Immunophenotypic Characterization of Peripheral Blood T-Lymphocytes and Their Subpopulations in Tuberculosis Patients before and after Treatments

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ABSTRACT

Tuberculosis is a chronic mycobacterial infection. The main effector cells against mycobacterium tuberculosis are CD4+ T lymphocytes. Our objective in this research was to evaluate the quantity of T lymphocytes and their subpopulations before and after treatments with combination of 4 drugs (Rifampcin, Isoniaside, pyrasinamide, Ethambutal) for 2 months directly in sputum-positive tuberculosis patients. Twenty patients as cases and twenty healthy people were selected as controls. Flow cytometry was used for TCD3+, TCD4+ and TCD8+ lymphocytes by using monoclonal antibodies. Our results indicated that there was alteration in cell mediated immunity during tuberculosis showing itself as decrease in TCD3+ and TCD4+ lymphocytes and increase in TCD8+ lymphocytes. The changes in TCD3+ and TCD4+ but not in TCD8+ were reversible after 2 months of treatment.

Keywords: Cell Mediated Immunity; Immunophenotyping; T Lymphocytes; Tuberculosis

INTRODUCTION

Tuberculosis is a chronic mycobacterial infection that is caused mostly by mycobacterium tuberculosis and rarely by mycobacterium bovis. This disease is characterized by cell-mediated hypersensitivity against mycobacterium. Tuberculosis usually involves lungs but virtually any organ can be involved.[1-4]

Currently, 1/3 of the world's population are infected with tuberculosis bacillus with 8 million new cases and 3 million death due to tuberculosis estimated annually.[5-10] For better fighting against this disease, knowledge of pathogenic mechanisms and immune response provoked against this disease are essential.[10-12]

Immune responses against tuberculosis are cell mediated immunity and humoral immunity responses have no important role in spite of the fact that antibodies can be detected in the serum of these patients.[1,9]

Cellular immunity has the main role in defense against tuberculosis and many types of T lymphocytes including αβ receptor T lymphocytes ($CD4^+$ or $CD8^+$), cytotoxic T lymphocytes and γδ receptor T lymphocytes play a role in this regard.[1,11,13-16]

Without any doubt, the main effector cells against mycobacterium tuberculosis are $CD4^+$ T lymphocytes.[9,11,13,16,17] There are numerous researches about tuberculosis with many conflicting data about it.[18-23]

Our objective in this research was to evaluate the quantity of T lymphocytes in peripheral blood and their subpopulations before and after treatment in sputum-positive tuberculosis patients in the hope to answer the following questions:

1. Is there any change in the percent of T-lymphocytes and its subpopulations in the patients with tuberculosis?

2. Is there any change in T lymphocytes and their subpopulations after 2 months treatment of the tuberculosis with combination of four drugs (Rhifampsin, Isoniaside, pyrasinamide, Etambutal)?

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MATERIALS AND METHODS

This research was a case control study. Cases were sputum positive tuberculosis patients who were admitted to Isfahan University of Medical Science clinics and their diseases were confirmed by clinical, radiological and sputum smear examinations. Patients with infectious respiratory disease, immunodeficiency disease (HIV), those who had used immunosuppressive drugs or those who had close contact with tuberculosis patients were omitted from the study.

Twenty patients were selected. Thirteen patients were male, and seven were female, and the mean age of patients was 38.6 years.

Twenty healthy people without any history of immunosuppressive therapy, tuberculosis or history of exposure to tuberculosis that their age and sex matched with case were selected as controls. Blood samples were collected on EDTA from the patients and controls. Monoclonal antibodies including: Anti-CD3, Anti-CD4 and Anit-CD8 (IQ Company, Netherlands) were separately added on blood samples from each individual.

