The value of serum interferon-γ level in the differential diagnosis of active and inactive pulmonary tuberculosis

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ÖZET

Serum interferon-γ düzeyinin aktif ve inaktif pulmoner tüberküloz ayırıcı tanısındaki değeri

İnterferon (IFN)-γ, Mycobacterium tuberculosis'e karşı koruyucu immünitede önemli rol oynamaktadır. Pulmoner tüberkülozlu hastaların etkilenen akciğerlerinde ve kanında IFN-γ düzeylerinin arttığı görülmüştür. Bu çalışmada, serum IFN-γ düzeyinin aktif ve inaktif pulmoner tüberküloz ayırıcı tanısındaki yerinin araştırılması amaçlanmıştır. Çalışmaya yeni tanı almış aktif pulmoner tüberkülozlu 47 hasta, inaktif pulmoner tüberkülozlu 21 hasta ve 20 sağlıklı kontrol dahil edildi. Alınan serum örnekleri IFN-γ analizine kadar -70°C'de saklandı. Ortalama IFN-γ düzeyleri yeni tanı almış aktif pulmoner tüberkülozlu hastalarda 9.3 ± 4.6 pg/mL, inaktif pulmoner tüberkülozlu hastalarda 9.8 ± 3.8 pg/mL ve sağlıklı kontrollerde 10.2 ± 3.4 pg/mL bulundu. Üç grubun IFN-γ düzeyleri arasında istatistiksel olarak anlamlı bir fark bulunmadı (p= 0.4). Serum IFN-γ düzeyi, aktif ve inaktif pulmoner tüberkülozun ayırıcı tanısında değerli bulunmamıştır.

Anahtar Kelimeler: İnterferon-γ, serum, tüberküloz.

SUMMARY

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Interferon (IFN)-γ plays a pivotal role in protective immunity against Mycobacterium tuberculosis. Elevations of IFN-γ have been found in the affected lung and bloodstream of patients with pulmonary tuberculosis. In the present study, we aimed to investigate the role of serum IFN-γ level in the differential diagnosis of active and inactive pulmonary tuberculosis. Fourty seven patients with newly diagnosed active pulmonary tuberculosis, 21 patients with inactive pulmonary tuberculosis, and 20 healthy volunteers were enrolled in the study. Serum samples were collected from each subject and stored at -70°C until the analysis of IFN-γ. The mean value of IFN-γ levels were 9.3 ± 4.6 pg/mL in patients with newly diagnosed pulmonary tuberculosis, 9.8 ± 3.8 pg/mL in patients with inactive tuberculosis, and 10.2 ± 3.4 pg/mL in healthy controls. The comparison of IFN-γ levels of the three groups was not found statistically significant (p= 0.4). Serum IFN-γ level was not found to be valuable in the differential diagnosis of active and inactive pulmonary tuberculosis.

Key Words: *Interferon-γ, serum, tuberculosis.*

Tuberculosis is an important global health problem, as one-third of the world`s population is estimated to be infected with *Mycobacterium tuberculosis*, and 8 million new active cases occur annually (1). Early detection and treatment of active pulmonary tuberculosis cases are significant measures in the fight against tuberculosis.

The diagnosis of pulmonary tuberculosis depends on the demonstration of acid-fast bacilli on sputum smears and isolating *M. tuberculosis* in sputum cultures, in patients with clinical and radiological findings consistent with tuberculosis. However, failure to isolate *M. tuberculosis* is not a rare condition. In United States about 17% of the reported new cases of pulmonary tuberculosis have negative cultures (2). Low bacillary populations, temporal variations in the number of bacilli being expelled, and errors in specimen processing all may result in failure to isolate organisms from patients who have active tuberculosis (3).

Cellular immunity plays an important role in response to infection with *M. tuberculosis*. Resistance to infection with *M. tuberculosis* is mediated by macrophages, T cells, and their interaction, and is dependant on the interplay of cytokines produced by each cell (4). Infection is chiefly controlled by the activation of macrophages through type 1 cytokine production by CD4+ T lymphocytes. IFN-γ has been shown to be the central of this process (5). Elevations of IFN-γ have been found in the affected lung (6-8) and bloodstream (8-12) of patients with pulmonary tuberculosis.

In the present study, in order to investigate the role of serum IFN-γ level in the differential diagnosis of active and inactive disease, we determined serum IFN-γ levels in patients with newly diagnosed pulmonary tuberculosis and compare them with the levels in patients with inactive tuberculosis and healthy controls.

