European Review for Medical and Pharmacological Sciences 2018; 22: 1061-1065

Study on the relationship between changes of immune cells and TNF-α in peripheral blood of patients with multidrug-resistant and extensively drug-resistant tuberculosis

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Abstract. – OBJECTIVE: To investigate the relationship between changes in CD4- and CD8-positive immune cells and TNF-α in the peripheral blood of patients affected by multidrug-resistant and extensively drug-resistant tuberculosis.

PATIENTS AND METHODS: 179 patients suffering from tuberculosis treated in the Chest Hospital of Hebei from April 2010 to December 2015 were selected for the study. There were 47 cases affected by drug-resistant tuberculosis and 132 cases affected by non-drug-resistant tuberculosis. The control group included 183 healthy subjects examined during the same period. ELISA was used to compare and analyze serum levels of TNF-α, CD4- and CD8-positive cell levels, and CD4/CD8 ratio in the two groups.

RESULTS: CD4- and CD8-positive cell count, CD4/CD8 ratio, and serum TNF-α were significantly higher in patients with drug-resistant tuberculosis compared with healthy controls and the non-drug-resistant tuberculosis patients ($p < 0.05$). There was a positive correlation between TNF-α level and CD4/CD8 ratio ($r=0.892$, $p < 0.05$). Before treatment, the differences in the levels of TNF-α in the different groups of drug-resistant patients were insignificant ($p > 0.05$). After treatment, the levels of TNF-α in the different groups of drug-resistant patients were decreased, except for patients with extensively drug-resistant tuberculosis, whose levels were significantly decreased compared with before treatment ($t = 0.648$, $p > 0.05$). The differences in the levels of TNF-α in the other groups of patients before and after treatment were statistically significant ($t = 8.497, 6.258, 5.346$, $p < 0.05$, fully sensitive tuberculosis single drug-resistant tuberculosis, multidrug-resistant tuberculosis, respectively).

CONCLUSIONS: The level of TNF-α plays a critical role in the evaluation of the severity of patients with drug-resistant tuberculosis and it has a clinical value.

Key Words:
Drug-resistant pulmonary tuberculosis, Tumor necrosis factor, Extensively drug-resistant.

Introduction

Drug-resistant tuberculosis refers to *Mycobacterium tuberculosis* infection that is confirmed *in vitro* to be resistant to more than one kind of first-line anti-TB drug and is not simultaneously resistant to isoniazid and rifampicin[1]. In recent years, because of the increase in drug-resistant tuberculosis, its low cure rate, and the high rates of recurrence and mortality, curing this disease has become a global issue. Many studies have been conducted domestically and abroad on CD4- and CD8-positive cells and tumor necrosis factor-α (TNF-α) in the peripheral blood of patients with pulmonary tuberculosis[2-4]. However, the correlation of CD4- and CD8-positive cells and TNF-α in the peripheral blood of patients with different drug resistances to tuberculosis has not been reported.

In recent years, there have been many works on drug resistance in tuberculosis and changes of serum levels of TNF-α. In our investigation, the levels of TNF-α in peripheral blood of patients with drug-resistant tuberculosis and non-drug-resistant tuberculosis were analyzed. We explored the correlation between the levels of TNF-α and immune cells in peripheral blood of tuberculosis patients with different drug resistances.

Patients and Methods

Research Methods

A total of 4 ml of peripheral blood was drawn from drug-resistant tuberculosis patients, 2 ml

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of which was anticoagulated with EDTA-K2 (Yangze, Pharm, Co., Ltd, Taizhou, China) to measure CD4- and CD8-positive cells. The remaining 2 ml of peripheral blood was not anticoagulated and used to measure serum TNF-α. Serum TNF-α kits and CD4- and CD8-positive cell detection kits were from Shanghai Semi-Bio Technology Co., Ltd (Shanghai, China), and counted with a Nikon H600L fluorescence microscope (Tokyo, Japan). Serum TNF-α was measured by ELISA kit (Abcam, Cambridge, MA, USA) using an Olympus AU400 fully-automated biochemical analyzer (Tokyo, Japan).

