

# THE PERCENTAGE OF T-LYMPHOCYTE AND NKT CELLS IN PERIPHERAL BLOOD OF PATIENTS WITH ACTIVE AND INACTIVE PULMONARY TUBERCULOSIS

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## ABSTRACT

**Objective:** T-lymphocyte and natural killer T (NKT) cells have an important role in viral and other intracellular infections. In the present study, it was aimed to investigate whether a difference of T and NKT cells ratio between active and inactive pulmonary tuberculosis (PT) patients.

**Material and Method:** Seventy five active and 25 inactive Pulmonary tuberculosis (PT) patients and 20 healthy individuals, having no disease were included in the study. Two mL venous blood samples were withdrawn from all patients and healthy subjects for studying T and NKT cells. Total T cells and NKT cells [CD3+, and CD3+CD(16+56)+] were established from EDTA blood. The blood cells were analyzed by Coulter EPICS XL-MCL (Beckman Coulter, USA) flow cytometry equipment.

**Results:** Percentages of total T cells were not found to be different between active and inactive PT cases, but they were significantly lower than in controls (64.9±9.6, 65.2±5.4, 73.2±7.1, respectively, p=0.001 for active and

p=0.003 for inactive patients compared with controls). NKT cells percentages were not statistically different between in patients with active and inactive. In addition, NKT percentages in active and inactive PT patients did not differ statistically than healthy subjects (5.25±3.93, 5.11±2.48, 5.91±6.09 respectively).

**Conclusion:** Many studies showed NKT cells can play an important role for protection from M. tuberculosis infections but in the present study, we could not found any difference regarding NKT cells among active, inactive PT patients and healthy controls. But percentages of total T cells were found significantly lower in patients with tuberculosis compared with controls.

The fewness of T-lymphocyte percentage in peripheral circulating blood in patients with tuberculosis compared to the healthy peoples that shows the importance of the T-lymphocyte in the pathogenesis of tuberculosis.

**Key Words:** T-lymphocyte, NKT cell, tuberculosis Nobel Med 2011; 7(2): 50-54

## AKTİF VE İNKTİF AKCİĞER TÜBERKÜLOZLU HASTALARIN PERİFERİK KAN T-LENFOSİT VE NKT HÜCRE YÜZDELERİ

### ÖZET

**Amaç:** T-lenfositleri ve doğal öldürücü T (NKT) hücreleri viral ve diğer hücre içi enfeksiyonlarda önemli bir role sahiptir. Mevcut çalışmada, aktif ve inaktif akciğer tüberkülozlu (AT) hastalarda T ve NKT hücrelerinin oranlarında bir farklılık olup olmadığının araştırılması amaçlanmıştır.

**Materyal ve Metod:** Çalışmaya 75 aktif ve 25 inaktif AT hastası ile herhangi bir hastalığı olmayan 20 sağlıklı birey alındı. T ve NKT hücrelerini çalışmak için tüm hastalardan ve sağlıklı bireylerden 2ml venöz kan örneği alındı. Total T hücreleri ve NKT hücreleri [CD3+, and CD3+CD(16+56)+] EDTA'lı kanda incelendi. Kan hücreleri Coulter EPICS XL-MCL (Beckman Coulter USA) akım sitometri cihazında analiz edildi.

**Bulgular:** Total T hücrelerinin yüzdesi, aktif ve inaktif

tif AT vakaları arasında farklı bulunmadı fakat kontrol grubundan önemli olarak daha düşüktü (64,9±9,6, 65,2±5,4, 73,2±7,1, sırasıyla, aktif için p=0,001 ve inaktif için p=0,003, kontrol grubuyla karşılaştırıldığında). NKT hücrelerinin yüzdeleri aktif ve inaktif AT hastaları arasında istatistiksel olarak farklılık göstermedi. İlaveten, NKT yüzdeleri, aktif ve inaktif AT hastalarında sağlıklı bireylerden istatistiksel olarak farklı değildi (5,25±3,93, 5,11±2,48, 5,91±6,09 sırasıyla).

