Tanaffos (2005) 4(15), 37-42

Diagnostic Value of Adenosine Deaminase Isoenzyme (ADA2) and Total ADA in Tuberculous Pleural Effusion

Zohreh Mohammadtaheri[1], Sirous Mashayekhpour[1], Foroozan Mohammadi[1], Davood Mansoori[2], Mohammad Reza Masjedi[3]

[1]Department of Pathology, [2]Department of Infectious Diseases, [3]Department of Pulmonary Medicine, NRITLD, Shaheed Beheshti University of Medical Sciences and Health Services, TEHRAN-IRAN.

ABSTRACT

***Background**: Determination of adenosine deaminase (ADA) activity is one of the most promising markers in diagnosing of tuberculous pleural effusion. ADA has two main isoenzymes: ADA1 and ADA2.The ADA2 is the predominant isoform in tuberculous pleural effusion, suggesting its important role as a diagnostic marker.*

This study was conducted to determine the diagnostic value of ADA and ADA2 measurement in tuberculous pleural effusion.

***Materials and Methods**: Total ADA and ADA2 isoenzyme activities were measured in 93 case of pleural effusion, including tuberculosis (26males/5females), malignancy (22males/8females), empyema and para-pneumonic (11males/4females), transudate (6males/4females), rheumatoid arthritis and idiopathic (4males/3females). ADA levels were determined by Giusti and Galanti methods. ADA2 was measured with a potent inhibitor of ADA1 isoenzyme.*

***Results**: Total ADA and ADA2 activities in tuberculous exudates were 96.6±29.1 and 74.4±29 U/L, respectively. With diagnostic thresholds of 46 and 42 U/L, the sensitivities of ADA and ADA2 for tuberculous exudates were 100% and 97%; their specificities 82 and 88%; and their efficiencies 88% and 93.5%, respectively. All tuberculous exudates, 2 neoplastic, 8 para- infective (including 4 empyemas) and one rheumatoid arthritis had total ADA levels >46 U/L; of these, only one lymphoma and one rheumatoid arthritis had ADA2/ADA activity ratio >50%.*

Considering simultaneous criteria of total ADA more than 46U/L, ADA2 >42 U/L and ADA2/ADA more than 50%, we had only two false positive results, rising the specificity up to 96%.

***Conclusion**: 1. ADA2 is a more efficient diagnostic marker for Tuberculous pleural effusion compared with total ADA.*

2. Overall, diagnostic value of ADA would be enhanced by the determination of its isoenzymes, especially for distinguishing between the tuberculous and para-infective effusions. ***(Tanaffos 2005; 4(15): 37-42)***

***Key words**: ADA, ADA2, Tuberculosis, Pleural effusion*

Correspondence to: Mohammadtaheri Z

Address: NRITLD, Shaheed Bahonar Ave, Darabad, TEHRAN 19569, P.O:19575/154, IRAN

Email address: mtaheri@nritld.ac.ir

INTRODUCTION

Diagnosis of tuberculous pleural effusion is difficult due to low sensitivity of the various standard diagnostic tools. The diagnosis can be established by demonstrating elevated levels of adenosine deaminase(ADA) or interferon gamma in the pleural fluid(1). However, other diseases can also have elevated ADA levels including; para-infective effusions, malignancies, and collagen vascular disease. For an adequate interpretation of pleural ADA, it is important to highlight the fact that ADA is represented by two isoenzymes: ADA1 (consisting of two dimmers) and ADA2. Both, deaminate adenosine and 2′deoxyadenosine to inosine and 2′deoxyinosine respectively. ADA1 is found in all cells with optimal pH of 7-7.5 and similar affinity for both substrates. ADA2 is not ubiquitous and exists only in monocyte-macrophages, with optimal pH of 6.5, and weak affinity for 2′deoxyadenosine (2,3). ADA2 is the predominant isoform in tuberculous pleural effusion, whereas ADA1 is elevated in empyema (3). This would suggest that ADA2 is the more efficient marker of tuberculous pleural effusion.

However, the diagnostic efficiency of this isoenzyme measurement is controversial and sufficient data is not available in Iran.

We studied the diagnostic value of total ADA and ADA2 isoenzyme in tuberculous pleural effusion.