A test tube with the size of 75×12 mm was chosen for every case or control and after adding 100 µl of whole blood and 10 µl of relevant monoclonal antibody to it, the contents of tubes were mixed for 10 minutes in darkness and again after adding 2 ml of lysin solution for RBC lysis, the contents of tubes were mixed. The tubes were incubated for another 10 minutes and centrifuged at 1500 rpm for 5 minutes. After removing supernatant, 2 ml of PBS buffer was added to tubes for washing the contents and again centrifuged at 1500 rpm for 5 minutes. Having removed the supernatant, 300 ml of PBS buffer was added to tubes and the cell suspension was analyzed by flowcytometer.

These steps were repeated again 2 months after starting the treatment in tuberculosis patients and the results were compared statistically.

T test was used to compare mean of TCD4+ and TCD8+ lymphocytes between cases and controls. T-paired test was used to compare mean TCD4+ and TCD8+ before and after treatments. $P<0.05$ was considered significant.

RESULTS

The results of the study of the normal people and the patients before treatment are shown in table 1. The mean of total lymphocyte percent, TCD3+ lymphocytes and TCD4+ lymphocytes of patients group were less than normal people and these differences were significant statistically.

In comparison with control group, the mean of $TCD8^+$ lymphocytes was significantly higher in the patients group ($P=0.02$). There was a significant decrease of TCD4+ lymphocytes and an increase of TCD8+ lymphocytes and a decrease of CD4/CD8 ratio (index of cell mediated immunity) in patients before treatment (Table 1).

Mean of total lymphocytes percent, TCD4+ lymphocytes and TCD3+ lymphocytes showed significant increase after 2 months treatment of tuberculosis ($p<0.05$) (Table 1). Increase of TCD4+ lymphocytes after treatment resulted a shift in CD4/CD8 ratio from 0.8 before treatment to 1.2 after treatment ($P=0.03$).

The results of our study in control group and patient group after treatment are also shown in table 1. The mean of total lymphocytes percent showed an increase after treatment when compared with the same group of patients before treatment of tuberculosis and it was not significantly different from normal people ($P>0.05$) that showed its appropriate level. The mean of TCD3+ and TCD4+ lymphocytes percent showed an increase after treatment when compared with the same group of patients before treatment of tuberculosis which were not significantly different from normal people ($p>0.05$).

These results showed that TCD3+ lymphocytes reached to the appropriate level after treatment but TCD4+ lymphocytes and CD4/CD8 ratio were still under appropriate levels as compared with normal group.

Table 1. Results of statistical analysis in patients group before and after treatments and control group.

Cells	Patients before Treatment (no=20)	Control Group (no=20)	P-Value	Patients after Treatment	P-Value (after vs. before Treatment)	P-Value (after Treatment vs. Control group)
Lymphocyte %	17.2±7.4	27.9±8.1	P=0.02	24.1±7.1	P=0.04	P>0.05
CD3%	60.9±13.1	70.2±6.7	P=0.04	69.6±15	P=0.047	P>0.05
CD4%	25.8±9.6	41.8±3.1	P=0.035	37.1±9.5	P=0.045	P=0.048
CD8%	33.2±8.4	28.5±2.9	P=0.02	32.5±8.6	P>0.05	P=0.02
CD4/CD8	0.8±0.4	1.5±0.2	P=0.015	1.2±0.4	P=0.03	P=0.03

There was no significant change in TCD8+ lymphocytes before and after treatment and as a result, there was a significant difference between TCD8+ level in control group and its level in patients after treatment.

DISCUSSION

Our objective in this study was to evaluate some of the immunologic factors in patients with tuberculosis before and after treatments. We evaluated three different markers (CD3, CD4, CD8 and CD4/CD8 ratio) in peripheral blood of patients with tuberculosis before and after treatments. Our results showed that cell mediated immunity was altered in pulmonary tuberculosis and the decrease in total number of lymphocytes and T lymphocytes are seen. The decrease of T-CD4+ lymphocytes maybe due to compartment from peripheral blood to lungs.[9] It was also demonstrated that anti-tubercolous chemotherapy was effective in reversing this condition after 2 months. In the patients group, increase in T-CD8+ lymphocytes along with decrease in T-CD4+ lymphocytes before treatment, resulted in a decrease of CD4/CD8 ratio, which was also observed in our study.

