

Tanaffos (2002) 1(4), 45-50

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Comparison of Lymphocyte Number and Their Subsets in Patients with Diabetes Mellitus Type II, Tuberculosis and Concomitant TB and Diabetes

Davood Mansoori¹, Hamid Reza Jamaati², Siamak Arami¹, Maryam Zadsar¹, Ladan Abbasian¹, Ali Reza Esteghamati³, Ali Akbar Velayati⁴

¹ Department of Infectious Diseases, ² Department of Pulmonary Medicine, NRITLD, Shaheed Beheshti University of Medical Sciences and Health Services, ³ Department of Endocrinology, Tehran University of Medical Sciences and Health Services, ⁴ Department of Pediatrics, NRITLD, Shaheed Beheshti University of Medical Sciences and Health Services, TEHRAN-IRAN

ABSTRACT

Background: Diabetics are prone to tuberculosis infection in part due to cellular immunity dysfunction, but the precise mechanism has not been fully understood yet.

Materials and Methods: This study was performed in NRITLD between August 1999 and August 2001 to elucidate the quantitative status of cellular immunity. We measured the number of B lymphocytes, T lymphocytes, Natural Killer (NK) cells, and their sub-units in three groups of patients with diabetes mellitus (DM), pulmonary tuberculosis (TB) and pulmonary tuberculosis associated with DM (TB+ DM); and compared their results with age - matched healthy controls.

Results: The number of these cells had not significant quantitative difference in TB and also DM patients comparing with healthy controls. A significant difference in total number of T lymphocytes and sub- units of helper T cell was observed in patients with concomitant TB and DM.

Conclusion: These results indicate that patients with diabetes mellitus type II do not quantitatively develop cellular immunity dysfunction, a finding that may be seen in patients with concomitant TB and DM. (*Tanaffos* 2002; 1(4): 45-50)

Key words: Tuberculosis, Cellular immunity, Diabetes Mellitus, Lymphocyte

INTRODUCTION

Diabetics are prone to bacterial infections in part, and the presence of diabetes mellitus plays an important role in the development of pulmonary tuberculosis. The reported relative risk for incidence of pulmonary tuberculosis among diabetics is 2-3

times higher than normal population (1). In a comprehensive study, it was shown that the development of both bacteriologically-confirmed and all types of pulmonary tuberculosis were 3.47 and 5.15 times higher in diabetics than those in the matched controls respectively. This figure was even higher at the age of 30-49 than in 50's or older (2). Increased incidence of atypical radiological images

Correspondence to: Siamak Arami

Tel.: +98-911-2115183

E-mail address: siamakarami@hotmail.com

of pulmonary tuberculosis in diabetic patients was also observed (3). The prevalence of tuberculosis reactivation was shown to be higher in diabetics (4). Other studies indicated that the prevalence of tuberculosis in diabetics and also of diabetes mellitus in tuberculous patients were increased (5,6). Diabetic patients may suffer from advanced forms of tuberculosis and its higher mortality rates (7-9). Increased incidence of tuberculosis in diabetic patients can be ascribed to the cellular immunodeficiency. These include decreased cytokine levels, (10) decreased number of T-cells and NK cells, (11,12) decreased blast cell transformation, mitogen-induced proliferation, reduced complement receptor 3 (CR₃) expression on monocytes surface, reduced IL₂ receptors on lymphocytes (13) and deficiency of T lymphocytes with CD₃ and CD₅₆ surface markers (14). Considering the controversies around the issue of immunodeficiency in diabetics and their higher risk of being afflicted with tuberculosis, we evaluated lymphocytes count and their subsets in these patients.

MATERIALS AND METHODS

Our study involved a total of 101 patients referred to the NRITLD, the major referral tuberculosis center in Iran, between August 1999 and August 2001. Patients were categorized into three groups and 37 other healthy individuals were considered as controls. They were as follows:

- 1) 42 patients with type II diabetes mellitus (36 females and 6 males with age range of 29-80) which were categorized as optimal and sub-optimal control of diabetes status.
- 2) 43 patients with pulmonary tuberculosis and positive sputum smear (15 females and 28 males with age range of 16-78).
- 3) 16 patients with concurrent type II diabetes mellitus and pulmonary tuberculosis (13 females and 3 males with age range of 48-78).

- 4) 37 healthy controls without diabetes, tuberculosis or any other disease (21 females and 16 males with age range of 22-73).

