ADENOSINE DEAMINASE MEASUREMENTS AND T-LYMPHOCYTE COUNTS IN THE BLOOD AND PLEURAL FLUID OF MALIGNANT AND TBC PLEURISY PATIENTS

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SUMMARY: Adenosine deaminase (ADA), absolute lymphocyte, % and absolute T-lymphocyte values in the blood and pleural fluid have been evaluated in 16 tb pleurisy, 14 malignant pleurisy patients and in 17 controls. A very significant ($p<0.001$) pleural lymphocytic infiltration has been observed in patients of the tuberculous pleurisy when compared to the peripheral blood. The minimum ADA activity in pleural fluid has been determined as 38.8 U/L in tb pleurisy patients while it is 28.4 U/L as the maximum in the patients of malignant pleurisy ($p<0.001$). In the same respect, pleural fluid/blood ratios of ADA levels are 7.14 and 2.03. Considering histopathological diagnosis as the reference, ADA measurements have been found highly sensitive and specific. Thus ADA measurement both in the blood and in pleural fluid could provide a higher success in the differential diagnosis of the tb pleurisy. Routine application of this test might be of clinical profit.

Key Words: Tuberculous pleurisy, adenosine deaminase.

INTRODUCTION

In the clinical evaluation of exudative pleural effusion, the first choice in our country is tuberculous (tb) or malignant pleurisy. In both diseases the increase of leukocytes in the pleural effusion is characterized by lymphocytic infiltration. This point, is of diagnostic value especially for tb although it is not specific (1, 2). With present facilities, ethological diagnosis of pleurisy is almost impossible in 20% of the patients (3, 4). ADA is a valuable diagnostic criterion which catalyses the hydrolyzing of adenosine to inosine and ammonia in the catabolism of the purins. In the recent years, this criterion has been in wide use for differential diagnosis of tb and malignant pleurisy (3-13).

In this study, the ADA levels have been measured both in blood and pleural effusion, and percentage by E-Rosette and absolute count of T-lymphocytes have been detected in the same material on the rational of linkage between the ADA activity and the proliferation and differentiation of T-lymphocytes.

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MATERIALS AND METHODS

The study comprises 16 pleural tb + 14 malignant pleurisy cases and 17 healthy controls who were free of other infections, malignancy, diabetes, chronic drug use etc. These 2 subgroups are aged between 17-85 (average 44.8±22.9 and 6 females+24 males) and 20-68 years (average 37.8±16.2 and 5 females+12 males) respectively. In the patient group, PPD skin test, pleural fluid aspiration and biopsy procedure from the parietal pleura by Cope's needle have additionally been performed to the routine hematological, biochemical, radiological investigation for the purpose of differential diagnosis. The sputum, pleural fluid and the biopsy material have also been examined microscopically, microbiology and histopathologically. In some instances, broncoscopy has also been conducted.

Leukocyte counts of the case and control groups in the pleural fluid and peripheral blood was carried out in the same day that the samples had been collected. The percentage values of lymphocytes in the blood smear, has been transformed to number/mm3. A similar way has been followed for pleural fluid by smearing the sediment of 10 ml pleural effusion after 5 mins centrifugation at 1500 rpm (1, 2).

Leukocytes have been separated by Boyum's method and Jondal's method has been applied in order to determine the cells

which form "rosette" with sheep erythrocytes existing in the medium (14, 15). By counting 300 cells, the % ratio of T-lymphocyte has been assessed. The normal ranges have been considered as 50-70 % for peripheral blood.

The following formula has been used for the calculation of absolute number of lymphocytes:

Absolute T-lymphocyte = (leukocyte count) X (%lymphocyte) X (T-lymphocyte)

The ADA activities in the sera and pleural fluid have been studied by G. Guisti's colorimetric method after 10 mins centrifugation of the samples at 3000 rpm (16).

Table 1: Comparisons of blood parameters between Tb cases and control groups.

Compared parameters	Tb group (n=16)	Control group (n=17)	Comparisons (unpaired t-test)
ADA (U/L)	10.02±2.98	5.02±2.21	t=5.444, p<.001
Absolute lymph	1832±1087	2124±512	t=0.976, p>.05
% T-lymphocytes	38.06±13.30	66.6±8.37	t=7.323, p<.001
Absolute T-lymp.	682.4±490.5	1416±378	t=4.791, p<.001

Table 2: Comparisons of blood parameters between pleurisy patients and control cases.

