Serum Interleukin-2 and Neopterin Levels as Useful Markers for Treatment of Active Pulmonary Tuberculosis

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Macrophages and T cells are responsible for the main immune response to tuberculosis by secreting many cytokines and other substances. The aim of this study was to determine the effects of multidrug treatment on serum levels of interleukin-2 (IL-2), secreted by activated T cells, and of neopterin, secreted by macrophages and monocytes, in patients with pulmonary tuberculosis. The study included 30 patients with active pulmonary tuberculosis, confirmed by the detection of acid-fast bacilli in direct sputum smears and/or sputum cultures. The serum levels of IL-2 and neopterin were measured before and during the treatment and compared with 15 patients with inactive pulmonary tuberculosis and 15 healthy controls. Serum IL-2 and neopterin levels were higher in patients with active tuberculosis (164.53 ± 58.91 pg/ml and 69.54 ± 29.42 nmol/l, respectively) than those in inactive tuberculosis (95.43 ± 31.17 pg/ml and 10.71 ± 1.78 nmol/l) or controls (79.20 ± 14.81 pg/ml and 9.50 ± 2.27 nmol/l) (p < 0.001 for each parameter). No significant differences were found in IL-2 and neopterin levels between inactive tuberculosis and control subjects. The IL-2 levels remained elevated in active tuberculosis at 2nd month of treatment (p < 0.001) and decreased to the control levels after 4th month. Neopterin levels were significantly higher in active tuberculosis than those in inactive tuberculosis or controls at the 2nd and 4th months of treatment. These findings indicate that measurements of serum IL-2 and neopterin levels are useful in following up the treatment and immune response to tuberculosis.

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Therefore, a better understanding of the pathogenesis of this condition and the development of novel prevention and management strategies are necessary.

In tuberculosis, the host-pathogen interaction is initiated by inhalation of *Mycobacterium tuberculosis* followed by phagocytosis by alveolar macrophages. Granuloma formation takes place within 2-6 weeks, induced by the cellular immune response through the accumulation of lymphocytes and macrophages at the site of the lesion (Raja 2004). T-cells play a key role in the response against the bacillus. Interleukin-2 (IL-2), released by activated T-cells, is important in the activation and proliferation of these cells (Johnson et al. 2003). IL-2, together with interferon-γ, is released by T-helper (Th)-1 cells, and these two cytokines have roles in the protective immune response (Schluger and Rom 1998).

Neopterin, a biochemical marker related to the cellular immune response, is associated with the activity of monocytes and macrophages. It is mainly produced by these cells and subsequently released into the body fluids. Measurement of neopterin in body fluids not only gives information about the actual cellular immune response, but is also helpful in monitoring diseases (Berdowska and Zwirska-Korczala 2001).

In this study, we aimed to assess cellular immunity using serum IL-2 and neopterin levels in patients with pulmonary tuberculosis, and to observe the changes in these markers during standard tuberculosis therapy.

**MATERIALS AND METHODS**

**Cases**

The study included 45 patients comprising 30 active and 15 inactive cases of pulmonary tuberculosis and 15 healthy subjects.

**Cases with active pulmonary tuberculosis.** This group consisted of 30 patients (17 males, 13 females, mean ± s.d. age 39.53 ± 16.59 year) diagnosed during January to September 2004 as pulmonary tuberculosis in either Firat (Euphrates) University Medical Faculty, Department of Chest Diseases, Elazig-Turkey or the Elazig Tuberculosis Dispensary. The diagnoses of tuberculosis were confirmed by positive detection of acid-fast bacilli (AFB) in direct sputum smears and/or sputum cultures from cases showing clinical and radiological findings that suggested tuberculosis. Patients with non-pulmonary tuberculosis, or with an additional systemic disease (such as diabetes mellitus or an autoimmune or immunological disease), or who had developed an additional infection in the previous 15 days, were excluded from the study. All the cases were HIV(−). Ziehl-Neelsen staining was used to detect *M. tuberculosis* in sputum, and Lowenstein-Jensen medium was used for culturing. Following the diagnosis, the patients began quadruple antituberculosis therapy including rifampicin (RMP), isoniazid (INH), pyrazinamide (PRZ) and ethambutol (EMB) or streptomycin (SM). After a two-month course, drugs other than RMP and INH were discontinued and the therapy was continued with remaining two drugs for an additional four months. No adverse chemotherapeutic effects were observed during the total 6 months of treatment. Five milliliters of venous blood were taken from each patient at the beginning of treatment and after the 2nd, 4th and 6th months of the therapy course.