None of our cases had congenital immunodeficiency, cirrhosis, collagen vascular disease, renal failure, malnutrition, cancer, and other immunocompromising disease. Serologic tests for HIV were done for all cases and were negative. None of the cases had received any immunosuppressive drug. The following tests were performed for all cases: lymphocytes count in peripheral blood, determination of surface markers by using murine monoclonal antibodies and, flow-cytometric method (FAC scan). The determined markers were CD₃ (pan T-lymphocytes), CD₄ (helper T-lymphocytes), CD₈ (cytotoxic T lymphocytes), CD₅₆ (NK lymphocytes), CD₁₉ (B-lymphocytes), and CD₂₅ (activated T lymphocytes=IL₂ receptors). Standard measurement of glycosylated hemoglobin (HbA_{1c}) was performed to reflect the status of metabolic control in diabetics. Levels of HbA_{1c} higher than 10% were considered as sub-optimal control and lower levels as optimal.

Mean number of surface markers in 4 groups was statistically analyzed by analysis of variance and P-values less than 0.05 were considered significant.

RESULTS

The results are listed in table 1. Mean of absolute values for number of surface markers did not show any significant difference between optimally and sub-optimally controlled diabetics. Mean of absolute value for total T-lymphocytes showed significant increment in diabetics comparing with the other three groups ($p < 0.05$); this value was significantly lower in the group with concurrent diabetes and tuberculosis as compared with control group ($p < 0.05$).

Number of helper T-cells (CD₄⁺) was significantly lower in the group with concurrent diabetes and tuberculosis as compared to the other three groups ($p < 0.001$). Helper T cells were reduced in group 3

Table 1. Comparison of lymphocytes number & their subsets in patients with TB*, DM**, TB+ DM and control group in NRITLD August 1999- August 2001.

	CD ₃	CD ₄	CD ₈	CD ₁₉	CD ₂₅	CD ₅₆	CD ₄ /CD ₈
** DM(1)	1873.79±76.23	1094.33±58.83	734.07±38.25	415.94±47.17	79.81±13.48	256.6±26	1.62±0.09
* TB(2)	1546.25±62.16	936.78±48	619.55±37.86	303.89±28.07	152.87±28.21	295.6±29	1.74±0.14
TB+DM(3)	1310.62±106.54	712.46±64.2	599.68±66.97	304.95±35.37	85.53±27.95	242.9±42	1.47±0.21
Control(4)	1614.56±25.40	1063.09±21.10	580.15±19.37	352.92±16.45	--	--	1.94±0.10
	1,2 (S)	3,1 (S)	1,4 (S)				
P value	1,2 (S) p<0.05	3,2 (S) p<0.001	1,2 (S) p<0.001	NS	1,2 (S) p<0.05	NS	NS
	1,4 (S)	3,4 (S)					
	3,4 (S)	1,2 (S)					

* Tuberculosis **Diabetes Mellitus S: Significant NS: Non-significant

with pulmonary tuberculosis only when compared with diabetic group ($p < 0.001$). Mean value for cytotoxic T-lymphocytes (CD₈⁺) in diabetic group was significantly higher than tuberculosis and control groups ($p < 0.05$). Regarding B-lymphocytes (CD₁₉⁺) NK cells (CD₅₆⁺) and ratio of helper to cytotoxic T cells (CD₄⁺/CD₈⁺), there was not significant difference among all the four groups. However, NK cells status was not assessed in control group. Mean value for activated T- lymphocytes characterized as cells with IL₂ receptor (CD₂₅⁺) was significantly lower in diabetic group as compared with tuberculosis group ($p < 0.05$). It should be noted that this marker was not determined in control group.

DISCUSSION

There was no difference between tuberculosis group and controls regarding any of the studied cell populations, which was in accordance with our previous study (15). However, in some other investigations, reduced total T-cell count without any significant reduction in cytotoxic T cell count has been reported in tuberculosis patients as compared to healthy controls (16).