MATERIALS and METHODS

Patients

Fourty seven patients (38 male, 9 female) with newly diagnosed active pulmonary tuberculosis (group 1), 21 patients (13 male, 8 female) with inactive pulmonary tuberculosis (group 2), and 20 healthy volunteers (17 male, 3 female) (group 3) were enrolled in the study. All patients were HIV negative. To eliminate the possible influence of other diseases, we excluded patients with a history of autoimmune diseases, malignancy, history of recent trauma or surgery, and pregnancy. Patients who had miliary tuberculosis and who had already received antituberculosis therapy or were on steroid or other immunosuppresive therapy were also excluded from the study.

The diagnosis of pulmonary tuberculosis was suggested by a positive sputum smear for acid fast bacilli (AFB), typical paranchymal tuberculosis on chest radiography, and later confirmed by a positive culture for *M. tuberculosis*. The diagnosis of inactive pulmonary tuberculosis was done based on a positive history of tuberculosis therapy, sequel lesions on chest radiography, and negative sputum smears and cultures for *M. tuberculosis*. In all of the subjects a detailed medical history was obtained and all of them underwent physical examination, chest radiography, and blood analysis for IFN-γ. Informed consent about cytokine measurements was obtained from each subject.

Methods

Venous blood samples (6 mL) were collected from each subject and serum was processed within 30 minutes and stored at -70°C until the analysis of IFN-γ. The blood samples were obtained before the initiation of antituberculosis chemotherapy from active pulmonary tuberculosis patients. IFN-γ levels were measured with an ELISA system (cytElisa) according to the instructions of the manufacturer. The detection limit of the assay was 8 pg/mL to 500 pg/mL.

Statistical Analysis

Analysis of data was done using statistical package program for the social sciences for windows, release 10.0. The results were expressed as mean ± SD. Groups were compared with non parametric Kruskal Wallis and Mann Whitney U tests. A p value less than 0.05 was considered significant.

RESULTS

Group 1 was consisted of 47 patients with active pulmonary tuberculosis (38 male, 9 female) with a mean age of 38 ± 16 (range:17-80) group 2 was consisted of 21 patients with inactive tuberculosis (13 male, 8 female) with a mean age of 44 ± 12 (range: 25-70) and group 3 was consisted of 20 healthy volunteers (17 male, 3 female) with a mean age of 38 ± 7 (range: 22-54) years.

The mean value of IFN-γ levels were 9.3 ± 4.6 pg/mL (range: 4-25.6, 95% CI of mean: 7.9-

10.6 pg/mL) in group 1, 9.8 ± 3.8 pg/mL (range: 4.06-15.9, 95% CI of mean: 8-11.6 pg/mL) in group 2, and 10.2 ± 3.4 pg/mL (range: 4-15.9, 95% CI of mean: 8.6-11.8 pg/mL). Mean ages, sex distribution, and serum IFN-γ levels of the study groups are listed in Table 1. Median and interquartile range of IFN-γ levels of the study groups are shown in Figure 1. The median IFN-γ level of group 1 was 8.4 pg/mL, group 2 was 10.6 pg/mL, and group 3 was 10.3 pg/mL. The comparison of IFN-γ levels of the

Table 1. Mean ages, sex distribution, and serum IFN-γ levels of the study groups.

	Group 1 (n= 47)	Group 2 (n= 21)	Group 3 (n= 20)
Mean age (years)	38 ± 16	44 ± 12	38 ± 7
Sex (Male/Female)	38/9	13/8	17/3
IFN-γ (pg/mL)*	9.3 ± 4.6	9.8 ± 3.8	10.2 ± 3.4

* IFN-γ levels were not found significantly different among the study groups (p= 0.4).