Significant increase in T-CD8+ lymphocytes in the patients before treatment, which did not change significantly in response to treatment, was observed in this study. These T lymphocytes may include T suppressor subgroup that necessitates the use of cytokine evaluation for confirmation.

$TCD8^+$ lymphocytes, in addition to their role in controlling mycobacterial infection through immunologic recognition and responses to mycobacterium and secreting gamma interferon and TNF, have the ability to shift immunologic response towards Th1.

Tsao and his colleagues divided the patients in to 3 groups of mild, moderate and severe based on the severity of their diseases. This study included evaluation of the both peripheral blood and bronchoalveolar lavage fluid (BALF). Decrease in TCD4+ lymphocytes in peripheral blood and its increase in BALF along with increase in TCD8 lymphocytes in peripheral blood and a decrease in BALF were seen in this study. These changes were more significant in the patients with more severe from of disease. The results of Tsao's study on peripheral blood are compatible with our results except that he did not evaluate patients after treatment.[24]

Rodrigues and his colleagues evaluated $CD4^+$ and $CD8^+$ lymphocytes in 3 groups of patients including patients with active tuberculosis, newly treated patients and healthy people. They concluded that $CD4^+$ and $CD8^+$ lymphocytes decreased in active tuberculosis in comparison with healthy people, resulting no changes in CD4/CD8 ratio. In the treated patients, there was an increase in $CD4^+$ and $CD8^+$ lymphocytes.

Rodriguez's study has some conflicts with our study. In contrary to Rodriguez's study, $CD8^+$ lymphocytes were increased in our study causing CD4/CD8 ratio to be decreased. Meanwhile, we evaluated the same group of patients before and after treatment but Rodrigues evaluated 2 different groups of patients at the same time; one group were patients who didn't take any treatment, and the other group were patients who were under treatment for 6 months.[16]

Onwubalili and his colleagues studied patients with various kinds of tuberculosis and concluded that TCD4+ lymphocytes and total lymphocytes count (TCD3+) were decreased but there was no change in TCD8+ lymphocytes compared to control group. Following 6 weeks anti-tuberculosis treatment, an elevation in total T lymphocytes and TCD4 lymphocytes was observed.[19] Unlike Onwubalili, we studied patients with only pulmonary tuberculosis and found an increase in TCD8 lymphocytes.

Swaminathan and his colleagues studied children with age range of 1 to 12 years old suffering from tuberculosis. They showed a decrease in $TCD4^+$ lymphocytes with an increase in $TCD8^+$ lymphocytes. They reported an elevation in $TCD4^+$ lymphocytes after treatment for tuberculosis.[22] Unlike their study, we evaluated adult patients with tuberculosis.

Jones and his colleagues evaluated 85 patients with tuberculosis including patients with sputum positive pulmonary tuberculosis, sputum negative pulmonary tuberculosis and extrapulmouary tuberculosis. Decrease in total lymphocytes count, CD4+ and CD8+ lymphocytes but without any change in CD4/CD8 ratio were reported by these researchers.[21] Jones proposed that significant decrease in CD4+ lymphocytes was related to only sputum positive pulmonary tuberculosis but was not seen in sputum negative or extrapulmouary tuberculosis. This decrease in TCD4+ lymphocytes that points out to pulmonary tuberculosis was also confirmed by Taso.

Jones's study is different from our study because he included extrapulmouary and sputum negative tuberculosis in their study. However, decrease in $CD4^+$ lymphocytes and total lymphocytes count was seen in sputum positive tuberculosis as in our study. Unlike our study, there was no increase in $CD8^+$ lymphocytes in Jones's study.

The result of our study and other studies confirm alteration in cells mediated immunity during infection with mycobacterium tuberculosis.

Although decrease in $CD4^+$ lymphocytes that are the main cells in defence against tuberculosis, has been established, there is no consensus about a decrease or increase of $CD8^+$ lymphocytes in tuberculosis.

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