**Cases with inactive pulmonary tuberculosis.** This group consisted of 15 pulmonary tuberculosis patients (8 males and 7 females, mean ± s.d. age 38.80 ± 16.26 year). Initial diagnoses were established by positive cultures. These patients were subsequently followed up by the Elazig Tuberculosis Dispensary. No radiological changes were observed during the six months follow-up period. Moreover, three sputum smears and a culture were prepared for each case, and all were negative.

**Healthy individuals (control group).** This group consisted of 15 healthy individuals (6 males, 9 females, mean ± s.d. age 35.07 ± 4.65 year) age and sex matched with the other groups.

Five milliliters of venous blood were taken from each of the inactive tuberculosis patients and healthy controls.

Since Bacillus Calmette-Guerin (BCG) is routinely used as a vaccine in accordance with the National Vaccination Program, all the cases in the study including the healthy controls were positive for purified protein derivative (PPD). The PPD test was performed using the Mantoux method with intradermal injection of 5 IU PPD (BB-NCIPD Ltd., Sophia, Bulgaria) on the medial aspect of forearm. An induration greater than 10 mm in diameter was accepted as positive.
Levels of IL-2 and neopterin

Blood samples obtained from the subjects were centrifuged for 10 min at 5,000 rpm and stored at -80°C prior to analysis. Serum IL-2 (Bender MedSystems GmbH, Vienna, Austria) and neopterin (DRG Instruments GmbH, Germany) levels were measured by ELISA in accordance with the recommendations of the manufacturers.

The requisite information was given to the patients and written consent was obtained from all patients.

Statistical analysis

Statistical analyses were carried out using the SPSS® v11.0 program pack. Values obtained from the cases were given as means ± S.D. Kruskal-Wallis and Mann-Whitney’s U-tests were performed for between-group comparisons. For patients with active tuberculosis, the values obtained at the beginning and after 2, 4 or 6 months of therapy were compared using the Wilcoxon Signed Ranks Test. Values of *p* < 0.05 were accepted as statistically significant.

RESULTS

There was no difference among the three study groups with respect to age or gender (*p* > 0.05). The PPD values of active (17.16 ± 2.96 mm) and inactive (15.93 ± 1.53 mm) tuberculosis patients were significantly greater than those of the control group (12.86 ± 1.40 mm) (*p* < 0.001 for both). The mean serum IL-2 and neopterin levels of each of these groups are shown in Table 1.

| Table 1. Serum IL-2 and neopterin levels in patients with tuberculosis and healthy subjects. |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Active tuberculosis (before treatment) (n = 30) (Mean ± S.D.) | Inactive tuberculosis (n = 15) (Mean ± S.D.) | Control (n = 15) (Mean ± S.D.) |
| IL-2 (pg/ml) | 164.53 ± 58.91 | 95.43 ± 31.17 | 79.20 ± 14.81 |
| Neopterin (nmol/l) | 69.54 ± 29.42 | 10.71 ± 1.78 | 9.50 ± 2.27 |

In patients with active tuberculosis, the mean serum IL-2 and neopterin levels were significantly higher than the patients with inactive tuberculosis or the healthy controls (*p* < 0.001 for both). There was no difference in IL-2 or neopterin levels between the inactive tuberculosis and control groups (*p* > 0.05) (Table 1).

The mean serum levels of IL-2 and neopterin in the patients with active tuberculosis after therapy were compared with the values in the other two groups (Table 2 and Fig. 1). The levels of IL-2 remained elevated at the 2nd month (*p* < 0.05 for active vs inactive, and *p* < 0.001 for active vs control). In addition, the neopterin levels were higher in the active tuberculosis patients at the 4th month than those in the inactive and control groups (*p* < 0.001 for both) (Fig. 2). By the 6th month, no differences were observed between the groups for these two parameters (*p* > 0.05). The mean serum IL-2 levels after 2 months of treatment differed significantly from the baseline value in patients with active tuberculosis (*p* < 0.001), and there were also significant differences between the levels at the 2nd and 4th months (*p* < 0.01), but not between the 4th and 6th months (*p* > 0.05). Also, the mean serum neopterin level was progressively reduced during the therapy course (between baseline and 2nd month values, *p* < 0.01); between 2nd and 4th month values, *p* < 0.001; and between 4th and 6th month values: *p* < 0.001). Serum IL-2 and neopterin levels of each

