.1 Isolation of Lymphoblasts

The physicians in the department of Pediatric Hematology-Oncology at the Soroka University Medical Center (SUMC) provided the blood samples from two children who had B-and T-type ALL. Standard chemotherapy treatment protocol was followed for both patients. The blood was processed immediately for the isolation of the lymphoblasts. The lymphoblasts were isolated from the blood as per the procedure reported earlier (17).

2.2 FTIR Microspectroscopy

FTIR-measurements were performed in transmission mode using the FTIR microscope IRscope II with sensitive MCT detector, which is coupled to the FTIR spectrometer (BRUKER EQUINOX model 55/S OPUS software). The microscope is also equipped with a CCD-camera for the visible range of the spectrum, and a fully computerized X-Y stage, which allows measurement of large number of spectra, which can be used, for creating FTIR chemical-maps. The measured spectra covering the wavenumber range 600-4000 cm$^{-1}$. Since the ordinary glass slides have strong absorption in the wavelength range of our interest, zinc selenide crystals, which are highly transparent to IR light, were used. During each measurement, the measured sites will be circular of about 50 µm diameter at most. Such area contains enough lymphocytes to obtain good quality spectra with high signal to noise ratio. The spectra taken were average of 128/256 scans to increase the signal to noise ratio. Baseline correction for all the spectra were done using rubber band baseline correction method and the spectra was amide I normalized after baseline correction for the entire spectrum. For each sample, the spectrum was taken as the average of ten different measurements. The signal to noise ratio was calculated for all the measurements and only spectra with high signal to noise ratio (≥1000) were used for further data analysis. The error bars shown in figures 2-5 represent the maximum standard deviation (SD) obtained in all the measurements.

3. RESULTS AND DISCUSSION

Figure 1 a & b show the microscopic FTIR spectra of lymphocytes isolated from the blood of two children having B and T-type ALL respectively. The spectra were recorded along with age-matched four healthy controls. In the case of patients, the spectra were recorded before and during the chemotherapy treatment. In the case of B-type ALL patient, the salient features are as follows. The spectra obtained for the average of four controls (Figure 1a : A) and before the treatment (Figure 1a :B) were similar with minor changes in the absorbance of symmetric (1000-1100) and asymmetric (1200-1245) regions of the phosphate group and also in the amide II region arising from the proteins. The chemotherapy treatment cased drastic molecular changes in the cells, which could be observed in the spectra. For clarity reasons, only the spectra obtained after 15 (C) and 30 (D) days of treatment were presented. The spectra (Figure 1a : C) and (Figure 1a : D) clearly showed differences in the absorbance of phosphate bands corresponding to the nucleic acids. Also the band at 965 cm$^{-1}$ accounting for the symmetric stretching vibration of the phosphodiester bonds in nucleic acids showed marked changes in the intensity before and after the treatment. In addition, spectral pattern changes were observed in the phosphate bands. No significant changes in the amide II band were observed. Figure 1b shows the microscopic FTIR spectra of controls, before treatment and during the chemotherapy treatment. Similar to the B-type ALL, the spectra of lymphocytes isolated from T-type ALL patient before treatment (Figure 1b : B) did not show intensity difference in the phosphate region in comparison to the controls (Figure 1b : A). However, in the region between 1400-1600 cm$^{-1}$, there were notable changes in the intensity between the controls and the sample before treatment. Decrease in protein concentration was evident from the lower intensity for amide II band. During the chemotherapy treatment, significant changes in the spectra were observed. The spectra measured on the 11th day of treatment showed sudden decrease in the absorbance in the entire region (900-1800 cm$^{-1}$). In addition, broadening of the spectra was observed in the phosphate region. The spectra collected on the 22nd day of treatment also showed dramatically lower phosphate content with much less absorbance in the region between 1000-1200 cm$^{-1}$ compared to spectrum before treatment (day zero). The changes were specific in biomolecular composition, as the spectral crossover could be observed from 1300-1600 cm$^{-1}$. The protein content remained constant during the chemotherapy
treatment. The absorbance changes in the higher wavenumber region (2500-3500) followed a trend similar to the 800-1800 cm\(^{-1}\) region for both patients.

The variation of phosphate level obtained by measuring the absorbance at 1084 cm\(^{-1}\) is presented in Figure 2 for both patients. The ionized PO\(_4^{2-}\) and sugar moiety of the base in nucleic acids give rise to the absorption at 1071, 1084 and 1095 cm\(^{-1}\) (18) apart from other phosphate containing metabolites. In both patients, we observed significant decrease in phosphate level after four days of the treatment. B-type ALL patient had a steady decrease in the phosphate content in contrast to T-type ALL patient in which the decrease was sharp after the 7\(^{th}\) day of treatment.

The cholesterol content in the biological samples can be measured by estimating the methylene band of cholesterol at 1476 cm\(^{-1}\) and this observation was confirmed by measuring the spectrum of pure cholesterol under same conditions (19). Our results on cholesterol content shown in Figure 3 revealed that in B-type ALL patient, the cholesterol level decreased in the first two days and later increased, and finally returned to day 2 stage after 30 days. But in the case of T-type ALL patient the cholesterol increased during therapy and stabilized on day 22.