MATERIALS AND METHODS

We studied 93 consecutive patients with pleural effusion who were admitted to our center. The diagnostic criteria were as follows: *Congestive heart failure*: compatible clinical and radiological findings; *Tuberculosis*: presence of acid-fast bacilli in pleural fluid or, sputum, or presence of necrotizing granuloma in biopsy specimens; *Malignancy:* neoplastic cells found in biopsy or cytology; *Parapneumonic*: association with pneumonia; *Lung abscess or bronchiectasis and empyema*: presence of purulent fluid, or positive culture of pleural fluid; *Rheumatoid arthritis* with clinical history, and *idiopathic cases* with no specific cause were identified. On the basis of these diagnoses, 5 groups were identified as follows: Group 1, tuberculosis (n: 31, 26 males/ 5 females, aged 15-82yrs); Group 2, malignant exudates (n:30, 22 males/ 8 females, aged 28-86yrs); Group3, para-infective exudates, including empyema and parapneumonic exudates (n:15, 11 males/ 4 females, aged 20-83yrs); Group4, transudate (n:10, 6 males/ 4 females, aged 47-84yrs); Group 5, others (n:7, 4 males /3 females, aged 47-80 yrs).(table-1)

Table 1. Etiology of pleural effusions.

Etiology	Sex M: F ratio	Age years	No
Tuberculosis	5.2	15 -82(mean 42)	31
Malignancy	2.7	28-86(mean56.7)	30
Mesothelioma			2
Lymphoma			1
Synovial sarcoma			1
Metastatic carcinoma			26
Para-Infective	2.7	20-83(mean49)	15
Empyema			4
Para pneumonic			11
Transudative	1.5	47-84(mean70)	10
CHF			9
COPD			1
Others	1.5	47-80(mean62)	7
Idiopathic			5
Rheumatoid arthritis			2

Pleural fluid was obtained and centrifuged for 10′ at 1000 xg and the supernatants were removed and Stored in -70°C. ADA activity (U/L at 37°C) was

determined calorimetrically by Giusti and galanti methods (4). Adenosine was used as substrate and the release of ammonium ion was determined by reaction with 1.5cc of phenol nitropruside (106 mM phenol plus 0.17 mM sodium nitropruside) in the presence of 1.5 mL of sodium hypochlorite (11mM NaCl plus 125mM NaOH), for 30 minutes and absorption of 628 nm, then being read by a spectrophotometer. For ADA2 activity, the same technique was used with increasing, Erythro-9-(2-hydroxy -3-nonyl) adenine (EHNA) which is a potent inhibitor of only ADA1 isoenzyme and a concentration of 200 µmol/L was used in the reaction solution.

To control the presence of ammonium present before addition of exogenous adenosine, untreated samples were run in parallel. The results were expressed in U/L.

Statistical Analysis

The variables of ADA and ADA2 were expressed as mean ±SD for each disease groups.

The statistical significance of differences between means was estimated by wilcoxon ranks sum test due to the absence of normal distribution. The Roc curve analysis was used for sensitivity and specificity. Correlation between variables was estimated with r (Pearson correlation coefficient) and or spearman. $P<0.05$ was considered as significant.

RESULTS

Table-2 lists the mean and standard deviation of total ADA and ADA2 in pleural fluids for each group of patients. The inter-mean differences between the tuberculosis group and other groups estimated with Mann Whitney U test were statistically significant for two variables; ADA and ADA2 ($p<0.05$). The correlation coefficient (r value) between total ADA and ADA2 in tuberculosis group was 0.9 ($p <0.05$).

Figure 1 shows the total pleural ADA activities of each group of patients. All the tuberculous effusions had total ADA activities of 46U/L or more, whereas two of 30 malignant cases, 8 of the 15 cases of para-infective effusions, 1 case in others group, and none of the transudate group had ADA activities above this level.

