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Lymphocyte Subgroups in Pleural Fluid and Peripheral Blood of Tuberculous Pleurisy Patients with Positive and Negative PPD Reactions

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ABSTRACT

Background: *The local cellular immunity is responsible for the pathogenesis and development of disease in the affected organ. In tuberculous pleurisy lymphocytes are principally involved in cellular immunity. This immune response is specially mediated by Th1 and its related cytokines. In laboratory investigations all of the interleukins associated with Th1 axis, such as interferon gamma, are 20-60 times higher in pleural fluid as compared to blood. This significant increase in lymphocytes of the pleural fluid as compared to blood is a strong evidence for the compartmentalization of cytokines of Th1 axis in pleura, resulting in an appropriate and satisfactory immune response to mycobacterial infections. PPD reaction test (Mantoux test) is a delayed hypersensitivity reaction in skin in which cellular immunity and Th1 axis are responsible. It seems that compartmentalization of CD4+ T cells is effective in response to Mantoux test in patients suffering from tuberculous pleurisy.This study was conducted either to prove or refuse this hypothesis.*

Materials and Methods: *This was a 2-year cross-sectional study in which we studied the status of lymphocyte subgroups by flowcytometry in peripheral blood and pleural fluid of patients with pleurisy in which lymphocytes are principally involved. We also evaluated and compared its relation with Mantoux reaction test.*

Results: *Overall, 36 patients with pleurisy in which lymphocytes were principally involved in their pleural fluid entered the study. Out of them, 25 suffered from tuberculous pleurisy. In the tuberculous pleurisy group (25), 17 (68%) had positive Mantoux test while in 8 cases (32%), this test was negative. There were 31 males (86%) and 5 females (14%) with the age range of 17 to 60 yrs and the mean age of 35 years. In the PPD negative group the CD4+ count was significantly high in the pleural fluid. However, in PPD positive cases the CD4+ count was less significant but was still significantly higher in pleural fluid as compared to peripheral blood ($p< 0.05$). In PPD negative group CD8+ lymphocytes were significantly in a higher level than in blood. This was not seen in PPD positive group.*

Conclusion: *Cellular immunity is the main local response in these patients. As it is seen majority of cells present in the pleural fluid are CD4 and CD8 lymphocytes. CD19+ lymphocytes are in minority. The dominance of CD4+ lymphocytes in pleural fluid of PPD negative and PPD positive groups is a sign of Th1 compartmentalization in this disease. But, the important difference between these two groups is the dominance of CD8+ lymphocytes in peripheral blood of PPD negative cases and the CD4+/CD8+ ratio in pleura and blood of PPD positive cases are close to each other. It means that the difference between the CD4+ and CD8+ counts in the pleural fluid and blood is significantly higher in PPD negative group compared to the PPD positive one.*

This difference indicates the effective role of compartmentalization of active CD4+ lymphocytes of pleura in response to Mantoux test, as some studies suggest that cutaneous anergy is due to aggregation of active lymphocytes in pleural fluid. Secondly, according to the accumulation of lymphocytes in pleura and considerable difference in the type of lymphocytes in peripheral blood and pleura, obtaining Paraclinical data related to disease (specially ADA) only by evaluating the blood is not appropriate. This fact is confirmed in other studies as well. ***(Tanaffos 2005; 4(13): 57-62)***

Key Words: *Lymphocyte, Pleural fluid, Tuberculous pleurisy, Mantoux test*

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INTRODUCTION

The local cellular immunity response is responsible not only for the development of tuberculosis but also for its prognosis in the affected organ (1,2).

In the non-cavitary and smear negative forms, lymphocytes are principally involved in local cellular immunity. This immune response is specifically mediated by Th1 and its related cytokines (1,3). In laboratory investigations, all of the interleukins associated with Th1 axis, such as interferon gamma, are 20-60 times higher in pleural fluid as compared to blood. This points towards the fact that active lymphocytes not only have the ability to produce more of these substances but also to increase in number (4,5,6,7).This significant increase in lymphocytes of the pleural fluid as compared to blood is a strong evidence for the compartmentalization of cytokines of Th1 axis in this fluid, resulting in an appropriate and satisfactory immune response to mycobacterial infections (5,8, 9 ,10). Researches in which subgroups of lymphocytes have been studied showed the differences present in systemic and local immune responses to mycobacterial antigens. They demonstrated that CD4+ active lymphocytes are higher in pleural fluid as compared to blood, while CD8+ lymphocytes are higher in blood in comparison with pleural fluid. This fact further confirms the compartmentalization of CD4+ T cells in pleural fluid (8). This issue is an important element in response to Mantoux test in patients suffering from tuberculous pleurisy (11,12). In some of the conducted studies it has been suggested that the anergy observed in PPD skin test is the result of aggregation of active lymphocytes, mostly CD4+ T cells in the pleural space (13).

In this research, we have studied and compared the status of different lymphocytes i.e. CD3+, CD4+, CD8+ and CD19 in blood and pleural fluid as well as its relation with PPD test in patients suffering from tuberculous pleurisy.

MATERIALS AND METHODS

This cross sectional study was conducted over a two-year period. Overall, 36 patients with pleurisy (due to different reasons) were admitted in the pulmonary ward of Masih Daneshvari hospital from October 2001 to September 2003. The inclusion criterion consisted of having exudative and lymphocytic pleural fluid.