Compared parameters	Cancer cases (n=14)	Control cases (n=17)	Comparisons (unpaired t-test)
ADA (U/L)	9.15±2.35	55.02±2.21	t=5.002, p<.001
Absolute lymph	1708±749	2123±512	t=1.763, p>.05
% T-lymphocytes	45.7±15.4	66.6±8.37	t=4.543, p<.001
Absolute T-lymp.	809±495	1416±378	t=3.774, p<.001

Table 3: Comparisons of blood and pleural fluid parameters of Tb pleurisy patients.

Compared parameters	Blood values (n=16)	Pl. fluid values (n=16)	Comparisons (unpaired t-test)
ADA (U/L)	10.02±2.987	1.47±22.37	t=10.895, p<.001
Absolute lymph	1832±1087	4354±1843	t=4.715, p>.001
% T-lymphocytes	38.1±13.3	58.13±16.82	t=3.744, p<.001
Absolute T-lymp.	682±490.5	2440±1257	t=5.211, p<.001

Table 4: Comparisons of blood and pleural fluid parameters in Malignant pleurisy patients.

Compared parameters	Blood values (n=14)	Pl. fluid values (n=14)	Comparisons (unpaired t-test)
ADA (U/L)	9.15±2.35	18.6±5.18	t=6.216, p<.001
Absolute lymph	1708±749	2365±2773	t=0.856, p>.05
% T-lymphocytes	45.7±15.4	42.6±17.4	t=0.494, p<.05
Absolute T-lymp.	809±495	980±1090	t=0.537, p<.05

Table 5: Comparisons of blood parameters between Tb and malignant pleurisy patients.

Compared parameters	Tb pleurisy cases (n=16)	Malign. pleurisy cases (n=14)	Comparisons (unpaired t-test)
ADA (U/L)	10.02±2.98	9.15±2.35	t=0.893, p<.05
Absolute lymph	1832±1087	1708±749	t=0.367, p>.05
% T-lymphocytes	38.06±13.3	45.7±15.4	t=1.444, p<.05
Absolute T-lymp.	682±491	809±495	t=0.698, p<.05

RESULTS

As presented in Table 1, when the parameters compared belong to the blood of the tb case and control group, statistically significant differences were observed except absolute lymphocyte. This statement is also correct when the comparison was made between the control and malignant pleurisy patients' blood parameters (Table 2).

Very significant differences have been found between the parameters of blood and the pleural effusion in tb pleurisy patients. The statistical report is in Table 3. In Table 4, the parameters of blood and pleural effusion belong to the patients of malignant pleurisy have been analyzed and 3 parameters have not revealed significant differences except ADA averages.

Tables 5 and 6 summarize the statistical analysis and the inferences between two case subgroups in terms of tb pleurisy and malignant pleurisy. There have been significant differences at varying degrees between pleural parameters.

In Figure 1, the minimum and maximum values of ADA measurements in the study subgroups have been represented. It is observed that average ADA value of tbc pleurisy patients is the most prominent one among all other subgroups.

Dual correlation analysis realized between various combinations of subgroups have been presented in Table 7. According to the r values, all of the simple correlations are highly weak excluding non-significant ADA % rosette r.

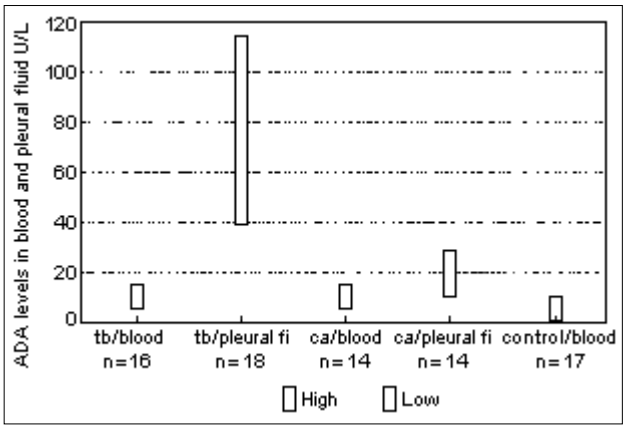
The specificity and sensitivity of ADA activity both in pleural fluid and blood have been taken into account by considering the histopathological diagnosis as the reference. When 38 U/L ADA value in pleural fluid considered as the reference limit, diagnostic specificity is 100% and sensitivity is 0% versus histopathological diagnosis. By starting from 10 various levels of pleural ADA levels, the specificity has been unchanged while sensitivity varies. The highest sensitivity rate has been calculated as 93.8% at the limit ADA value of 40 U/L.