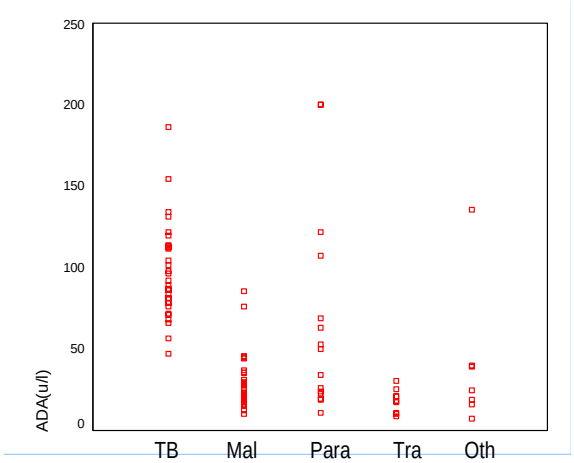

Figure 1. Total adenosine deaminase activity (ADA) of pleural fluids; Tuberculosis (TB), Malignancy (Mal), Para infective (Para), Transudate (Tra), Others (Oth).

Figure 2 shows the pleural ADA-2 activities of each studied group. All the tuberculous effusions had pleural ADA2 activities of 36 U/L or more, whereas 27 of the 30 malignant cases, 11 of the 15 para-infective cases, including all para-pneumonic effusions, and 6 of the 7 others group had ADA2 lower than this level.

Table 2. Total adenosine deaminase (ADA) and adenosine deaminase isoenzyme 2(ADA2) activities of pleural fluids in diagnosed subgroups.

DISEASES	ADA(u/l) Mean±SD	ADA-2,D(u/l) Mean±SD
Tuberculosis	96.6 ± 29.1	74 ± 28.6
Malignancy	28.8 ± 17.1	20.0 ± 10.3
Para-infective	68.0 ± 62.6	24.3 ± 17.4
Transudates	18.2 ± 6.9	13.4 ± 4.4
Others	40.2 ± 43.7	30.1 ± 36.2

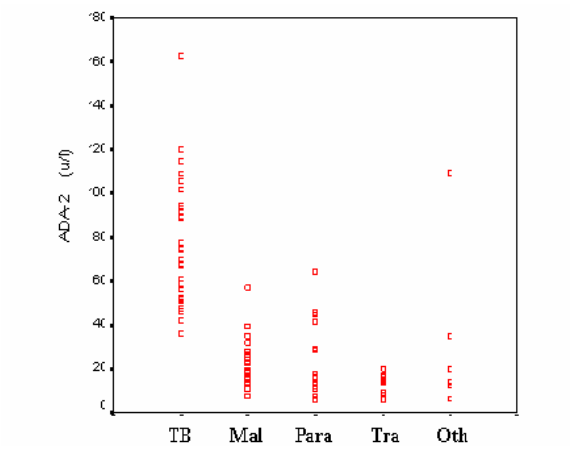

Figure 2. Adenosine deaminase 2 (ADA2, D) activity of pleural fluids: Tuberculosis (TB), Malignancy (Mal), Para infective (Para), Transudate (Tra), Others (Oth)

With diagnostic thresholds of 46 and 42 U/L, the sensitivities of ADA and ADA2 for tuberculosis were 100% and 97% respectively; their specificities were 82% and 88%; and their efficiencies were 88% and 93.5%, respectively.

The 5.5 percent difference between the efficiency of ADA and ADA-2 was significant.

All tuberculous exudates, 2 neoplastic, 8 para-infective effusions (including 4 empyemas) and one rheumatoid arthritis had total ADA levels >46 U/L; of these, only one lymphoma and one rheumatoid arthritis had ADA2/ADA activity ratio >50%.

Considering simultaneous criteria of ADA more than 46 U/L and ADA2/ADA more than 50%, we had only two false positive results (one lymphoma and one rheumatoid arthritis) which increase the specificity up to 96%.

DISCUSSION

In this study all tuberculous pleural effusions had high total ADA activity, mostly due to ADA2 isoenzyme (median activity of 70%). Most of the false positive results belonged to para-pneumonic effusions including empyema; of which, all had ADA2/ADA ratio lower than 50%.

High levels of ADA in pleural fluids of patients with TB pleurisy have been confirmed by all authors with cut off value between 40-70 u/l, with sensitivity and specificity ranging from 88 to 100%, and 81-97%, respectively (5-9).Depending on the chosen cut off point, the sensitivity and specificity is different. However, in the countries with high prevalence of TB pleuritis, the high sensitivity is more important. Using Roc curve, and choosing 46U/L as a cut off point, we found sensitivity of 100% and specificity of 82%. High levels of ADA can also be found with less frequency in patients with other diagnoses, particularly empyema, parapneumonia, lymphoma, carcinoma, and collagen vascular disease.