| Table 2. Serum IL-2 and neopterin levels of patients with active tuberculosis before and after the treatment of 2, 4 and 6 months. |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Initial (Mean ± S.D.) | 2nd month (Mean ± S.D.) | 4th month (Mean ± S.D.) | 6th month (Mean ± S.D.) |
| IL-2 (pg/ml) | 164.53 ± 58.91 | 111.52 ± 36.42 | 94.86 ± 33.70 | 90.25 ± 30.80 |
| Neopterin (nmol/l) | 69.54 ± 29.42 | 54.16 ± 19.25 | 25.60 ± 9.12 | 12.89 ± 6.80 |
patient with active tuberculosis are summarized in Table 3.

**DISCUSSION**

This study demonstrated that mean serum IL-2 and neopterin levels are higher in patients with active tuberculosis than in patients with inactive tuberculosis or in healthy controls, but these values decline with therapy to the levels similar to those displayed by the inactive tuberculosis and healthy control groups after treatment of six months.

Clinically, the presentation of tuberculosis is more severe in individuals sustaining functional T-cell impairment such as people of advanced age, corticosteroid users and AIDS patients.
contrast, the risk of tuberculosis is not enhanced in individuals with humoral immune defects (sickle cell anemia, multiple myeloma, etc.). These two observations are consistent with the fact that successful elimination of *Mycobacterium tuberculosis*, an intracellular pathogen, depends on the interaction between infected macrophages and antigen specific T-cells (Stenger and...
It is well known that CD4+ Th cells, which participate in the cellular immune response, are divided into two subgroups on the basis of the cytokines they produce. Th1 cells are involved in cellular immunity and in phagocytosis-associated inflammatory processes, principally by producing interferon (IFN)-γ, IL-2 and tumor necrosis factor (TNF)-β (Romagnani 2000).

Many studies have focused on the detection of cytokine levels in tuberculosis cases. In one of these, cytokine release was studied in CD4+ culture media obtained from 20 patients with active pulmonary tuberculosis and 30 healthy PPD (+) individuals. This study demonstrated that in cases with active disease, IL-2 gene expression is substantially higher than in the healthy control group (Lai et al. 1997). Another study using cell cultures demonstrated that although the activity of IL-2 is higher in newly-diagnosed active pulmonary tuberculosis patients than in a healthy control group, it is reduced in advanced and resistant cases, indicating that IL-2 has an important role in regulating the immune response (Shiratsuchi et al. 1987). In addition, a study scrutinizing the association between the severity of tuberculosis and serum cytokine levels emphasized that levels of soluble IL-2 receptor (sIL-2R) are higher in the patient group than in the control group, but no relationship between sIL-2R levels and disease severity was revealed (Kart et al. 2003). Sánchez et al. (1994) IL-2 levels in cultures of peripheral blood mononuclear cells were higher in active pulmonary tuberculosis patients than in PPD (+) healthy control subjects. Interestingly, in that study, the cell cultures were stimulated with PPD, and while a highly significant increase in IL-2 production over baseline values was noted in the control group, there was no apparent change from the baseline values in the patient group. As a result, the authors reported that stimulation caused a Th1-predominant immune response to develop in healthy PPD (+) controls.

In contrast to the publications mentioned above, Zhang et al. (1995) studied the levels of IFN-γ and IL-2 in peripheral blood mononuclear cells from tuberculosis patients and 30 healthy individuals. The IFN-γ levels were higher in the tuberculosis cases but the groups did not differ with respect to IL-2 levels. Barnes et al. (1993) found that in cases with tuberculous pleurisy, substantially more IL-2 was produced by pleural fluid mononuclear cells than by peripheral blood mononuclear cells. This study suggested that like IFN-γ, IL-2 might facilitate the elimination of the mycobacteria by potentially acting as a T-cell development and activation factor. All these data support the idea that Th1 cells have a predominant role in the immune response to active tuberculosis. On the basis of these results, the use of IL-2 in tuberculosis therapy was suggested, and studies giving both favorable and unfavorable results followed; it was noted that IL-2 may be a potential immunotherapy agent (Johnson et al. 1995, 1997, 2003). In our study, serum IL-2 levels were also found to be higher in cases with active pulmonary tuberculosis than in cases of inactive tuberculosis or healthy controls. The increased levels of IL-2, as a cytokine originating from Th1 cells in tuberculosis, suggest that the cellular response has an important role in the pathogen-host interaction in this disease.

