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# Comparison of red blood cells from gastric cancer patients and healthy persons using FTIR spectroscopy

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ABSTRACT

In this paper, FTIR spectroscopy was used to compare gastric cancer patients' red blood cells (RBCs) with healthy persons' RBCs. IR spectra were acquired with high resolution. The A1653/A1543 (the protein secondary structures), A1543/A2958 (the relative content of proteins and lipids), A1106/A1166 (the structure and content changes of sugars) and A1543/A1106 (the relative content of proteins and sugars) ratios of gastric cancer patients' RBCs were significantly different from those of healthy persons' RBCs. Curve fitting results showed that the protein secondary structures and sugars' structures had differences between gastric cancer patients' and healthy persons' RBCs. Additionally, FTIR spectroscopy could obtain 95% sensitivity, 70% specificity, 84.2% accuracy and 80.9% positive predictive value in combination with canconical discriminant analysis. The above results indicate FTIR spectroscopy may be useful for diagnosing gastric cancer.



## 1. Introduction

Cancers have become common and frequently-occurring diseases in the world, which threatens human beings' life and health seriously. According to "Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012" released in 2015, about 14.1 million new cancer cases were found while 8.2 million cancer patients died in 2012 [1]. The cause of cancer may be related to chemical/physical carcinogenic factors, viral and bacterial infections, genetic factors, immune factors and so on [2]. Although the pathogenesis of most cancers is uncertain, it is generally accepted that early diagnosis and timely treatment are very important for controlling cancers [3].

Gastric cancer ranks fifth in common malignancies, which caused 723000 deaths worldwide in 2012 [1]. At present, gastroscope combined with pathology biopsy is the gold standard for diagnosis of gastric cancer. However, it has several shortcomings [3,4]: ① It brings pain to patients. ② It goes through a series of steps such as tissue fixation, section, staining and reading, which take a few days to obtain the results. ③ It depends on pathologists' individual professional ability. Hence, new methods for rapid and simple diagnosis of gastric cancer are needed urgently.

At the early stage of cancer, pathological changes in morphology can not be revealed, however, the main biochemical components of cells/tissues (proteins, lipids, carbohydrates, nucleic acids, etc.) change significantly in contents, structures and conformations [3]. As Fourier transform infrared (FTIR) spectroscopy can detect these changes sensitively and quickly, it has been widely used in cancer research recently [3,5–7]. There were also some reports on FTIR spectroscopic study of gastric cancer. For example, Tong et al. compared IR spectra of gastric cancer tissues and corresponding normal tissues, and they found obvious spectral differences in shape, frequency and intensity of bands [8]. Sheng et al. compared IR spectra of gastric cancer patients' and healthy persons' serum, and they found H2959/H2931 might be a standard for distinguishing gastric cancer patients from healthy persons [9]. Bai et al. investigated IR spectra of gastric cancer patients' lymph nodes, and they found FTIR spectroscopy might be a new tool for judging lymph node metastasis of gastric cancer rapidly in situ and in vivo [10]. These reports showed FTIR spectroscopy might be a power tool for investigation of gastric cancer.

Red blood cells (RBCs) are an important part of blood which can reflect life and health condition of human body [11,12]. Hence,

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investigation of RBCs using FTIR spectroscopy may provide a novel method for diagnosing gastric cancer. Feng et al. compared gastric cancer patients' and healthy persons' RBCs' IR spectra, and they found the $S_{1540}/S_{1084}$ (proteins/nucleic acids and ribosomes) ratios of gastric cancer patients' RBCs were higher than those of healthy persons' RBCs [12]. Although this study was interesting, it had several shortages such as relatively few samples (only 3 gastric cancer samples and 3 normal samples) and influence of intense $H_2O$ absorption bands.

In this study, FTIR spectroscopy was used to investigate gastric cancer patients' and healthy persons' RBCs. The sample number was larger compared to Feng's study, furthermore, the effect of $H_2O$ decreased greatly by drying RBCs under vacuum. The differences were compared between gastric cancer patients' and healthy persons' RBCs in lipids, proteins, sugars, etc. The aim of this work is to investigate the feasibility of discriminating gastric cancer patients from healthy persons using RBCs' IR spectra.

## 2. Materials and methods

### 2.1. Sample

Gastric cancer patients' blood samples (before operation) and healthy persons' blood samples were gathered from The First Affiliated Hospital of Anhui Medical University. The disease group (40 gastric cancer patients were included) should meet the following inclusion criteria: (1) They were older than 18 years. (2) They were definitely diagnosed as gastric cancer. The control group (30 healthy persons were included) should meet the following inclusion criteria: (1) They were older than 18 years. (2) They didn't have cancer, gastric disease, cardiovascular disease, cerebrovascular disease, inflammation, obviously abnormal liver and kidney function, etc. Moreover, there was no significant difference in gender and age between the disease group and the control group.