As an another parameter, the ratio of blood and pleural fluid ADA levels of tb pleurisy patients, has been

Table 6: Comparisons of pleural parameters belong to Tb and malignant pleurisy patients.

Compared parameters	Tb pleurisy cases (n=16)	Malign. pleurisy Cases (n=14)	Comparisons (unpaired t-test)
ADA (U/L)	71.5±22.4	18.6±5.18	t=9.177, p<.001
Absolute lymph	4354±1843	2365±2773	t=2.279, p>.05
% T-lymphocytes	58.1±16.8	42.6±17.4	t=2.470, p<.002
Absolute T-lymp.	2440±1257	980±1090	t=3.406, p<.001

Figure 1: The distribution ranges of ADA level in the study sub groups (min. and max.)



examined. The sum of ADA levels which belong to 16 tbc pleurisy cases equals to 1143.5 U/L. This sum is 160.32 U/l for the blood ADA so that there has been a coefficient of 7.143 between 2 sums.

In addition more card of ADA levels below 38.79 U/L have been previously exists in pleural fluid of the tb pleurisy patients. On the contrary, the maximum pleural ADA level belong to malignant pleurisy patients is about 28.43 U/L (Figure 1).

DISCUSSION

The ADA initially, was proposed as all adjunct to clinical diagnosis (17). Giblet *et al.* (18) observed the relationships between immunological dysfunctions and the ADA deficiency by pointing out the 'ADA deficiency' concept for the first time in 1972.

Later investigators focused their efforts on the ADA measurements in the pleural and cerebro-spinal fluid. Many subsequently found elevated ADA activity in the pleural fluid of tb pleurisy patients and emphasized the value of this finding in the differential diagnosis of tbc and malignant pleurisies (4, 5,7-13). The minimum ADA levels have still been subject to debate probably due to different detection methods. Petterson *et al.* reported about the correlation between the ADA and lysosim

Table 7: Simple correlation analysis between dual subgroups.

Subgroups correlated	Simple r coefficient	Significancy of r value
Tb-pleural fluid ADA/% rosette	+.341	t=1.355, p>0.05
Tb-pleural fluid ADA/absolute T-lymph.	+.204	t=0.780, p>0.05
Tb-blood ADA/rosette	+.908	t=0.368, p>0.05
Tb-blood ADA/absolute T-lymph.	+.247	t=0.954, p>0.05
Ca-pleural fluid ADA/ % rosette	+.546	t=2.258, p>0.05
Ca-pleural fluid ADA/absolute T-lymph.	+.087	t=0.303, p>0.05
Ca-blood ADA/ %rosette	+.032	t=0.112, p>0.05
Ca-blood ADA/absolute T-lymph.	+.257	t=0.921, p>0.05
Control-blood ADA/% rosette	+.284	t=1.145, p>0.05
Control-blood ADA/absolute T-lymph.	+.005	t=0.019, p>0.05

enzymes and suggested that high ADA levels could be explained by the sequestration of active lymphocytes into the pleural cavity + monocytes' maturation towards macrophages (3, 10).

In our study, minimum pleural ADA level is 38.79U/L and this one is significantly higher than those in the blood ($p<0.001$). Pleural/blood ADA ratio is 7.143 for tbc pleurisy patients while it's 2.033 in the malignant pleurisy patients. This finding clearly emphasizes that one should get the ADA levels detected both in the pleural fluid and in the blood.

In this study, no correlation between T-lymphocyte count and ADA levels have been observed. The helper T-lymphocytes are the dominant ones in pleural fluid of the tbc pleurisy patients and this fact suggests that the ADA enzyme has been existing at higher amounts in these cells than those in others (19-25). In our study, however, there has been no possibility of assessing any causative relationship at least at this phase. We think that our findings need to be supported by other reports giving information concerning T4/T8 ratios and the ADA enzyme levels. We suggest that simultaneous measurement of pleural and blood ADA levels might be a very useful way in differential diagnosis of tbc pleurisy. Because considering this is a non-invasive, practical, easy and inexpensive method and much more sensitive and however specific procedure compared to pleural biopsy. It could increase the clinical versatility to if utilized as a routine procedure especially in the countries were tbc is prevalent.

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