The bands at 1025 and 1045 cm\(^{-1}\) in the IR spectra are responsible for the vibrational modes of -CH\(_2\)OH groups and the C-O stretching vibration coupled with C-O bending of the C-OH groups of carbohydrates (includes glucose, fructose and
glycogen etc.) (20). The ratio of areas of the bands at 1045/1545 gives an estimate of the carbohydrate levels presented in Figure 4. The carbohydrate content steadily decreased in the B-type ALL patient during the course of treatment. There were fluctuations observed in this case. But in the T-type ALL patient, the carbohydrate level initially increased and decreased rapidly from 7th day of treatment.

Earlier reports suggest that the amide I/II intensity ratio increase with DNA content of the epithelial cells (21), whereas in the case of RBC (Red Blood Cells), the intensity ratio of amide I/II is nearly the same as any other pure protein spectrum. The ratio of area under amide I/II band is shown in figure 5 a & b. Similar to phosphate and carbohydrate contents, the DNA content decreased in both patients during the chemotherapy treatment. After 11 days of therapy, the DNA content reached saturation. The rate of decrease was different in B and T-type ALL cases.

Figure 2: Phosphate content is presented as the absorbance at 1084 cm\(^{-1}\) arising from symmetric stretching vibration of the phosphate group in the nucleic acids. In figures 2 through 5, day -1 is the average of four controls and day 0 stands for the day before treatment.
Figure 3: Cholesterol content in the cells was measured using the absorbance at 1467 cm\(^{-1}\) for a) B-type ALL  b) T-type ALL patients.

Figure 4: Total carbohydrate level in the cells was measured by the ratio at 1045/1545 cm\(^{-1}\) for a) B-type ALL  b) T-type ALL patients.
Figure 5: Ratio of integrated absorbance under Amide I/II is presented as the indirect measure of DNA content from cells a) B-type ALL b) T-type ALL patients. Integrated absorbance calculations were performed using ORIGIN software.

4. DISCUSSION

ALL is the major leukemia type among children and research till today focused on the aspects of its causes, diagnosis and treatment. However, following the molecular mechanisms of drug action during the treatment is still to be understood in detail. Our studies gain special significance giving the nature that biomolecular changes occur upon the action of various drugs during therapy using an advanced optical technique. Earlier reports showed that in the case of CLL (Chronic Lymphoblastic Leukemia) in adults, the FTIR spectra could be used to distinguish normal cases from patients (22). In our study, only T-type ALL could be differentiated from the controls by means of absorbance changes in the 1300-1600 cm$^{-1}$, which include substantial decrease in protein content for patients. However, FTIR-MC was sensitive to biomolecular changes in mononuclear cells upon the action of drugs.

Battery of drugs administered for leukemia patients are known to inhibit the DNA and RNA synthesis. Particularly, the effects of MTX (Methotrexate), L-Asparaginase and Doxorubicin are well documented in the literature (23). In our study, we observed the decrease in DNA and RNA immediately after the beginning of the chemotherapy treatment with MTX for both patients. The reduction in DNA content was confirmed by two different methods of analysis such as amide I/II absorbance ratio and monitoring the absorbance at 1084 cm$^{-1}$, which corresponds to phosphate group of the nucleic acids. Also the spectra showed significant decrease in absorption at 965 and 1245 cm$^{-1}$ arising from phosphodiester bonds in the nucleic acids. The difference in the rate of decrease between B and T-type ALL patients is not clearly understood.
Hyperglycemia in children with ALL is a well-established fact resulting in obesity before and post therapy periods (24). The chemotherapy treatment decreased the carbohydrate content in both B and T-type ALL patients. Reduction in the energy metabolism leads to lower Acetyl-CoA levels, which is the precursor of cholesterol synthesis. Our results also showed decrease in cholesterol content in B-type ALL after 12 days of treatment. Contrary to B-type ALL, the cholesterol level in T-type ALL continued to increase during treatment. It is possible that cholesterol content in T-type ALL may follow the same trend after 22 days as observed in B-type ALL patient. Studies are in progress in our laboratory on the comparison of vast amount of clinical data available on patients with FTIR data, which is expected to offer a complete picture in the near future.

5. SUMMARY AND CONCLUSIONS

Our results on follow-up of B and T-type ALL patients during chemotherapy using FTIR-MC has opened an area called “Optical Follow-up”. The results indicate that FTIR-MC could differentiate controls and T-type ALL patients before the commencement of treatment. In addition, our method was sensitive to monitor the biomolecular changes due to the action of drugs. There were notable spectral pattern changes before and after the treatment. Analysis of the results showed decrease in nucleic acids content during the treatment in both patients. Also, total carbohydrate and cholesterol levels were observed to decrease in agreement with literature. In conclusion, FTIR-MC will be a novel state-of-the-art technology in the hands of physicians in the future.

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