Neopterin, produced by guanosine triphosphate as a response to lymphocyte-derived IFN-γ and released from activated macrophages, is a biochemical marker of monocyte/macrophage activity. The amount released from activated macrophages correlates with reactive oxygen species (ROS) (Murr et al. 1999; Berdowska and Zwirska-Korczala 2001; Hoffmann et al. 2003). Abnormal neopterin levels in body fluids have been reported in many diseases, i.e., infections, autoimmune diseases, malignancies, cardiac and renal failure, coronary arterial diseases and myocardial infarction (Berdowska and Zwirska-Korczala 2001). Neopterin levels in tuberculosis have been studied in a variety of fluids such as serum, bronchio-alveolar lavage (BAL) and urine to obtain information about the immune response in this disease. Serum neopterin levels are reported to be significantly higher in active tuberculosis than in healthy control groups (Vanham et al. 1996; Immanuel et al. 2001, 2005). In a study aimed at achieving a better understanding of the local cellular immune response, neopterin levels...
were measured in BALs from pulmonary tuberculosis patients, and the serum and BAL levels of neopterin were found to be higher than in pulmonary cancer patients or healthy individuals. This difference was even more marked in advanced tuberculosis patients (Mohamed et al. 2001). In another study, Baganha et al. (1992) found that neopterin levels in the pleural fluids from 10 cases of tuberculosis and 15 of malign pleurisy were higher in the tuberculous pleurisy patients. As a result, neopterin was considered a useful marker for detecting the etiology of pleurisy. Moreover, it has been suggested that measurements of urinary neopterin levels in pulmonary tuberculosis patients might reflect disease activity earlier than cultures (Yuksel et al. 2003).

In our study, as in previous studies on pulmonary tuberculosis, mean serum neopterin levels were higher in the active tuberculosis group than in the inactive tuberculosis cases or healthy controls. As with IL-2, high levels of neopterin, of which the main source is activated macrophages, demonstrate the importance of the cellular immune response in tuberculosis. In some research, both IL-2 and neopterin have been measured in peripheral blood mononuclear cell cultures. However, as in the study by Immanuel et al. (1997), values obtained from cell cultures stimulated with PPD or bacteria may not show any difference between patient and control groups. In view of these data, our measurements were performed on serum.

A limited number of studies have investigated the relationship between tuberculosis therapy and neopterin as well as IL-2. Measurements of serum IL-2 and IFN-γ levels in 18 active tuberculosis patients at the initial phase and during the 2nd month of therapy showed an apparent decrease of these two cytokines after treatment. Since there was no control group, these authors could not state whether the cytokine levels had regressed to values in healthy individuals (Berktaş et al. 2004). In our study, IL-2 levels were also significantly reduced by the 2nd month of treatment but had returned to healthy control values by the 4th month. Serum neopterin levels in tuberculosis patients decrease steadily from the baseline point to the end of antituberculous therapy, and this diminution is more prominent in patients with moderately severe tuberculosis. These results also suggest that immune activation is more powerful in patients with moderately severe tuberculosis. This study emphasizes that measurement of serum neopterin levels can help to evaluate the response to therapy (Immanuel et al. 2001). Similarly, Hosp et al. (1997) concluded that neopterin could be used to monitor the response to therapy. Another study showed that measuring neopterin levels using a noninvasive method in pediatric patients with pulmonary tuberculosis might be useful for assessing the degree of disease activity and the response to therapy (Horak et al. 1998). Immanuel et al. (2005) showed that serum neopterin levels in HIV seropositive and HIV seronegative patients with tuberculosis are higher than in a control group. In HIV seropositive patients with CD4 < 200/mm³ in the peripheral blood, these levels were higher before treatment and decreased more markedly during treatment. The authors therefore proposed that measurement of neopterin levels can also be used as a technically easy and cheap test, particularly for early diagnosis and treatment of tuberculosis in immunocompromised patients. Our results also indicate that the serum levels of IL-2 and neopterin could be helpful in assessing the immune response to treatment.

In conclusion, serum IL-2 and neopterin levels are elevated in cases with active tuberculosis, but are markedly reduced by effective and appropriate therapy. Thus, these two parameters are useful in evaluating current therapy. Moreover, a therapy course of 6 months in the treatment of tuberculosis seems to be enough for immunological improvement.

References


