The Relationship between Age and Pleural Fluid Adenosine Deaminase Activity in Pleural Tuberculosis

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흉막 결핵에서 연령과 흉수 Adenosine Deaminase 활성도와의 연관성

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연구 배경 : Adenosine deaminase (ADA)는 퓨린 (purine) 대사에 작용하는 효소로서 림프구, 특히 T-림프구의 증식과 분화에 관여하며, 결핵성 흉수의 진단에 있어서 중요한 생화학적 표지자 중의 하나이다. 한편, 노인의 경우, T-림프구의 수와 기능의 감소에 의하여 면역 기능이 감소하는 것으로 알려져 있다. 이에 저자 등은 노인 결핵성 흉수 환자에서 흉수 내의 ADA 수치가 젊은 환자에서보다 감소하는지를 조사하였다.

방 법 : 4년 동안 세브란스 병원에서 1) 흉수 결핵균 배양 양성 또는 2) 흉막 조직 검사상 결핵에 합당한 소견을 보여 결핵성 흉수로 진단받은 환자 80명을 대상으로 후향적으로 조사하였다. 65세를 기준으로 두 군으로 분류하였으며, 연령과 흉수 ADA 수치의 연관 관계를 독립 표본 t-검정 및 선형 회귀 분석을 이용하여 연구하였다.

결 과 : 80명의 환자 중 65세 이상은 21명 (26.3%)이었다. 흉수 내의 ADA 수치는 65세 이상 및 이하 군에서 각각 71.2 ± 27.6 IU/L, 68.5 ± 25.8 IU/L 이었다 (p=0.69). 선형 회귀 분석에서도 연령과 흉수 내의 ADA 수치는 상관 관계를 보이지 않았다 (r^2=0.05, p=0.59).

결 론 : 본 연구의 결과에 의하면, 결핵성 흉수의 진단에서 흉수 ADA 수치를 보조 지표로 사용하는 데 있어서, 노인 환자에서도 젊은 환자와 동일한 임상적 유의성을 가지고 동일한 결정 수치 (cut-off value)를 적용할 수 있을 것으로 판단된다. *(Tuberc Respir Dis 2005; 58: 459-464)*

Key words : Age, Adenosine deaminase, Pleural effusion, Tuberculosis

INTRODUCTION

For most cases of tuberculous pleural effusion, the number of the organisms in the pleural effusion is very small, so the conventional methods for the detection of *M. tuberculosis* are often of no use. The culture is positive in less than 25% of cases[1] and even the pleural biopsy shows granulomatous inflammation only in approximately 60% of cases[2,3]. Even though the combination of the microscopic examination and the culture of pleural biopsy specimens was reported to increase the diagnostic rate up to 90%[4], it is time-consuming. The diagnosis of the cases with tuberculosis at an earlier stage would be advantageous because they are less contagious[5,6] and have lower morbidity and mortality[7]. The early diagnosis of pleural tuberculosis has been greatly improved by the use of the biochemical markers such as adenosine deaminase (ADA), interferon-γ, and lysozyme[2,8-10]. Among them, the determination of the ADA level in pleural fluid appears to be the most promising for the diagnosis of pleural tuberculosis because of its ease, rapidity, and cost-effectiveness. ADA is found in most cells, but its major role is concerned with the proliferation and differentiation of lymphocytes, especially T-lymphocytes. For this reason, ADA has been thought of as a marker of the

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Received : Feb. 14. 2005
Accepted : Apr. 13. 2005

cell-mediated immunity including the delayed hypersensitivity reaction which is the key mechanism of tuberculous pleural effusion.