Patients

Patients suffering of tuberculosis who underwent treatment in the Chest Hospital of Hebei from April 2010 to December 2015 were randomly selected. All patients met the requirements of the *Guidelines on Tuberculosis Diagnosis and Treatment* formulated by the Chinese Society for Tuberculosis, Chinese Medical Association (5). Among them, 47 cases had tuberculosis with drug resistance and 132 cases had tuberculosis without drug resistance. Among the 47 cases affected by drug-resistant tuberculosis, there were 21 cases affected by single drug-resistant tuberculosis, 16 cases affected by multidrug-resistant tuberculosis, and 10 cases affected by extensively drug-resistant tuberculosis. These patients were further classified according to the results of acid-fast bacilli (AFB) infection testing. Among them, seven cases were AFB-negative (–), three cases were weakly AFB-positive (±), 10 cases were AFB-positive (+), 16 cases were AFB-positive (++), six cases were AFB-positive (+++), and five cases were AFB-positive (++++). The control group consisted of 183 healthy subjects examined during the same period. Patients with tuberculosis were aged from 20-84 years old (mean: 56.38 ± 14.14 years). The healthy controls were aged from 21-90 years old (mean: 57.33 ± 15.42 years).

Selection Criteria

Inclusion criteria: All patients were diagnosed with tuberculosis bacillus infection through X-ray examination and tuberculin test, and met the relevant standards of the *Guidelines on Tuberculosis Diagnosis and Treatment* formulated by the Chinese Society for Tuberculosis, Chinese Medical Association: 1. No history of infection within the past 3 months; 2. No history of tuberculosis; 3. No acquired immunodeficiency syndrome; 4. No autoimmune diseases; 5. Have not been recently infected with hepatitis virus; 6. Did not suffer from high blood pressure, malignancy, or other diseases; 7. Did not use hormones recently; 8. Did not consume excessive alcohol recently. Exclusion criteria: Diseases with similar symptoms were excluded, such as pneumonia and lung cancer. The study was approved by the Ethics Committee of our hospital. All subjects signed the informed consent.

Treatment Methods

Patients with tuberculosis infection received conventional treatment for tuberculosis, i.e., therapy combining isoniazid, rifampicin, ethambutol hydrochloride tablets, and pyrazinamide tablets. Three months after treatment, blood samples were collected for testing.

Statistical Analysis

The SPSS19.0 software (SPSS Inc., Chicago, IL, USA) was used for data analysis. Analyses of quantitative data were by ANOVA. Quantitative data were compared by a χ^2-test. If data did not meet the conditions, Fisher's method was used to calculate the exact probability. $p<0.05$ was considered statistically significant. Correlation analyses were by Pearson correlation analysis and the test level was a=0.05 (bilateral).

Results

Comparison of General Parameters Between Patients and Healthy Controls

There were no significant differences in age, sex ratio, BMI, or other basic parameters between the non-drug-resistant tuberculosis group, the drug-resistant tuberculosis group, and the healthy control group ($p>0.05$). Baseline parameters were therefore comparable (Table I).

Serum Levels of CD4 and CD8-positive Cells, CD4/CD8, and TNF-α

The number of CD4-positive cells was 481.5 ± 293.2/μl, the number of CD8-positive cells was 376.6 ± 239.6/μl, and the CD4/CD8 ratio was 1.46 ± 0.60 in drug-resistant tuberculosis patients. The serum level of TNF-α was 17.4 ± 8.1 μg/l. These results were significantly higher than those of the healthy control group and non-drug-resistant tuberculosis group ($p<0.05$) (Table II).

Table I. Comparison of general parameters data between patients and healthy controls.

Group	Number of cases	Sex (male/female)	Age (years)	BMI (kg/m^2)
Patients with drug-resistant tuberculosis	47	22/25	56.8±3.2	22.7±3.2
Patients with non-drug-resistant tuberculosis	132	73/69	59.4±6.9	23.0±1.8
The healthy controls	183	92/91	62.3±6.4	22.1±2.1
Value of f/χ^2	-	0.43	0.43	0.28
Value of *p*	-	0.68	0.68	0.76

Table II. Serum levels of CD4, CD8, CD4/CD8, and TNF-α.