### 2.2. Sample preparation

2 ml fresh anti-coagulation and 2 ml HES-TBD (hydroxyethyl starch 550) were mixed, and then 2 ml saline for injection was added. The supernatant was discarded after natural sedimentation for 30 min at room temperature. After transfer of 100 μl RBCs into a 2 ml EP tube, the RBCs were washed with saline. The wash step was carried out as following: The RBCs were mixed with 1 ml saline and centrifuged for 3 min, and the supernatant was removed after centrifugation. After 5 times washing, the RBCs were mixed with 500 μl saline homogeneously. Then 10 μl RBCs solution was dropped onto a $BaF_2$ window and smeared evenly. The $BaF_2$ window was dried under vaccum to obtain a film for FTIR spectroscopic measurement.

### 2.3. FTIR spectroscopic measurement and data procession

All IR spectra were measured by an IRAffinity-1 FTIR spectrometer produced by SHIMADZU Corporation (operation software: IRsolution). Instrument parameters were set as follows: the scan region was 4000–800 $cm^{-1}$, the scan times were 64, the spectral resolution was 8 cm-1 (Note: 10 μl saline was dropped onto a blank $BaF_2$ window and dried naturally to obtain a background for FTIR spectroscopic examination.)

All spectral data were translated from IRsolution files to JCAMP data which could be processed by OPUS 5.5 software. Then all spectra were cut (range: 3670-920 $cm^{-1}$), baseline corrected (64 points' rubberband correction) and min-max normalized (to amide I absorbance). For statistics, the peak-area ratios were analyzed by origin 6.0. The differences between the disease group and the control group were examined by the independent *t*-test, and the difference could be considered significant if $P < 0.05$. Canonical discriminant analysis (CDA) was done by SPSS 20.0.

## 3. Results

### 3.1. IR spectra of serum

The average IR spectra of gastric cancer patients' and healthy persons' RBCs were shown in Fig. 1. The main bands were assigned in Table 1[13–18]. These bands were mainly related with proteins (bands around 3296, 3065, 1653, 1543, 1300 and 1245 $cm^{-1}$), lipids (bands around 2958, 2936, 2872, 1745, 1451 and 1395 $cm^{-1}$), sugars (bands around 1166 and 1052 $cm^{-1}$), etc. From Fig. 1 and Table 1, no obvious peak shift could be observed between gastric cancer patients' RBCs (A) and healthy persons' RBCs (B).

### 3.2. Comparison of the peak-area ratios

The peak-area ratios were used to compare differences between gastric cancer patients' and healthy persons' RBCs. Five peak areas (A2958, A1653, A1543, A1166, and A1106) were measured (Table 2) and then the A1653/A1543, A1543/A2958, A1106/A1166 and A1543/A1106 ratios were calculated. The results were expressed as mean ± SD (Table 3).

#### 3.2.1. A1653/A1543

The 1700-1600 $cm^{-1}$ region owes to amide I band while the 1585-1488 $cm^{-1}$ region owes to amide II band. A1653/A1543 is related with the protein secondary structures [19,20]. The average A1653/A1543 value was 1.9933 for the disease group while it was 1.8887 for the control group. The *P* value ($2.6922 \times 10^{-4}$) was lower than 0.05, indicating the A1653/A1543 values of the disease group were significantly higher than those of the control group [21].

#### 3.2.2. A1543/A2958

The 2990-2900 $cm^{-1}$ region mainly belongs to asymmetric $CH_3$ and $CH_2$ stretching of lipids. Since amide II band represents proteins, A1543/A2958 may be used to evaluate the relative content of proteins and lipids. The average A1543/A2958 value was 6.2488 for the disease group while it was 4.6799 for the control group. The *P* value ($1.1072 \times 10^{-7}$) was lower than 0.05, indicating the proteins level relative to the lipids level was significantly higher in gastric cancer patients' RBCs.

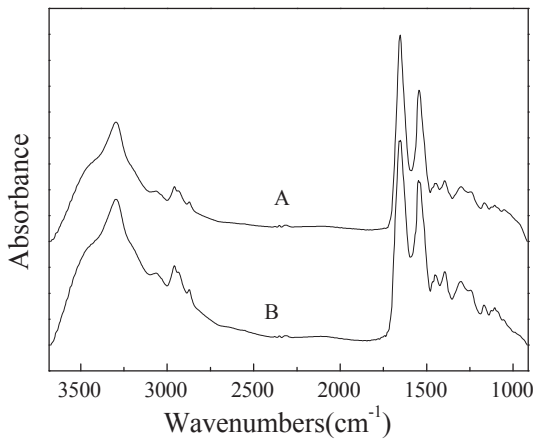


**Fig. 1.** Average IR spectra of gastric cancer patients' RBCs (A) and healthy persons' RBCs (B).