Meanwhile, the diagnostic criteria of tuberculous pleurisy were mainly based on the pleural fluid examination i.e. being exudative with majority of cells being lymphocytes (greater than 50%) and monocytes as well as an ADA level>45 u/liter.

Also, other criteria are as follows: granuloma with or without caseation in pleural biopsy along with positive smears, PCRs' and (positive) cultures of pleural fluid and sputum. Meanwhile, flowcytometry (evaluating the lymphocyte subpopulation by monoclonal antibody panel) of peripheral blood and pleural fluid was performed. In the standard flowcytometry CD3+(total T cells), CD4+(helper T cells), CD8+(cytotoxic) and CD19+(B lymphocytes) were evaluated. All patients underwent standard PPD test and the induration was measured after 48 hours. An induration of more than 10 mm was considered as a positive PPD. The results of the tuberculous pleurisy group (25 cases) were compared with the exudative non-tuberculous pleurisy group (with the majority of lymphocytes). Also, their PPD reactions were studied. For statistical analysis, Wilcoxon Signed Rank test was used.

RESULTS

Out of total 36 patients, 25 (59.5%) suffered from tuberculous pleurisy. In the tuberculous pleurisy group (25), 17(68%) had positive Mantoux test while in 8(32%) this test was negative. In the PPD negative group the average count of CD4+, CD8+ and CD3+

lymphocytes of pleural fluid were 60.18%, 21.18% , and 79.35% respectively. The mean counts of the above cells in peripheral blood were 33.71%, 32.18% and 63.12% respectively. As it is seen, the average CD4+ count was significantly higher in pleural fluid as compared to peripheral blood.($P<0.05$)

Also, there was a significant difference regarding the average CD8+ count of both pleural fluid and peripheral blood, demonstrating a higher level of CD8+ cells in blood($P=0.006$).

According to the results, there is a significant difference in the ratio of CD4+ and CD8+ lymphocytes in blood and pleural fluid ($P=0.000$).

However, in the tuberculous pleurisy group having positive PPD, this significant difference exists only in case of CD4+ T cells of peripheral blood and pleural fluid in such a way that CD4+ T cells are more in pleural fluid than in peripheral blood. Meanwhile, no significant difference exists in other cases. As it is observed, there is no significant difference between the average CD4+ counts of peripheral blood and pleural fluid($P>0.05$) in both subclasses of nontuberculous pleurisy group(PPD positive and negative). Meanwhile in tuberculous pleurisy group majority of the lymphocytes present in the peripheral blood and pleural fluid are CD4+, and CD8+ T lymphocytes. In all of the groups under study i.e. PPD positive and negative cases of tuberculous and non-tuberculous pleurisy, the difference observed was only in CD3+, CD4+ and CD8+ lymphocytes. Meanwhile, no difference could be detected in the B lymphocytes (CD19) of pleural fluid and peripheral blood in the above mentioned groups.

DISCUSSION

Based on the results of this research, it is clear that cellular immunity is the main local response in these patients. As it is seen, majority of cells present in the pleural fluid are CD4+ lymphocytes. The B lymphocytes (CD19) along with the remaining studied immune cells, are insignificant and do not show any difference compared to peripheral blood, a fact that is confirmed in most of the studies (1,14). In the PPD test, reaction to mycobacterium tuberculosis antigen is of delayed type hypersensitivity reaction; the mechanism of which is activated by Th1 and inhibited by Th2. The dominance of active CD4+ cells in pleural fluid of tuberculous pleurisy patients (having a high ADA level and tissue granuloma) could be due to influence and ascendancy of Th1 axis.

In contrast, the low number of active CD4+ lymphocytes in peripheral blood probably points towards the dominance of Th2 axis. Thus, a negative PPD reaction in tuberculous pleurisy patients is associated with significant decrease in the CD4+ lymphocytes of peripheral blood along with an increase in the pleural fluid. Also, there is a significant difference regarding the average ratios of CD4+/CD8+ lymphocytes in pleural fluid and blood in this group, as ratios of CD4+/CD8+ in pleural fluid and blood were 3.7 and 1.16 respectively with the difference being significant ($P=0.000$).

The same condition nearly exists in the patients suffering from PPD positive tuberculous pleurisy. However, the difference observed in this group was not as much as that seen in the PPD negative group ($P=0.016$).

The difference observed in the systemic and local responses to mycobacterial antigens, suggests a lymphocytic compartmentalization response to the tuberculous antigen (15,16)

In this study, evaluation of lymphocyte subpopulation showed the presence of large number of CD4+ and CD8+ lymphocytes in pleural fluid and

peripheral blood respectively, confirming the compartmentalization phenomenon in tuberculous pleurisy.

Thus, we can conclude that primary cutaneous anergy seen in tuberculous pleurisy is due to aggregation of active lymphocytes activated by mycobacterium antigen present in the pleural fluid. This fact could not be detected in non tuberculous lymphocytic pleurisy patients (17, 18).

Also, the present study showed that in the control group (non-tuberculous lymphocytic pleurisy patients) there was no significant difference between the CD4+lymphocytes of peripheral blood and pleural fluid (P>0.05) especially compared to the two previous groups.

Since tuberculous pleurisy is a local clinical finding, the immune responses observed are mainly localized in the pleural space. Thus, it seems that in the tuberculous patients, laboratory investigation of the immune cells present in peripheral blood is invaluable and only by evaluation of the local immune system, valuable details could be reached.

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