Meanwhile, in one of these studies, B cell lymphocytes were found to be lower in tuberculosis patients (17) which is in contrast to our previous (15)

and present experience. There have been many studies focused on the difference of immunologic markers in diabetics and controls, though the results of such studies were not always the same. Some studies (14,18,19) have shown that there is no significant change in total T-cell population (CD₃⁺) in type II diabetics and control group, which is what we have reached in our study, too. Such studies were also similar to ours in observing no difference in total B cell count between diabetics and controls. It has also shown a significant lower level of NK lymphocytes with T- lymphocyte markers (CD₃⁺, CD₅₆⁺) in diabetics than controls (18,19). Regarding the cytotoxic effect of this group of cells, the lower levels of this subset of NK cells could explain susceptibility of diabetics to infection. Activated T lymphocytes (CD₂₅⁺T lymphocytes) showed significant decreased in group 1 (diabetics) compared to that of group 2 (tuberculosis). Since CD₂₅ markers had not been evaluated in control group, it was impossible to compare the results with healthy individuals in this study. Chang and his colleagues reported reduced levels of activated T-lymphocytes (CD₂₅⁺) in patients with type II diabetes mellitus compared to healthy controls (13). They claimed that it is the basis for sub-optimal proliferation of T- lymphocytes stimulated by mitogens (13). Number of

T-helper cells in diabetics showed no reduction compared to controls but it was higher than that of tuberculosis as well as concurrent tuberculosis and diabetes groups. Chang and his colleagues demonstrated that there exists no difference in CD_4^+ lymphocytes count between diabetics healthy controls. Additionally, our results show a significant elevation of cytotoxic T-cell lymphocytes in diabetics compared to controls, which is consistent with their findings (13). The present study is also comparable to the report of Chang and his colleagues regarding CD_4/CD_8 ratio, as in both studies revealed no significant difference between patients with type II diabetes and controls (13). However, Pometkin et al. reported that this ratio would elevate at the onset of type I diabetes mellitus (12). The number of NK lymphocyte (CD_{56}^+) population was not different among diabetics and other groups (except for controls, since NK cells were not counted in healthy controls). The same result was obtained by Tsujino et al. (14). Significantly reduced levels of total T lymphocytes (CD_3^+) in patients with concurrent diabetes and tuberculosis (group 3) in comparison with healthy controls (group 4) and diabetic group (group 1) are a notable finding in our study. There has been a similar report that described lower levels of T lymphocytes in patients with concurrent diabetes and tuberculosis (20).

Although in our study total T lymphocyte count in patients with concurrent diabetes and tuberculosis was lower than those with tuberculosis alone, the difference was not statistically significant. In the present study, helper T cell count showed significantly diminished levels in group 3 compared to all other three groups. It may be an evidence of suppressor effect of concurrence of diabetes and tuberculosis on the number of helper T cells. Another study (19) demonstrated lower secretion of interferon gamma and IL-12 (Th1 cytokines) after stimulation with BCG vaccine in patients with concomitant

diabetes and tuberculosis than in tuberculosis patients without diabetes, which this finding may be similar to our result. Regarding other markers, our study demonstrated that there was no real difference between group 3 (patients with diabetes and tuberculosis) and other three groups. Despite marked reduction of CD_4^+ T lymphocytes, the ratio of CD_4/CD_8 in groups 3 had not significantly decreased comparing to healthy controls. This may be explained by non-significant rise in the number of suppressor cytotoxic (CD_8^+) T cells in this group. Lymphocyte populations with the mentioned surface marker did not have much difference between well-controlled and poor controlled diabetic patients, and we considered all diabetics, regardless of their disease control state-together in the same group. Nevertheless, another study showed that the level of glycosylated hemoglobin (HbA_{1c}) was directly associated with the rate of progression of tuberculosis, extent of lung destruction, drug resistance, and expectorating large amounts of TB bacilli in sputum (20).

To summarize the results, we concluded that the number of B and T lymphocytes as well as their subset were not different between tuberculosis patients and healthy controls, as is the case with diabetic patients. However, concurrency of tuberculosis and diabetes causes reduction of total T lymphocytes particularly T-helper sub-population. It still calls for more extensive and precise studies. More evaluations including lymphocyte transformation, profile of cytokines secreted by lymphocytes, function of cytotoxic T cells and NK cells, number of T cells with gamma/delta receptors, and their function in the aforementioned three groups are recommended for a better definition of cellular immunity function. However, we can suggest that patients with type II diabetes mellitus show no significant quantitative reduction in T lymphocytes and its subsets with no explanation for the increased

incidence of infection with intracellular organisms, especially mycobacterium tuberculosis. Probably evaluation of type 1 diabetic patients with the same methodology may result in different results.

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