Immunosenescence, the progressive decline in the immune function that develops with aging, has largely been attributed to the alterations in the T-cell immunity[11-14]. The loss of the effective immune activity within the T-cell compartment is partly caused by the thymic involution[15]. Substantial changes in both the number and the function of T-cells have been reported with an advancing age[16,17]. For the number of T-cells, one of the most consistent changes with an advancing age, is a decrease in the proportion of naive T-cells with a concomitant increase in the proportion of activated/memory T-cells. The observed functional changes include decreased responses to T-cell receptor stimulations, impaired T-cell proliferative capacities, decreased number of interleukin-2-producing CD4+ T-cells, and decreased interleukin-2 receptor expressions. These latter findings probably explain the loss of the proliferative capabilities of T-cells from the aged individuals[18].

In this study, we hypothesized that the pleural fluid ADA activity might be lower in the elderly patients and investigated whether we should apply a different cut-off value to them for the diagnosis of pleural tuberculosis.

METHODS

Study Population and Samples

We retrospectively reviewed the patients older than 18 years who were diagnosed with tuberculous pleural effusion at the Severance Hospital, over a 4-year period (June 2000 - June 2004). The diagnostic criteria used were as follows: positive *M. tuberculosis* culture of pleural effusion, and/or histopathologic finding consistent with tuberculosis on pleural biopsy. Among them, the immunocompromised patients due to the underlying diseases such as diabetes mellitus, liver cirrhosis, uremia, malnutrition, leukemia, and lymphoma, were excluded. The patients taking immunosuppressive medications such as corticosteroid, cyclosporine, cyclophosphamide, azathioprine, and mycophenolic acid, were also excluded.

We reviewed the pleural effusion ADA level, the differential cell count from pleural effusion and peripheral blood, the pleural effusion lactate dehydrogenase (LDH) level, and the pleural effusion/serum LDH ratio.

AFB Smear and Culture of *Mycobacterium tuberculosis*

The Ziehl-Neelsen staining and the 3% Ogawa medium culture were performed with the pleural effusion specimens and the sputa.

Determination of Adenosine Deaminase (ADA) Activity in Pleural Effusion

The ADA activity was determined by the colorimetric method described by Giusti[19]. The ADA level below 45 IU/L was considered as negative in this study[20,21].

Statistical Analysis

The pleural effusion ADA activity, pleural effusion LDH level, pleural effusion/serum LDH ratio, pleural effusion leukocyte and lymphocyte count, peripheral blood leukocyte and lymphocyte count from the patients older than 65 years were compared with those from the younger patients by student's t-test. The sensitivity of the pleural effusion ADA activity was compared between the two age groups by Fisher's exact test. The relationship between age, pleural

effusion lymphocyte count, and pleural fluid ADA activity was examined using multiple linear regression analysis. The differences were considered statistically significant if p-value was less than 0.05.

RESULTS

Subjects' Characteristics

A total of 80 patients were included, who consisted of 22 females and 58 males. The patients' age ranged from 19 to 85 years. The pleural effusion *M. tuberculosis* culture was positive in 30 (37.5%) cases. Pleural biopsy was not performed in 8 (10.0%) patients due to a small amount of pleural effusion. Out of the 72 biopsy-performed cases, 62 (86.1%) showed the pleural histopathology consistent with tuberculosis. The pleural effusion ADA activity was between 10.4 and 133.0 IU/L.

The subjects included 21 patients older than 65 years and 59 patients younger than 65 years. The female patients were 4 (19.0%) of 21 and 18 (30.5%) of 59 in each group (p >0.05). The pleural effusion *M. tuberculosis* culture was positive in 7 (33.3%) of 21 and in 23 (39.0%) of 59 patients, respectively (p >0.05). Pleural biopsy was performed to 19 (90.5%) of 21 and to 53 (89.8%) of 59 patients in each group. The pleural biopsy pathology was consistent with tuberculosis in 16 (84.2%) of 19 and in 46 (86.8) of 53 biopsy-performed patients, respectively (p >0.05). The pleural effusion ADA level was above 45 IU/L in 18 (85.7%) of 21 and in 50 (84.7%) of 59 patients (p >0.05). The lactate dehydrogenase (LDH) level in the pleural effusion specimen was 1008±542 IU/L and 1020±552 IU/L, respectively. The pleural fluid to serum LDH ratio was 2.29±1.50 and 2.54±1.58 in each group (p >0.05) (Table 1).