For an adequate interpretation of pleural ADA, it is important to highlight the fact that ADA has two main isoenzymes; ADA1 and ADA2. Both deaminate mainly two nucleosides: adenosine and 2′deoxyadenosine. ADA1 is ubiquitous and guarantees the downregulation of both nucleosides. ADA2 coexists with ADA1 only in monocytes-macrophages. It is necessary to consider ADA1 and ADA2 as a system that acts in homeostasis of adenosine and 2′deoxyadenosine in monocyte-macrophages (10). Both isoenzymes have similar affinity for the substrate adenosine, whereas ADA2 is very week for the substrate 2′deoxyadenosine. Through the simulation model STELLA II, Gakis (10) has demonstrated that in monocytes-macrophages the adenosine level is always low, whilst, in certain conditions, the 2′deoxyadenosine level rises dramatically owing to an increase of ADA2. This occurs when these cells are infected by intracellular microorganisms and while the parasite is still alive. 2′deoxyadenosine is deleterious for nucleic acid and ADA1-ADA2 homeostatic system may be a tool in the production of a weapon of monocytes-macrophage against *Mycobacterium tuberculosis*. The ADA of TB pleuritis mainly represents the

activation or turn over of monocyte-macrophages, more closely related to ADA2 (11-15). In this regard, it seems necessary to define the contribution of the ADA isoenzymes for diagnosis of tuberculous pleuritis.

In our study, all the tuberculous effusions had total ADA activities above 46U/L (sensitivity of 100%). This level was also present in 11 of non-tuberculous effusions mostly para-infective exudates (8 cases). ADA2 measurement increased specificity and considering ADA2 of more than 42U/L, only empyematous exudate (4 cases), one lymphoma and one RA had this level of activity.

Para-infective effusions including empyema had a high ADA activity and ADA1 was the predominant isoform due to significant numbers of polymrphonuclear. All the empyema in this study had ADA2/ADA ratio of lower than 50%.

Considering ADA2/ADA ratio more than 50%, all para-infective groups were excluded and only two false positive results were present, which increase specificity up to 96%.

If ADA assay is not performed for empyema fluid, one can avoid most false positive results due to an elevated ADA1 as shown by other authors.

In these conditions; however, ADA2 activity is lower than 50% of total ADA activity.

In malignant effusion, only one lymphoma had total ADA and ADA2 above the mentioned cut off, as in other studies (4, 16). Although, none of the other malignancies had this high level of activity, as previously described by others (7, 8, 10, 17). However, cytological examination can differentiate malignancy from tuberculosis.

Also, high ADA and ADA2 activities were noted in rheumatoid arthritis. The diagnosis of RA is solely based on clinical history and exclusion might be easy.

Using a sensitive test for early diagnosis and treatment is very important, regarding high prevalence of TB in our country. Determination of total ADA and then, its isoenzymes in selective cases gives the highest specificity and sensitivity. Those studies which do not recommend ADA2 measurement belong to the countries in which the disease is less prevalent such as the USA (18). The high cost of the test is another reason for the ADA2 measurement not to be in routine uses (19).

CONCLUSION

In tuberculous pleural effusion, there is a high ADA activity which is mostly due to ADA2 isoenzyme (median of 70% of total activity).

Determination of the individual isoenzymes could help in distinguishing between the various causes of increased ADA activity in effusions, especially between tuberculosis and para-infective causes. Overall, diagnostic value of ADA measurement would be enhanced by the determination of its isoenzymes.

