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A study of structural differences between TBM patients' and non-TBM persons' CSF using UV–Vis absorption spectroscopy

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ABSTRACT

Tuberculous meningitis (TBM) is a very common infectious disease in the central nervous system. The delay of diagnosing and treating TBM will lead to high disability and mortality of TBM. Hence, it is very important to promptly diagnose TBM early. In this work, we proposed a new method for diagnosing TBM with CSF samples by using UV–Vis absorption spectroscopy. CSF samples from TBM patients and non-TBM persons were compared, and the sensitivity, specificity, accuracy, positive predictive value reached 83.6%, 69.8%, 77.2%, 76.1% respectively. Our work indicated investigation of CSF using UV–Vis absorption spectroscopy might become a potentially useful method for TBM diagnosis.

1Introduction

According to global tuberculosis report of WHO in 2014, more than 8 million persons were infected by *Mycobacterium tuberculosis* (*M. tuberculosis*) annually [1,2]. Tuberculous meningitis (TBM) is a very common infectious disease in the central nervous system, and it occurs especially in developing countries and becomes a serious public health problem. The mortality of TBM reaches 30% while the disability of TBM exceeds 51%, which result from late diagnosis and treatment of TBM [3–5].

It is very important to choose appropriate anti-TB chemotherapy at early stage, which is based on promptly diagnosing TBM early. The "Golden standard" for rapid diagnosis of TBM is to find *M. tuberculosis* in cerebrospinal fluid (CSF). The fastest and most inexpensive method is direct Ziehl–Neelsen staining concentrate CSF to look for acid-fast bacilli (AFB). Unfortunately, this method has a low sensitivity (10–30%, only few reached 58%) [6,7]. Although a modified Ziehl–Neelsen staining method showed its ability to detect AFB easily, it was still needed to accumulate more confirmed experimental data [8,9]. Another method is culture, but it is very slow (>2 weeks) and insensitive (<50%). Recently, a new diagnostic technique has been developed by amplification of *M. tuberculosis* DNA in CSF using polymerase chain reaction (PCR). Although this method is highly sensitive, it still has some significant limitations: (1) False positive rate and negative rate vary in different laboratories (3–77%); (2) The detection of viable or dead bacteria can not be identified [10–12]. Now T-spot is a new method with higher sensitivity and specificity for detecting *M. tuberculosis*, which detects interferon γ released from specific T lymphocytes by ELISPOT to confirm infection of *M. tuberculosis*, but it can not distinguish active TB from latent TB and it can not concentrate enough active cells from CSF [13]. Hence, researchers are still trying to explore better methods to diagnose TBM early.

Since spectroscopic technologies can provide a highly sensitive method for analyzing the structures, chemical compositions and contents of material effectively and accurately, they have been widely used to detect changes of human beings' cells, tissues and body fluids caused by diseases at molecular level. For example, FTIR spectroscopy [14–16], UV–Vis absorption spectroscopy [17–19], Raman spectroscopy [20,21] and fluorescence spectroscopy [22] have been applied in disease research. These studies showed spectroscopic technologies had several advantages such as non-invasive, non-destructive, highly automated, highly sensitive and

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so on, suggesting they might be potentially useful tools for diagnosing diseases at early stage. Among these spectroscopic technologies, UV–Vis absorption spectroscopy is the most familiar and cheapest. UV–Vis absorption spectroscopy was used to investigate CSF of cerebral hemorrhage patients, and the results indicated that the contents and species of biological macromolecules changed during disease process [17]. In this study, UV–Vis absorption spectroscopy was applied to comparison of TBM patients' and non-TBM persons' CSF. The goal of our study is to explore a new and quick method for distinguishing TBM patients' CSF from non-TBM persons' CSF.

2. Materials and methods

2.1. Materials

This study was approved by the Ethics committee of Anhui Medical University. All patients provided written consents for offering CSF. Totally, 114 CSF samples were obtained from The First Affiliated Hospital of Anhui Medical University. TBM patients should meet the inclusion criteria as following: (1) They were ≥18 years old; (2) They were diagnosed as TBM; (3) They were able to give informed consents. The non-TBM persons in control group should meet the inclusion criteria as following: (1) They were ≥18 years old; (2) They didn't have any neurological diseases; (3) They were able to give informed consents. The age of TBM and control groups was not obviously different. For each CSF sample, it was divided into two parts, one was processed for cytological examination, another was stored at −80 °C before UV–Vis absorption spectroscopic examination (Fig. 1).

