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Original article

Adenosine deaminase isoenzymes estimation - as a diagnostic tool for tuberculous pleural effusions

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Abstract

Objective: To assess the efficacy of ADA isoenzyme estimation over that of total ADA level in pleural fluid and serum as a more efficient diagnostic indicator in tuberculous pleural effusions in high prevalence country like India. **Methods**: The efficacy was analysed in total thirty four patients of pleural effusions. Total ADA was estimated by Guitsi and Galanti Calorimetric method and ADA isoenzymes with and without EHNA[Erythro-9-(2-hydroxy-3-nonyl) adenine] a potent ADA_1 inhibitor using the same method. **Results**: The results demonstrated a statistically significant values of ADA_2 in serum ($P < 0.001$), pleural fluid ($P = 0.000$) and significant value for the ratio of pleural fluid ADA_2/serum ADA_2 ($P < 0.001$) and pleural fluid ADA_1/ADA_2 ($P < 0.005$). The sensitivity and specificity values of pleural fluid ADA_2 is 81.8 % and 91.6 % (cut off value 60 IU/L for Tuberculous effusions), serum ADA_2 95.4 % and 66 % (cut off value 70 IU/L for tuberculous effusions). ADA_2 is an isoenzyme, which is significantly raised in tuberculous pleural effusions both in the serum and pleural fluid. **Conclusion**: Estimation of ADA isoenzymes is redundant as a diagnostic aid over total ADA estimation in view of the limited improvements both in specificity and sensitivity patterns and also in term of cost-benefit ratio.

Keywords: Adenosine deaminase; Isoenzymes; Tuberculous pleural effusions

INTRODUCTION

Tuberculous pleural effusion is a global problem and its special reference to the high prevalence country like India cannot be ignored. Tuberculous pleural effusions pose a diagnostic problem for the clinician before starting the specific therapy.

Adenosine deaminase (EC 3.5.4.4) called ADA[1], catalyses the pathway from adenosine and deoxyadenosine to inosine and deoxyinosine. ADA exists in 3 molecular masses or isoenzymes ADA_1, ADA_{1+CP}, ADA_2. These isoenzymes are coded by different genes. ADA_1 is monomeric protein with a molecular mass of about 35 kDa (gene assignment chromosome 20); ADA_{1+CP} (molecular mass about 280 kDa) is composed of two ADA_1 molecules (dimer) connected via a connecting protein (CP)-a glycoprotein (gene assignment, chrome 2 and 6). The third isoenzyme ADA_2, appears to be coded by separate gene locus of unknown chromosomal position ($\approx$100 kDa)[2]. The isoenzymes are divided based on the different kinetic properties.

Putting one of the properties to use i.e. selective inhibition of ADA_1 by Erythro-9(2-hydroxy 3-nonyl) adenine (EHNA), an electrophoretic technique was developed by Buel E[3] and later on modified by Ungerer *et al*. He studied using this principle and esti-

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mated that ADA_2 is the predominant (mean 88 %) contributor of total ADA in tuberculous pleural effusions and ADA_1 and ADA_{1+CP} in parainfective etiologies (mean 70 %). ADA_2 activity principally reflects that of monocytes and macrophage turnover and has been evaluated as a diagnostic tool in the tuberculous pleural effusions. These studies were done in view of the varied cut-off points and positive predictive values, sensitivities and specificities and associated limitations of ADA estimation in diagnosing tuberculous pleural effusions[4-20] and varying positive predictive value with age of patients[21]. The estimation of ADA isoenzymes has been proved as an efficient biochemical parameter for the diagnosis of tuberculous pleural effusions than total ADA levels[22-27].

With different studies taking different combinations of obtained parameters for diagnosing the tuberculous pleural effusions, the present study tries to evaluate the utility of ADA isoenzymes in high prevalence country, India.

MATERIALS AND METHODS

Study design

Twenty two confirmed patients of tuberculous pleural effusions (median age of 35.5 years, range 18-62) were evaluated, 12 patients of confirmed malignant pleural effusions were taken as the comparative group as they constitute an important etiological cause of pleural effusions after tuberculosis (median age 54.7, range 28-74) The patients were confirmed by standard diagnostic criteria like cultures for tuberculous mycobacterium of pleural fluid and pleural biopsy for histopathological examination and culture.