REFERENCES

1. Light RW. Pleural Disease.4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001.
2. Ungerer JP, Oosthuizen HM, Bissbort SH, Vermaak WJ. Serum adenosine deaminase: isoenzymes and diagnostic application. ***Clin Chem*** 1992; 38 (7): 1322- 6.
3. Ungerer JP, Oosthuizen HM, Retief JH, Bissbort SH. Significance of adenosine deaminase activity and its isoenzymes in tuberculous effusions. ***Chest*** 1994; 106 (1): 33- 7.
4. Giusti G: Adenosine deaminase. In: Bergmeyer HU editor. Methods of enzymatic analysis, 2nd Ed, vol 2. WF700 L723p 2001. Pleural diseases / Richard W. Light. 4th ed. Philadelphia : Lippincott Williams & Wilkins, c2001. New York: Academic press inc; 1974, p.1092-9.
5. Burgess LJ, Maritz FJ, Le Roux I, Taljaard JJ. Combined use of pleural adenosine deaminase with lymphocyte/neutrophil ratio. Increased specificity for the diagnosis of tuberculous pleuritis. ***Chest*** 1996; 109 (2): 414- 9.

6. Banales JL, Pineda PR, Fitzgerald JM, Rubio H, Selman M, Salazar-Lezama M. Adenosine deaminase in the diagnosis of tuberculous pleural effusions. A report of 218 patients and review of the literature. ***Chest*** 1991; 99 (2): 355- 7.
7. Valdes L, Alvarez D, San Jose E, Juanatey JR, Pose A, Valle JM, et al. Value of adenosine deaminase in the diagnosis of tuberculous pleural effusions in young patients in a region of high prevalence of tuberculosis. ***Thorax*** 1995; 50 (6): 600- 3.
8. Valdes L, San Jose E, Alvarez D, Valle JM. Adenosine deaminase (ADA) isoenzyme analysis in pleural effusions: diagnostic role, and relevance to the origin of increased ADA in tuberculous pleurisy. ***Eur Respir J*** 1996; 9 (4): 747- 51.
9. Chen ML, Yu WC, Lam CW, Au KM, Kong FY, Chan AY. Diagnostic value of pleural fluid adenosine deaminase activity in tuberculous pleurisy. ***Clin Chim Acta*** 2004; 341 (1-2): 101- 7.
10. Gakis C. Adenosine deaminase (ADA) isoenzymes ADA1 and ADA2: diagnostic and biological role. ***Eur Respir J*** 1996; 9 (4): 632- 3.
11. Gakis C, Calia GM, Naitana AG, Ortu AR, Contu A. Serum and pleural adenosine deaminase activity. Correct interpretation of the findings. ***Chest*** 1991; 99 (6): 1555- 6.
12. Mohammed KA, Nasreen N, Ward MJ, Mubarak KK, Rodriguez-Panadero F, Antony VB. Mycobacterium-mediated chemokine expression in pleural mesothelial cells: role of C-C chemokines in tuberculous pleurisy. ***J Infect Dis*** 1998; 178 (5): 1450- 6.
13. Nasreen N, Mohammed KA, Ward MJ, Antony VB. Mycobacterium-induced transmesothelial migration of monocytes into pleural space: role of intercellular adhesion molecule-1 in tuberculous pleurisy. ***J Infect Dis*** 1999; 180 (5): 1616- 23.
14. Nicod LP. Cytokines. 1. Overview. ***Thorax*** 1993; 48 (6): 660- 7.
15. Pace E, Gjomarkaj M, Melis M, Profita M, Spatafora M, Vignola AM, et al. Interleukin-8 induces lymphocyte chemotaxis into the pleural space. Role of pleural macrophages. ***Am J Respir Crit Care Med*** 1999; 159 (5 Pt 1): 1592- 9.
16. Ocana I, Martinez-Vazquez JM, Segura RM, Fernandez-De-Sevilla T, Capdevila JA. Adenosine deaminase in pleural fluids. Test for diagnosis of tuberculous pleural effusion. ***Chest*** 1983; 84 (1): 51- 3.
17. Perez-Rodriguez E, Jimenez Castro D. The use of adenosine deaminase and adenosine deaminase isoenzymes in the diagnosis of tuberculous pleuritis. ***Curr Opin Pulm Med*** 2000; 6 (4): 259- 66.
18. Kataria YP, Khurshid I. Adenosine deaminase in the diagnosis of tuberculous pleural effusion. ***Chest*** 2001; 120 (2): 334-6.
19. Roth BJ. Searching for tuberculosis in the pleural space. ***Chest*** 1999; 116 (1): 3- 5.