**Sonuç:** Pek çok çalışmada NKT hücrelerinin *M. tuberculosis* enfeksiyonundan korunmada önemli bir rol oynayabileceği gösterilmesine rağmen bu çalışmada biz aktif, inaktif AT hastaları ve sağlıklı kontrol grubu arasında NKT hücrelerine ilişkin bir farklılık bulamadık. Fakat total T hücreleri kontrol grubu ile karşılaştırıldığında tüberkülozlu hastalarda önemli olarak daha düşüktü. Tüberküloz hastalarında periferik kanda total T-lenfositlerin düşüklüğü tüberküloz patogenezinde T-lenfositlerin önemini göstermektedir.

**Anahtar Kelimeler:** T-lenfosit, NKT hücresi, tüberküloz **Nobel Med 2011; 7(2): 50-54**

## INTRODUCTION

A hallmark of *M. tuberculosis* infection is the ability of most (90-95%) healthy adults to control infection through acquired immunity, in which antigen specific T cells and macrophages arrest growth of *M. tuberculosis* bacilli and maintain control over persistent bacilli. In addition to CD4<sup>+</sup> T cells, other T cell subsets such as  $\gamma/\delta$  T cell, CD8<sup>+</sup> and CD1-restricted T (NKT) cells have roles in the immune response to *M. tuberculosis*.<sup>1</sup> CD1-restricted T cells do not react with mycobacterial protein antigens in the context of MHC class I or class II molecules. Instead, these T cells react with mycobacterial lipid and glycolipid antigens bound to CD1 on antigen-presenting cells.<sup>2-5</sup> CD1-restricted T cells display cytotoxic activity and are able to produce IFN- $\gamma$ .<sup>6</sup> NKT cells functional response to specific stimulation is rapid and abundant production of cytokines type1 (Th1) (IFN- $\gamma$ ) and type2 (Th2) (IL-4).<sup>7</sup>

Therefore, these cells have important role for priming and regulation of immune system. NKT cells comprise a highly heterogeneous subpopulation of T cells that co-express TCR and NK cell markers such as NK cell adhesion molecule CD56 and the activation marker CD161.<sup>8,9</sup> T-lymphocyte subsets play a crucial role in immunity against mycobacterium infections.<sup>10,11</sup> In this study, it was aimed to investigate whether a difference of T and NKT cells ratio between active and inactive pulmonary tuberculosis (PT) patients.

## MATERIAL and METHOD

### Patients and study design

A total of 75 newly diagnosed active PT patients (mean±SD age, 35.76±15.8) who are admitted to Department of Chest Diseases of Firat Medical Center and Elazig Tuberculosis Dispensary were included in the study. 39 patients were women and 36 patients were men. Pulmonary tuberculosis was diagnosed by the following parameters; presence of symptoms of cough/expectoration, chest X-ray showing infiltrates and/or cavities, a minimum of one positive sputum smear and culture result for acid-fast bacilli. There was no extrapulmonary involvement in any patients. The exclusion criteria were as follows; HIV positivity, diabetes mellitus, pregnancy, and immunological or autoimmune diseases.

Twenty five inactive (40.23±20.5 year-old, 9 women, 16 men) PT cases that were admitted and treated in the Elazig Tuberculosis Dispensary and 20 healthy volunteer subjects as a control groups (38.20±17.6 year-old, 9 women, 11 men) were included in the study. Inactive PT patients had a history of previous episode of tuberculosis with documentation of a positive culture at the time of diagnosis. There were abnormal stable radiographic findings and no changes on their chest X-rays in last six months. Three sputum cultures for *M. tuberculosis* were negative →

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<b>Table:</b> Percentages of T and NKT cells (percentage of means ± SD) among active PT, inactive PT, and healthy subjects			
	<b>Active PT (n=75)</b>	<b>Inactive PT (n=25)</b>	<b>Healthy subjects (n=20)</b>
CD3 <sup>+</sup> CD (16+56) <sup>+</sup> (%) (NKT)	5.25 ± 3.93	5.11 ± 2.48	5.91 ± 6.09
CD3 <sup>+</sup> Cells (%) (T)	64.89 ± 9.59*	65.19 ± 5.45 <sup>#</sup>	73.24 ± 7.14* <sup>#</sup>
*p=0.001, <sup>#</sup> p=0.003			

in all patients.<sup>12</sup> Healthy subjects had no changes on chest X-rays and tuberculosis history or other underlying disease and they were currently taking any medication. Exclusion criteria for the healthy control groups included smoking, medication, pregnancy and any abnormalities in renal and liver function tests.