Group	Number of cases	CD4 (per μl)	CD8 (per μl)	CD4/CD8	TNF-α (mg/l)
Patients with drug-resistant tuberculosis	47	481.52±293.21	376.26±239.36	1.46±0.61	17.42±8.13
Patients with non-drug-resistant tuberculosis	132	430.33±188.21	320.32±182.19	1.34±0.13	10.73±6.52
The healthy controls	183	320.42±128.98	308.42±118.32	1.17±0.22	4.31±3.23
Value of f/χ^2	-	12.62	12.13	13.73	10.92
Value of *p*	-	0.008	0.007	0.005	0.014

Table III. Comparisons of immune cells and the levels of serum TNF-α in peripheral blood different acid-fast bacilli testing results.

Group	No.	TNF-α (μg/l)	CD4/CD8
AFB-negative (−)	7	34.83±4.28	1.03±0.04
Weakly AFB-positive (+)	3	42.33±6.58	1.13±0.34
AFB-positive (+)	10	67.23±4.39	1.21±0.21
AFB-positive (++)	16	82.33±5.48	1.33±0.43
AFB-positive (+++)	6	91.23±3.28	1.42±0.29
AFB-positive (++++)	5	104.32±24.33	1.53±0.38

Comparison of Immune Cells and Serum Levels of TNF-α in Peripheral Blood with Different Acid-Fast Bacilli Testing Results

After comparing immune cells and the serum levels of TNF-α in peripheral blood of drug-resistant tuberculosis patients with different acid-fast bacilli infection testing results, there was a positive correlation between the serum levels of TNF-α and the CD4/CD8 ratio in peripheral blood, y=0.0083x+0.7209 (r=0.0083, $p<0.05$) (Table III and Figure 1).

Changes in the Levels of TNF-α in the Different Groups of Drug-Resistant Patients Before and After Treatment

Before treatment, the differences in the levels of TNF-α in the different groups of drug-resistant patients were insignificant ($p>0.05$). After treatment, the levels of TNF-α in the different groups of drug-resistant patients were decreased, except for the patients with extensively drug-resistant tuberculosis, whose levels were not significantly decreased compared with those before treatment ($t=0.648$, $p>0.05$). The differences in the other patients before and after treatment were statistically significant ($t=8.497$, 6.258, 5.346, $p<0.05$, fully sensitive tuberculosis single drug-resistant

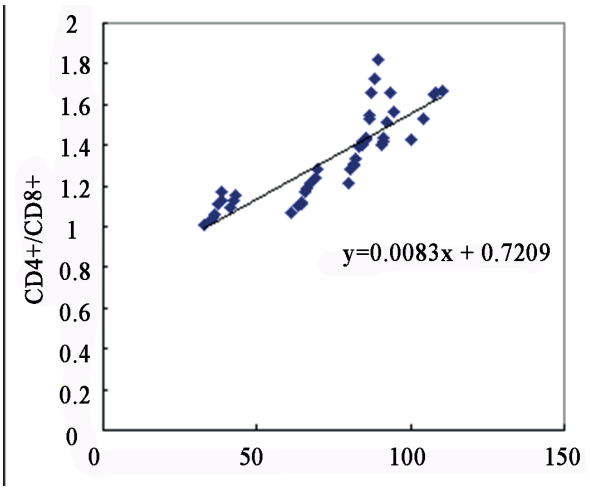

Figure 1. Correlation analysis of immune cells and serum levels of TNF-α and CD4/CD8 ratio in the peripheral blood of patients with different acid-fast bacilli infection testing results, y = 0.0083x + 0.7209, $p<0.05$.

tuberculosis, multidrug-resistant tuberculosis, respectively) (Table IV).

Correlation Analysis

The correlation coefficient between CD4-positive cell count and TNF-α level in the drug-resistant tuberculosis patients was r=0.066 (p=0.660); the correlation coefficient between CD8-positive cell count and TNF-α level in the drug-resistant tuberculosis patients was r=0.053 (p=0.722); the correlation coefficient between the CD4/CD8 ratio and TNF-α level was r=0.141 (p=0.345). The differences were not statistically significant (p>0.05).