Comparison of Pleural Effusion ADA Activity, Pleural Effusion Leukocyte and Lymphocyte Count, Peripheral Blood Leukocyte and Lymphocyte Count between Two Age Groups

The pleural effusion ADA level was 71.2±27.6 IU/L in the elderly group and 68.5±25.8 IU/L in the younger group (p=0.69). The pleural effusion leukocyte count was 1189±1892/μL and 2014±2158/μL, respectively (p=0.19). The pleural effusion lymphocyte count was 932±1500/μL and 1777±1568/μL in each group. It was slightly lower in the elderly group even though the result was not statistically significant (p=0.07). The peripheral blood leukocyte count was 7543±3180/μL and 6898±2215/μL, respectively (p=0.31). The peripheral blood lymphocyte count was 844±

Table 1. Clinical characteristics of the patients

	> 65 years old (n=21)	≤ 65 years old (n=59)	p-value
No. of females (%)	4 (19.0%)	18 (30.5%)	0.40‡
No. of cases with positive PE MTB culture (%)	7 (33.3%)	23 (39.0%)	0.79‡
No. of cases with positive pleural pathology (%)	16/19*(84.2%)	46/53† (86.8%)	0.72§
No. of cases with ADA >45 IU/L (%)	18 (85.7%)	50 (84.7%)	1.00§
PE LDH (IU/L) (mean±SD)	1008 ± 542	1020 ± 552	0.93‖
PE/Serum LDH ratio	2.29 ± 1.50	2.54 ± 1.58	0.56‖

LDH: lactate dehydrogenase, MTB: *Mycobacterium tuberculosis*, PE: pleural effusion
*16 of the 19 biopsy-performed patients showed the histopathologic findings consistent with pleural tuberculosis.
† 46 of the 53 biopsy-performed patients showed the histopathologic findings consistent with pleural tuberculosis.
‡ Pearson's chi-square test was used.
§ Fisher's exact test was used.
‖ Student's t-test was used.

Table 2. Comparison of ADA activity and cell counts between two groups*

	> 65 years old (n=21)	≤ 65 years old (n=59)	*p*-value
Pleural effusion ADA level (IU/L)	71.2 ± 27.6	68.5 ± 25.8	0.69†
Pleural effusion WBC count (/μL)	1189 ± 1892	2014 ± 2158	0.19†
Pleural effusion lymphocyte count (/μL)	932 ± 1500	1777 ± 1568	0.07†
Peripheral blood WBC count (/μL)	7543 ± 3180	6898 ± 2215	0.31†
Peripheral blood lymphocyte count (/μL)	844 ± 527	1251 ± 424	0.001†

*The values are expressed as mean ± standard deviation.
† Student's t-test was used.

527/μL in the elderly group and 1251±424/μL in the younger group (p=0.001) (Table 2). The multiple linear regression analysis of the relationship between age, pleural effusion lymphocyte count, and pleural fluid ADA activity did not show a significant correlation, either (r^2=0.05, p=0.59) (Fig. 1).

DISCUSSION

ADA catalyzes the conversion of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively[22]. Because ADA is primarily concerned with the proliferation and differentiation of T-lymphocytes[23], the pleural effusion ADA activity is thought to reflect the cellularity of activated T-lymphocytes in the pleural compartment. According to some previous reports, the serum ADA level was lower in the patients with severe combined immunodeficiency[24], and it was higher in the patients with increased cell-mediated immunity due to typhoid fever, infectious mononucleosis, viral hepatitis, chronic liver disease, etc. According to Hsu et al., the diagnostic value of ADA in the immunocompromised hosts with tuberculous pleural effusion was not as significant as in the immunocompetent hosts[25]. On the contrary, Riantawan et al. showed that the diagnostic value of the pleural effusion ADA activity was not different between the human immunodeficiency virus (HIV)-seropositive and the HIV-seronegative patients[26].