2.2. Methods

The UV absorption spectra were recorded by using Jena 205 UV–Vis spectrophotometer at room temperature. Firstly, 3 ml distilled water was injected into the sample cell and the reference cell respectively for obtaining baseline calibration. After adjusting for baseline, the 3 ml water in the reference cell was unchanged while 0.06 ml CSF and 2.94 ml water were mixed uniformly in the sample cell, and then the spectrum was measured. The instrument parameters were set as: scan range was 220–450 nm, delta lambda was 1 nm, the speed was 50 nm/s.

2.3. Statistics

The data such as peak position, vale position and the A_{max}/A_{min} ratio were analyzed by origin6.0 software and shown in Table 1. Moreover, the data were studied with canonical discriminant analysis (CDA) by using SPSS20.0 software.

3. Results

3.1. Cytological examination of CSF

All samples were examined immediately. The slides with monolayer cells were prepared by cytospin and then evaluated by Wright–Giemsa staining method. The type and sum of cells were

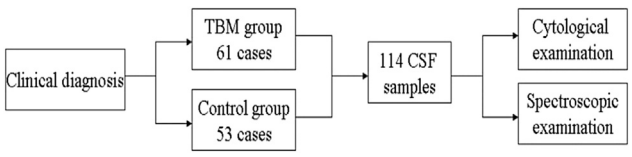

Fig. 1. A summary of CSF samples.

Table 1
Statistic data.

	Peak frequency	Vale frequency	A_{max}/A_{min}
TBM patients	279.49 ± 5.24	255.75 ± 3.18	1.813 ± 0.872
Non-TBM persons	276.92 ± 5.25	258.19 ± 6.14	1.350 ± 0.511
P value	0.01039	0.00781	$9.569*10^{-4}$

observed with a microscope. The TBM samples presented characteristics such as pleocytosis (>5 × 10^6/L), inflammatory neutrophilic granulocyte and active lymphocytes (Fig. 2A). The control samples had two characteristics: (1) The sum of cells on a slide was no more than 5 × 10^6/L; (2) All cells on the slide were small lymphocytes and monocytes (Fig. 2B). For all CSF samples, tumor cells and cryptococcus neoformans were not found.

3.2. Analysis of spectra

A typical UV absorption spectrum of CSF was shown in Fig. 3. It could be observed that there were a vale (around 254 nm) and a peak (around 278 nm) in the spectrum. Then secondary derivative spectrum was calculated (Savitzky–Golay, 9 points) (Fig. 4). From the secondary derivative spectrum, the following peaks were observed at 255 nm (phenylalanine residues), 260 nm (nucleic acids), 273 nm (tyrosine residues) and 281 nm (tryptophan residues) [23], which indicated the peak in UV absorption spectrum received contributions from proteins and nucleic acids.

3.3. Statistical results

3.3.1. Comparison of the peak and vale positions and A_{max}/A_{min}

In order to distinguish TBM samples from control samples, the peak and vale positions, A_{max}/A_{min} (intensity ratio of peak/vale) were counted. The data were expressed as mean ± standard derivation (SD) (Table 1). Then the differences between TBM group and control group were tested by independent *t*-test analysis, and the difference between TBM and control groups could be considered statistically significant if $P < 0.05$. For the peak position, the mean value was 279.49 for TBM samples while 276.92 for control samples. Independent *t*-test analysis showed *P* value of the peak position was 0.01039 ($P < 0.05$), indicating the peak positions of TBM samples were significantly higher than those of control samples. For the vale position, the mean value was 255.75 for TBM samples while 258.19 for control samples. Independent *t*-test analysis showed *P* value of the vale position was 0.00781 ($P < 0.05$), indicating the vale positions of TBM samples were significantly lower than those of control samples. For A_{max}/A_{min}, the mean value was 1.8137 for TBM samples while 1.3504 for control samples. Independent *t*-test analysis showed *P* value of A_{max}/A_{min} was $9.569*10^{-4}$ ($P < 0.05$), indicating the A_{max}/A_{min} ratios of TBM samples were significantly higher than those of control samples. In conclusion, A_{max}/A_{min} had the highest significant difference among the peak position, the vale position and A_{max}/A_{min}, and it might be a useful factor to distinguish TBM patients' CSF from non-TBM persons' CSF.

3.3.2. Canonical discriminant analysis

Canonical discriminant analysis (CDA) was carried out in order to discriminate TBM samples from control samples effectively [24]. Two functions were established from CDA, and they were listed as below:

$$Y_1 = 5.135X_1 + 7.763X_2 + 10.097X_3 - 1709.145$$

$$Y_2 = 5.426X_1 + 7.357X_2 + 10.444X_3 - 1709.163$$

Y_1 was CDA categorized TBM while Y_2 was CDA categorized