Acid-fast bacilli stains were performed by Erlich Ziehl-Neelsen method and cultures for *M. tuberculosis* were performed on Löwenstein-Jensen solid media.

The PPD skin test was performed by trained and experienced nursing personnel in the Elazig Tuberculosis Dispensary. Five tuberculin units of purified protein derivative were administered by intradermal injection on the volar surface of the left arm. The result was assessed after 48-72 hours and the diameter of the induration (not erythema) recorded in millimeters. PPD skin test conversion was defined as an induration of 10 mm or more increase from previous skin test. All active and inactive pulmonary tuberculosis patients had positive PPD skin test. Healthy control subjects had been vaccinated with BCG as part of the required Turkey National Vaccination Program and all were tuberculin positive.

The specialist filled questionnaire forms by asking about signs and symptoms of the disease. The Local Ethics Committee supplied ethical consent. Informed consents were taken from all individuals.

### Flow cytometric analysis of peripheral blood

Two milliliters of venous blood from the individuals diagnosed as tuberculosis and healthy subjects were taken into the tubes with EDTA in order to examine total T and NKT cells for flow cytometric analysis. Samples were taken before starting to antituberculous treatment in patients with active PT. All samples were studied within 2 hours. Cell surface expressions of lymphocyte antigens were examined by monoclonal antibodies staining of peripheral blood samples with two-color flow cytometry. Blood was collected in EDTA tubes from peripheral venous blood; 100 µL anticoagulated venous blood and 20 µL monoclonal antibodies [CD3-FITC (cat no, IM0452), CD16+56

PE (cat no, IM2026), isotype control IgG1 FITC/ IgG1 PE (cat no, A07794 ) and CD45-FITC / CD14-PE, (cat no, A07738)] (Immunotech Coulter Company, Marseilles, France) were added to the test tubes and vortex mixed. Test tubes were incubated for approximately 20 minutes in the dark at room temperature (20-25 °C). To extract leukocytes from whole blood for Immunophenotyping, the Immuno Prep Reagent Sytem (cat no. 7546946) for Coulter TQ-Prep Workstation was used automatically with the standard procedure (Beckman Coulter, Miami, Fla.). All samples were studied within 2 hours. Analytic flow cytometry was carried out by counting a total of 10.000 cells using the Coulter EPICS XL-MCL device (Beckman Coulter U.S.A) and the results were interpreted using isotopic controls after performing the voltage tunings required. Analyses were conducted using the Expo-32 analysis software program (A Beckman Coulter Company, Miami, Florida 33196-2500 USA). Lymphocyte populations are expressed as percentages of the total number of lymphocytes. The quality criteria involved the frequency above 95% of total lymphocytes in the analysis gate, homogenous CD45<sup>+</sup> lymphocyte population (minimum of 2000 events in the gate, CD45>95%).

### Statistical analysis

The data were expressed as their means ± standard deviations. Mann-Whitney U test for between groups, and Spearman's correlation analysis were performed by using SPSS 12.0 packet software. A p-value of 0.05 or less was considered statistically significant.

### RESULTS

Ages of participants were not statistically different between groups. Mean PPD response was higher statistically in active PT (15.35±2.78, range 11-24 mm) and inactive PT (13.92±2.25, range 10-17 mm) than in the healthy subject (11.80±1.64, range 10-16 mm) (p=0.000 for active PT, p=0.007 for inactive PT compared with healthy subjects). No correlations were observed between PPD with T-lymphocyte and NKT cells counts.

