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Continuous monitoring of WBC (biochemistry) in an adult leukemia patient using advanced FTIR-spectroscopy

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Abstract

Fourier transform infrared (FTIR)-spectroscopy has been found useful for monitoring the effectiveness of drugs during chemotherapy in leukemia patients. In the present work, spectral changes that occurred in the white blood cells (WBC) of an adult acute myeloid leukemia (AML) patient and their possible utilization for monitoring biochemistry of WBC were investigated. The phosphate absorbance from nucleic acids and the lipid–protein ratio in the WBC decreased immediately after treatment and then increased to levels of a control group. Similar observations were recorded in child patients with acute lymphoblastic leukemia (ALL) who were used as test cases. These parameters maybe used as possible markers to indicate successful remission and suggest that FTIR-spectroscopy may provide a rapid optical method for continuous monitoring or evaluation of a WBC population.

Keywords: Leukemia; WBC; FTIR-microscopy; Chemotherapy; Markers

1. Introduction

Leukemia is characterized by a large number of immature cells (blasts) in the blood. The cells are in a different growth stage compared to what are normally present in the blood. The changes in tissue biochemistry are reflected as spectral changes. This is the principle behind several diagnostic tools that are based on spectroscopic methods. Fourier transform infrared (FTIR)-spectroscopy is one such area that has seen rapid development in the past decade with a promise of easier, rapid and objective diagnosis [1]. FTIR-spectroscopy is also an effective and non-destructive method to monitor cellular changes [2,3]. Diseases of several organs have been identified using structure and quantity of biomolecules in biological samples such as proteins [4], nucleic acids [5] and lipids [6].

Chemotherapy decreases the blast count [7,8]. Chemotherapeutic drugs such as doxorubicin and vincristine interfere with cell division by binding to the DNA and targeting its synthesis or function [7]. In non-dividing cells; however, the lethal effects are due to their ability to interfere with DNA repair polymerases as well as lipid biosynthetic enzymes. Thus, the presence of blasts in the blood is used as an indicator of residual malignancy during treatment and care of leukemia patients after induction of chemotherapy. However, no studies have been carried out to examine the biochemistry of white blood cells (WBC) to see whether they become biochemically normal following chemotherapy, though conventional blood profiles are studied. Rapid and continuous monitoring of biochemistry of WBCs on a molecular level remains a challenge, which is also essential for the well being of the patient. Thus, we studied FTIR-spectroscopy as a potential reagent

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free supplementary technique for such routine monitoring of WBCs in patients being treated for leukemia.

FTIR-spectroscopy along with cluster analysis of the spectra has shown a promise as a tool for monitoring the effectiveness of drugs during chemotherapy in children [9,10]. However, no such report on the validation of the technique for monitoring biochemical changes in WBC during chemotherapy of adults is available. It is well known that the response of adults to leukemia is different from children and often more difficult. In the present work, we study the utility of FTIR-spectroscopy derived parameters for an adult, which were earlier found effective in monitoring changes in WBCs during chemotherapy in children. The patient in the present case study had low blast count in the peripheral blood (5%), which is only a minor deviation from normal values, thus, this patient might serve as an analogy for the effects of chemotherapy on WBCs in vivo in normal adults. The plasma and RBCs profiles before the treatment regimen were comparable to the normal average value with low WBC counts. For further validation of the FTIR-spectroscopy derived markers a comparison is made with the values obtained from a control population. The corresponding values of the diagnostic parameters from healthy persons are obtained in parallel to act as control references to ascertain the presence of residual malignancy or the return to normalcy. Thus, it becomes imperative that monitoring by simple blood count would not be possible, and an optical method for following the biochemical changes in WBCs would be more suitable. To further substantiate this hypothesis, cases of child leukemia patients under treatment were tested.

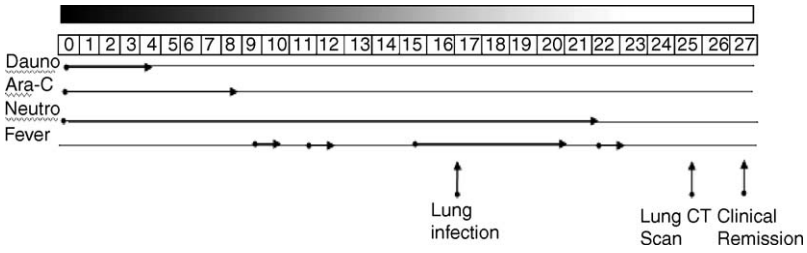
2. Materials and methods

The blood samples were collected from the adult patient with his consent. The adult was given a treatment regimen the "7 and 3" protocol composed of Arabinoside-C (Ara-C) for the first 7 days and Daunorubicin (Dauno) for the first 3 days as shown in Scheme 1. Blood from a group of normal people (eight healthy adults without any known clinical symptoms for any disease) was taken to serve as a control for comparison. Blood samples from two children under treatment for acute lymphoblastic leukemia (ALL) were taken after obtaining consent of their parents and used as test cases. Erythrocytes, plasma and WBCs from blood of leukemia patients and controls were separated using the method of Hudson and Poplack [11] within two hours of the collection of the blood samples. The WBCs were washed with normal saline (0.9 M sodium chloride) to remove any impurities from the plasma. The blood components were then diluted as required in normal saline. Two microlitres of each blood component was spotted on a zinc selenium slide to form approximately a monolayer of cells and air dried under the laminar flow to remove remaining water.

The FTIR measurements on samples were performed using the FTIR microscope IRscope II with liquid nitrogen cooled mercury-cadmium-telluride (MCT) detector, coupled to the FTIR spectrometer (Bruker Equinox model 55/S, OPUS software). To achieve high signal to noise ratio (SNR) 128 co-added scans were collected in each measurement in the wavenumber region 600–4000 cm^{-1} for Fourier transform processing. Five sites were measured on each sample containing on an average 100 cells as reported previously with an aperture of 1.5 mm that measures a circular area of 100 microns diameter [9,10]. The spectra were baseline corrected using OPUS software and were normalized to the amide II (~1545 cm^{-1}) absorbance band in the region 800–1800 cm^{-1}. The averages of the normalized spectra were used for subsequent analysis. For the higher wavenumber region, the spectra were cut in the region 2835–3000 cm^{-1}, baseline corrected and normalized to the CH_2 antisymmetric band (~2920 cm^{-1}), which has the maximum absorbance in this region. Other normalization methods such as amide I for the 800–1800 cm^{-1} region or vector normalization for the entire spectra have also been tested and gave negligible changes in the results. The various biochemical parameters studied were glucose [12], phosphate levels [9,10,13], nucleic acid ratio [14], lipid–protein ratio [15,16].

3. Results

During the period of study, the patient was treated with a chemotherapy regimen which included Daunorubicin and Ara-C (Scheme 1). These drugs affect all WBCs and cause biochemical changes and apoptosis. Representative normalized spectra of WBC from the adult leukemia patient on several days of treatment are presented in Fig. 1a along with the average spectra of WBC from healthy persons (dashed line) for identification of the major changes in the spectra dur-



Scheme 1. Treatment regimen protocol for the adult AML patient. Dauno-Daunorubicin, Arabinoside-C (Ara-C), Neutro-Neutrophils.