Results expressed as mean ± SD

Group 1: Newly diagnosed pulmonary tuberculosis patients

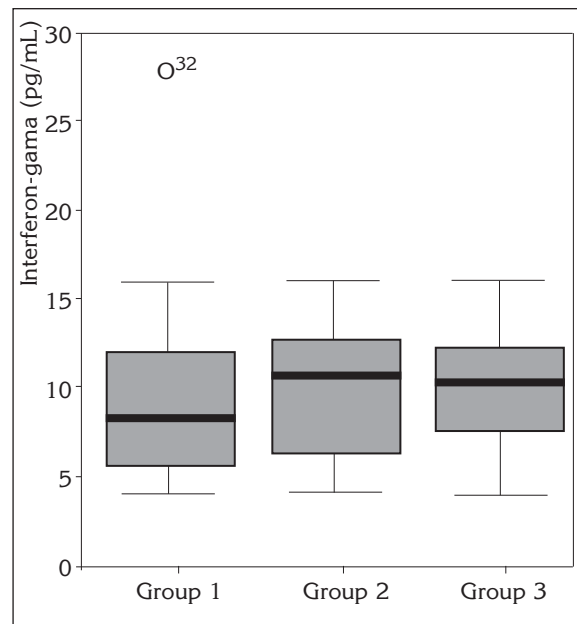Group 2: Inactive pulmonary tuberculosis patients

Group 3: Healthy control

Figure 1. Median and interquartile rangeof IFN-γ levels of the study groups are seen.

three groups was not found statistically significant (p= 0.4).

DISCUSSION

Tuberculosis is primarily acquired through inhalation of airborne droplets containing *M. tuberculosis*. When the inhaled bacilli reached the alveoli, they are ingested by mononuclear phagocytes which play a central role in antigen presentation and in eliciting IFN-γ production by Th1 lymphocytes. When mononuclear phagocytes are exposed to M. tuberculosis, antigen-presenting cells produce interleukin (IL)-12 and IL-18, which bind to their respective receptors and induce IFN-γ production (13,14). IFN-γ plays a pivotal role in protective immunity against *M. tuberculosis*. Mice with a targeted deletion of the IFN-γ gene have a markedly increased susceptibility to tuberculosis (15,16), and humans with a defective IFN-γ receptor develope severe mycobacterial infections (17,18).

Cytokines are thought to be transiently produced following antigenic stimulation in vivo. Thus, elevated cytokine levels are generated following prolonged stimulus, and this could be expected in patients with tuberculosis. Therefore, we conducted a study investigating the role of serum IFN-γ level in the differential diagnosis of active and inactive pulmonary tuberculosis. And we demonstrated that serum IFN-γ level is not useful for the differential diagnosis of active and inactive disease.

It can be easily hypothesed that serum IFN-γ level in a tuberculosis patient is due to leakage of this cytokine from tissue into the circulation, because of the increased local vascular permeability that favors diffusion of IFN-γ into the bloodstream. There are studies reporting elevations of IFN-γ in the affected lung (6-8) and bloodstream (8-12) of patients with pulmonary tuberculosis. Besides, mean serum IFN-γ concentrations are higher in patients with severe disease, who are likely to exhibit the most severe local inflammatory response and production of the highest concentrations of cytokines (9,12). Finally, the decrease of serum IFN-γ levels with the resolving of tissue inflammation during therapy also support this hypothesis (9,11).

We cannot find plausible explanations for the results of the present study. The unchanged levels of IFN-γ were not consistent with the data presented in some previous studies (8-12). But when we review the literature there is also conflicting data. In the literature, studies of the systemic cytokine response in patients tuberculosis have focused either on serum cytokine levels, or cytokine production by peripheral blood mononuclear cells. In studies determining serum concentrations, IFN-γ levels were found to be increased in patients with tuberculosis compared to healthy persons (8-12). In the studies determining 'ex vivo' cytokine production capacity of isolated peripheral blood mononuclear cells or CD4+ T cells after stimulation with *M. tuberculosis*, there are different results, reporting decreased (19-21) or unchanged (22) cytokine production. The explanation for these variable results is not clear, but differences in isolation and culture techniques, and differences in the stimulus used can be responsible. Besides 'ex vivo' stimulated production of cytokines does not necessarily provide insight into the actual status of the cytokine network in vivo.

Vankayalapati et al., were the first who studied both the serum cytokine concentrations and *M. tuberculosis*-stimulated cytokine production by peripheral blood mononuclear cells obtained from the patients with *M. tuberculosis* infection. They found that serum IFN-γ levels were higher in patients with tuberculosis than in healthy tuberculin skin test responders. In contrast *M. tuberculosis*-stimulated peripheral blood mononuclear cells recovered from tuberculosis patients produced less IFN-γ. They concluded that serum cytokine concentrations do not reflect cytokine production by peripheral blood cells (23).

In conclusion, the present study demonstrated that serum IFN-γ level is not valuable in the differentiating active pulmonary tuberculosis from inactive disease.

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