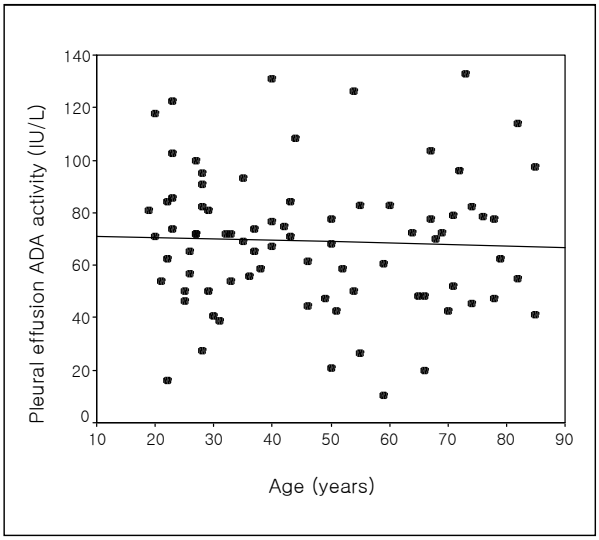

Figure 1. Relationship between pleural effusion ADA activity and age in the patients with tuberculous pleural effusion.
Multiple linear regression analysis did not show a significant correlation between age and pleural fluid ADA activity (r^2=0.05, p=0.59).

We partitioned the patients into two groups, one older than 65 years and the other younger. The results indicated that the pleural effusion ADA level did not correlate with the patient's age. The mean value and the sensitivity of the pleural effusion ADA level did not show statistically significant differences between the two age groups. The pleural effusion lymphocyte count was slightly lower in the elderly patients even though not statistically significant, and the peripheral blood lymphocyte count was much lower in the elderly patients with a statistical significance. This might suggest that the activated lymphocyte count in the pleural effusion is

similar between the two age groups in spite of the difference in the total lymphocyte count. In the future, therefore, it would be necessary to compare the activated lymphocyte counts between the two age groups, possibly by means of the stimulation of the lymphocytes with the mycobacterial antigens such as culture filtrate proteins and purified protein derivatives.

From these results, we can assume that it is reasonable to apply the same cut-off value of the pleural fluid ADA level with the same clinical significance, to both the elderly and the younger patients, for the diagnosis of pleural tuberculosis. Our results were in some accordance with Riantawan et al.'s study which showed that the pleural effusion ADA level of the HIV-seropositive patients did not differ from that of the HIV-seronegative ones[26].

In this study, we included only the cases of confirmed tuberculous pleural effusion with positive *M. tuberculosis* culture of pleural effusion and/or histopathologic finding consistent with tuberculosis on pleural biopsy. We excluded the patients with probable pleural tuberculosis who had one of the following: positive *M. tuberculosis* culture of biologic specimens other than pleural effusion, and/or positive response to anti-tuberculous medications without other possible causes of pleural effusion. And hence the *M. tuberculosis* culture-positive and the pleural pathology-positive rate were higher than those in the previous studies in which the cases with probable pleural tuberculosis were also enrolled.

A lot of Korean elderly people are thought to have been exposed to *M. tuberculosis* in their early lives and are suspected to be in the state of latent infection. This might have induced the activation of the memory T-lymphocytes when developing pleural tuberculosis in the elderly group. Even though the number of the naïve T-lymphocytes was decreased in the elderly, these memory T-lymphocytes might have proliferated and released ADA in the pleural space, to the same degree as in the younger patients.

In conclusion, it is assumed that we can apply the same cut-off value of the pleural effusion ADA activity with the same clinical significance, to both the elderly and the younger patients, for the diagnosis of pleural tuberculosis. Considering the limitations of a retrospective study, further prospective studies including much more cases will be necessary in the future.

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