Properties of ADA_1 and ADA_2 isoenzymes[28-31] were shown in Table 1. Total ADA was estimated by Guitsi and Galanti Calorimetirc method[32]. To estimate the levels of ADA_1 and ADA_2 isoenzymes, the ADA activity was measured by the same above technique with and without Erythro-9 (2-hydroxy 3-nonyl) adenine (EHNA). EHNA is a potent inhibitor of only ADA_1 isoenzyme and a concentration of 200 μmol/L was used in the reaction[29]. In its presence, only the ADA_2 isoenzyme is active. The ADA_1 activity is then calculated by subtracting the ADA_2 isoenzyme from the total activity (this method cannot differentiate between ADA_1 and ADA_{1+CP}).

Statistical analysis

The results were analysed using the non-parametric Wilcoxon Mann-Whitney *U*-test. The results of the parameters were then evaluated by looking at sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), percentage of false positivities and percentage of false negativities.

RESULTS

The mean values of the total ADA, ADA_2 both in pleural fluid and serum were significantly higher in tuberculous pleural effusions in comparison to malignant effusions ($P < 0.001$). Similarly the ratio of ADA_2 in pleural fluid and serum is found statistically significant ($P < 0.005$) in tuberculous pleural effusion (Table 2).

The sensitivity, specificity, positive predictive value, negative predictive values of different parameters is shown in Table 3. There is no marked variation in the sensitivity and specificity values of pleural fluid ADA_2 over that of the total ADA estimated when a higher cut off value is taken; in our study the values for total pleural fluid ADA were estimated when the cut off value of 70 IU/L is taken. This higher cut off value is taken, when compared to the previous studies to get a comparative estimation of the efficacy of isoenzyme estimation over that of the total ADA. Even though mycobacterium culture and histopathology of pleural biopsy is the specific test it has widely different and low sensitivities. Sensitivities of the other parameters using the isoenzymes were found to be higher than the total ADA even though a higher cut off value is taken for it (Figure1).

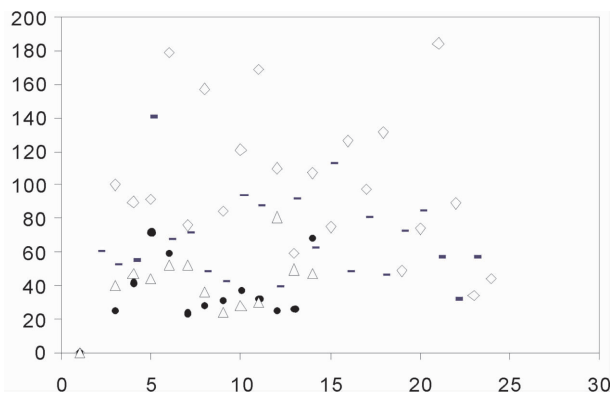

Figure 1 Distribution of ADA_2 in pleural fluid and serum of tuberculous and malignant pleural effusions.
· Serum ADA_2 of malignant pleural effusions
◇ Pleural fluid ADA_2 in tuberculous pleural effusions
△ Pleural fluid ADA_2 in malignant pleural effusions
- Serum ADA_2 in tuberculous pleural effusions

Table 1 Properties of ADA_1 and ADA_1 isoenzymes[28-31].

Properties	ADA_1	ADA_2
Site of distribution	> 90 % of Adenosine deaminase activity in human cells and tissues	Specially seen in spleen with 2 % of Adenosine deaminase activity and monocytes (18 %)
Molecular weight	ADA_1-35 kDa ADA_{1+CP}-280 kDa	100 kDa
Optimum pH	5.5-8.0	6.5
Km for Adenosine	50 μmol/L	2 000 μmol/L
Deaminating activity for 2′ deoxyadenosine versus adenosine	0.8 (higher)	0.2 (lower)
Inhibition by EHNA	Inhibited	Insensitive for inhibition
Gene assignment	ADA_1-chromosome 20 ADA_{1+CP}-chromosome 2 and 6	ADA_2-coded by separate gene locus of unknown chromosomal position

Table 2 Mean values of the different parameters in tuberculous and malignant pleural effusions (values in IU/L).