Discussion

Tuberculosis is caused by infection with acid-fast bacilli that have no endotoxin component and no capsule to prevent phagocytosis[6]. The mechanism of their toxicity is not clear and may be related to an active component, according to some studies[7]. In addition, some lipid components, such as sulfur lipids, are associated with the toxicity of acid-fast bacilli and can inhibit the combined activity of lysosomes and phagosomes, thereby promoting the growth and reproduction of acid-fast bacilli in macrophages[8]. The phospholipids of acid-fast bacilli can stimulate the formation of Langhans giant cells and promote monocyte proliferation and cellularization of epithelioid cells[9]. The bacterial component, Wax D, can stimulate immune cells to produce immune globulins, which play an important role in the formation of tuberculous cheese-like lesions and their evolution[10]. Some polysaccharides are also essential for acid-fast bacilli. These polysaccharides do not have biological activity but can bind with other substances to form complete acid-fast bacillus antigens and participate in the immune response.

M. tuberculosis infection primarily results in cellular immunity. T lymphocytes differentiate, proliferate, and synthesize and secrete a series of cytokines after antigen stimulation, thereby producing specific cellular immunity[11]. In this process, CD4-positive lymphocytes play important roles in secreting lymphokines, activating macrophages, and promoting them to completely kill *M. tuberculosis* cells[12]. Similarly, CD8-positive lymphocytes play roles in regulating immunity. They exert cytotoxic effects on cells, such as dissolving target cells that have lost immunocompetence after infection, or promoting apoptosis, TB exposure, and lesion removal. Because of the sensitization and enhanced phagocytic function of T lymphocytes, when suffering from tuberculosis, the cellular immune response is stimulated. Also, the acid-fast bacillus bacterial protein, as a complete antigen, also plays an important role in the body's immune response to tuberculin[13]. TNF-α, also known as cachectin, is produced mainly by activated macrophages, natural killer (NK) cells, and T lymphocytes. TNF-α is a cytokine that can directly kill tumor cells and has no toxicity on normal cells. It is also one of the most active factors known that directly kills tumor cells[14]. After entering the body, acid-fast bacilli can bind with specific receptors on tumor cells, and can transfer inside them. Lysosomes then phagocytose tumor cells. The stability of lysosomes is greatly reduced and a variety of enzymes are released, causing cell lysis[15]. If the body is infected by acid-fast bacilli, the levels of local TNF-α increase rapidly, and the number of acid-fast bacilli correlates positively with the concentration of TNF-α. Higher concentrations of TNF-α indicate stronger infectivity and result in the appearance of more typical lesions on X-ray[16]. Since TNF-α can kill tumor cells, appropriate serum levels of TNF-α can effectively exert an anti-tuberculosis effect and provide immune protection. However, excessively high concentrations of TNF-α can cause immune damage to normal tissues and aggravate the condition of patients[17,18]. In our study, we found that the levels of TNF-α in peripheral blood of patients with drug-resistant tuberculosis and non-drug-resistant tuberculosis were significantly higher than those in the control group (p<0.05). Furthermore, the levels of TNF-α in the drug-resistant group were higher

Table IV. The levels of TNF-α in different groups of drug-resistant patients before and after treatment.

Types of drug resistance	No.	TNF-α		Value of t	Value of p
		Before treatment	After treatment		
Fully sensitive tuberculosis	132	92.2±10.22	44.32±5.48	8.497	0.012
Single drug-resistant tuberculosis	21	92.33±10.32	65.22±9.43	6.258	0.018
Multidrug-resistant tuberculosis	16	95.22±3.23	66.32±4.32	5.346	0.023
Extensively drug-resistant tuberculosis	10	97.83±12.98	92.31±9.43	0.648	0.317

than those in the non-drug-resistant group. There was a positive correlation between the levels of TNF-α and CD4/CD8 ratio (r = 0.892, $p<0.05$) in the peripheral blood of the two groups. After treatment, the levels of TNF-α in the different groups of drug-resistant patients were decreased, except for patients with extensively drug-resistant tuberculosis, whose levels were not significantly decreased compared with those before treatment ($p>0.05$). The differences in the levels of TNF-α in the other groups of patients before and after treatment were statistically significant ($p<0.05$). These results suggest that, as a serological marker, TNF-α may be associated with the function of the immune system of patients with TB infection. Determining the level of TNF-α in the peripheral blood of patients with tuberculosis is clinically significant for promoting tuberculosis drug resistance.

Conclusions

We observed that the level of TNF-α plays a critical role in the evaluation of the severity of patients with drug-resistant tuberculosis and it has a clinical value.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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