Patients with active and inactive PT had lower T cell counts than in healthy subjects (p=0.001 and p=0.003 respectively). No statistically significant differences were observed concerning CD3<sup>+</sup>CD (16+56)<sup>+</sup> (NKT) and CD3<sup>+</sup> T-lymphocyte when compared groups of patients with active PT and inactive PT (p>0.05).

Mean percentage of T cells, and NKT cells values were given in Table. →

## DISCUSSION

The host immune response to *M. tuberculosis* has a critical role in preventing clinical evident disease following infection. Natural killer T (NKT) cells participate in host defense against microbial infection. Cell-mediated immunity is particularly important, and it is well documented that people with defective T-cell responses are at a higher risk for developing primary or reactivation tuberculosis.<sup>13-15</sup> T-lymphocyte subsets play a crucial role in immunity against mycobacterium infections.<sup>10,11</sup> In our study active and inactive PT patients had a significantly lower percentage of T cells in the peripheral blood than healthy control. In addition, in the present study, no significant differences were observed in the percentages of T cell in active and inactive PT patients.

NKT cells were made a small volume in all T cells but surprisingly they have very important functions.<sup>16</sup> CD4<sup>+</sup> NKT cells may help Th2 cells and they may play a regulatory role on physiology of B cell.<sup>17</sup> These cells have a role for making a balance between protective immunity and immunopathology.<sup>18</sup> Immune response to tuberculosis can be affected by NKT cells because these cells produce two most critical cytokines (IL-4 and IFN- $\gamma$ ).<sup>19,20</sup>

Although the roles of CD4<sup>+</sup> and CD8<sup>+</sup> T cells in tuberculosis are well known, the roles of NKT cells on this disease are still obscure. It has been shown that in murine and human system CD1d restricted cells recognize lipid, lipoglican, hydrophobic peptid antigens that derived *M. tuberculosis* cell walls.<sup>5,21</sup> In animal models, NKT cells have been found to be recruited by mycobacterial glycolipids, and these cells lead to developing of granulomatous lesions.<sup>21</sup> On the contrary, of HLA molecules, CD1 molecules have restricted and cell specific expression. CD1 proteins expressed on thymocytes promptly and out of thymus, it is presented on antigen presenting cells.<sup>22</sup>

CD1 restricted T cells which specific to mycobacterial antigens isolated in tuberculosis and it was found related to active cellular immune response.<sup>23</sup>

Sugawara et al.<sup>24</sup> performed a study in NKT knockout mice and they did not found in pulmonary granuloma size between NKT knockout and wild type mice. When they stimulated with Kurono strain in vitro, splenic cells from NKT knockout mice produced less IFN-gamma than did those from wild type mice. Therefore, they concluded that NKT cells play a detrimental role in late-phase mycobacterial infection. A study done by Chackerian et al.<sup>20</sup> showed that NKT cells are not required for optimum immunity to *M. tuberculosis*. In addition, they did not found absence of NKT cells did not impair immunity against tuberculosis. Additionally the authors gave  $\alpha$ -galactosylceramide to infected mice with tuberculosis to activate NKT cells. They observed that this substance modulated innate immunity and treatment with this substance reduced the bacterial burden in the lungs, diminished tissue injury, and prolonged survival of mice infected with tuberculosis.

In the present study, NKT cells were not different as statistically among all groups but T cells counts were lower in patients with active and inactive PT than in healthy subjects. Furthermore, no statistically significant differences were observed concerning CD3<sup>+</sup>CD (16+56)<sup>+</sup> and CD3<sup>+</sup> T-lymphocyte when compared groups of patients with active PT and inactive PT. This result shows us NKT cells in peripheral blood did not change even if the patient has active pulmonary tuberculosis.

As a conclusion, some studies showed NKT cells can play an important role for protection from *M. tuberculosis* infections, but in this study, any differences were observed for NKT cells between active, inactive tuberculosis and healthy controls. But percentages of total T cells were found significantly lower in patients with tuberculosis compared with controls.



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