Parameter	Malignant	Tuberculous	*P*
Serum ADA_1	6.23	7.70	<0.790
Serum ADA_2	39.16	68.79	<0.001 *
Total Serum ADA	45.38	76.50	<0.001 *
Pleural fluid ADA_1	10.99	14.82	<0.423
Pleural fluid ADA_2	44.25	102.09	0.000 *
Total Pleural fluid ADA	55.41	116.91	0.000 *
P/S ratio of ADA_1	2.04	3.08	<0.632
P/S ratio of ADA_2	1.02	1.50	<0.001 *
Serum ADA_1/ADA_2	0.17	0.13	<0.136
Pleural fluid ADA_1/ADA_2	0.27	0.18	<0.005 *

* = significant and others not significant
P/S ratio = pleural / serum ratio

Table 3 Yield of different parameters in diagnosing tuberculous pleural effusion, which were found to be statistically significant.

	Total ADA	Pl. fluid ADA_2	Serum ADA_2	P/S ratio of ADA_2	Pl. fluid ratio of ADA_1/ADA_2
Sensitivity	86.36 %	81.8 %	95.4 %	95.45 %	80.0 %
Specificity	91.66 %	91.6 %	66.0 %	75.00 %	83.3 %
P P V	95.00 %	94.7 %	84.0 %	87.50 %	88.8 %
N P V	78.57 %	73.3 %	66.6 %	60.00 %	71.4 %
% of F N	13.63	18.00	4.50	4.50	20.00
% of F P	8.33	8.30	25.00	25.00	16.60
Cut-off value for tuberculosis	≥70 IU/L	≥60 IU/L	≥40 IU/L	≥1.00	≤0.20

(Pl. fluid = Pleural fluid, P P V = positive predictive value, N P V = Negative predictive value, % of F N = percentage of false negatives, % of F P = percentage of false positives)

DISCUSSION

The diagnosis of tuberculous pleural effusion is best made on the conventional diagnostic techniques of pleural biopsy (culture and histopathology). ADA estimation has over taken other markers in the march as the cheap and easily performed tests with wide acceptability in India and other countries, amply demonstrated by a number of studies with wide range of specificities and sensitivities, and wide availability of the test in many laboratories.

The greatest ADA activity was found in lymphocytes and monocytes[28]. ADA is the major isoenzyme originating from lymphocytes or neutrophils and ADA_2 is an isoenzymes unique to monocyte/macrophage cell lineage[22,29] with the studies showing the ADA_2 is specific marker in the diagnosis of tuberculous pleural effusion than ADA[22-24,26,27].

All the pleural biochemical factors are taken as a ratio in comparison with serum so as to negate any confounding factor affecting the marker; therefore we estimated the serum levels of isoenzymes.

The sensitivity and specificity values have varied widely with different cut off values adopted for isoenzyme parameters (in pleural fluid and serum). The standard deviation from mean in case of isoenzymes was similar as in case of the total ADA levels, but did not offer any advantage over that of the total ADA estimation.

ADA_2 is the major contributor of the total ADA activity in tuberculous pleural effusions[22,29] and ADA_2 activity is unique to monocyte/macrophage lineage[28]. In the study it was found that in some cases the contribution of ADA_2 to the total activity of ADA in pleural fluid and serum as 100 %, with nil contribution by ADA_1 isoenzyme. Even though the correlation of ADA cell lineage is not probed in our study it poses the question - don't lymphocytes contribute in any way to the total ADA activity as seen in some cases, but ADA is raised in all lymphocytic pleural effusions. So what is the reason for the total absence of ADA_1 in few cases of tuberculous pleural effusion, which is predominant lymphocyte pleural effusion. The reason for this can be deduced from the explanations that have come out of the previous studies[8,17]. The ADA_1 contribution in the tuberculous effusions may be related to the subsets of T lymphocytes and their maturity, and this may dictate the level of ADA_1 in tuberculous effusion, with the rest being contributed by ADA_2 from the monocyte/macrophage. It has also been shown in the previous studies that the ADA in the tuberculous effusion correlated to the monocyte/macrophage cell lineage[25].

To conclude the study showed that ADA_2 is the predominant isoenzyme both in serum and pleural fluid, which is significantly raised in tuberculous pleural effusion. But the advantage in terms of sensitivity and specificity and in cost-benefit ratio in a developing country like India makes the estimation of ADA isoenzymes redundant and should not be done routinely.

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