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A Comparative Study And Evaluation Of The Diagnostic Utility Of Adenosine Deaminase (ADA) Activity In Pleural, Peritoneal And Cerebrospinal Fluid For The Diagnosis Of Tuberculosis

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Abstract:

Background *Tuberculosis is a major cause of effusion & meningitis. Diagnosis by conventional methods has either less sensitivity or time consuming. Detection of ADA enzyme activity has been proposed to be a useful surrogate marker for diagnosis of tuberculous effusion & TB meningitis.*

Objective *To Assess the value of ADA activity in aspirated pleural, peritoneal and cerebrospinal fluid for the diagnosis of TB infection.*

Methods *Total 150 fluid; 70 pleural, 16 peritoneal and 64 cerebrospinal fluid were evaluated for ADA activity along with routine biochemical, bacteriological and cytological examination. Findings are correlated with clinical and radiological evidences.*

Results *Among confirmed 51 TB pleural effusion: ADA activity found positive (>60 U/L) in 46 (90.2%) borderline (between 40 to 60 U/L) in 4. only 1 (1.96%) was negative (<40 U/L) .*

Among 2 TB peritoneal effusions: ADA activity found positive in both (100%)

Among confirmed 21TB meningitis cases: ADA activity found positive (>10U/L) in 20(95.2%) and only 1(4.8%) was negative (<10 U/L).

Conclusion *Diagnosis of tuberculous effusion by direct smear, culture, cytology and biochemical tests have either limited sensitivity or specificity while test for biochemical marker- ADA activity is simple, rapid, and economic and has significant role in early diagnosis of tuberculous effusion and TB meningitis.*

Introduction

Despite the discovery of tubercle bacillus more than a hundred years ago, and all the advances in our knowledge of the disease made since then, tuberculosis still remains one of the major health problems facing mankind, particularly in developing countries. Presently about one third of the world's population is infected with mycobacterium tuberculosis. It is estimated that currently there are about 10 million new cases of tuberculosis every year with 3 million deaths occurring world-wide [(1)]

Currently more people die of tuberculosis than from any other infectious diseases. nearly 95% of all tuberculosis cases and 98% of deaths due to tuberculosis are in developing countries and 75% of tuberculosis cases are in the economically productive age group[(2)]. In India, out of a total population over 1 billion, each year about 2 million develop active disease and up to half million die [(3)]. It also imposes a cost on our economy in terms of current and future output losses because of premature deaths & ill health[(4)].

To add to the exciting burden, the situation is compounded by large scale increase of new TB cases associated with increasing HIV-infection. India is estimated to have 3.5 million HIV patients and about 1.8 million of these are co-infected with TB [(5)].

Early diagnosis of tuberculosis and initiating optimal treatment would not only enable a cure of individual patient but will also curb the transmission of infection and diseases to others in community. Diagnosis of tuberculosis confirmed mainly by direct sputum examination of AFB and by culture. But diagnosis of tuberculosis pleural effusion and TB meningitis are difficult due to low sensitivity of the various standard diagnosis tools. The diagnosis can be established by demonstrating elevated level of Adenosine Deaminase (ADA) or interferon gamma in the aspirated fluid [(6)].

However the diagnoses of tuberculous effusion require investigation of fluid biochemistry, cytology and pleural biopsy. Positivity for AFB is very low and culture is time consuming. ELISA, PCR and interferon are very expensive test.

Adenosine Deaminase (ADA) has been proposed to be a useful surrogate marker for the diagnosis of tuberculosis in pleural, pericardial and peritoneal fluids. Studies have confirmed high sensitivity and specificity of Adenosine Deaminase for early diagnosis of extra pulmonary tuberculosis [(7, 8, 9)].

ADA is an enzyme widely distributed in mammalian tissue particularly in T lymphocytes.ADA is enzymes that catalyses conversion of Adenosine to inosine. Adenosine is a predominant T lymphocytes enzyme and its plasma activity is high in

diseases in which cellular immunity is stimulated. Increase level of ADA found in various forms of tuberculosis making it a marker for the same [10].

Materials And Methods

This study was carried out on 150 patients suffering from pleural, peritoneal effusion and meningitis who attended OPD or were admitted in P.D.U. Government Medical College & Hospital, Rajkot during April to July 2012. Detailed clinical history, physical examination and investigations e.g. smear for AFB, cytological examination, biochemical examination and wherever possible USG,X-ray chest ad other appropriate investigations including ADA level in aspirated fluid were carried out in all the patients. ADA estimation was done by commercially available kits based on colorimetric method with following principles.

Principle

Adenosine Deaminase hydrolyses adenosine to ammonia and inosine. The ammonia formed, further reacts with phenol and hypochlorite in an alkaline medium to form a blue indophenols complex with sodium nitropruside acting as a catalyst. Intensity of blue colored complex formed is directly proportional to the amount of ADA present in sample.

$$\text{Adenosine} + H_2O \xrightarrow[\text{ADA}]{} \text{Ammonia} + \text{Inosine}$$

$$\text{Ammonia} + \text{Phenol} \xrightarrow[\text{Alkaline medium}]{} \text{Blue Indophenol complex.}$$

Reference value

Specimen	Reference value (ADA)
Serum,plasma,Pleural, Pericardial Peritoneal fluid	Normal < 40 U/L, Positive > 60 U/L Suspect > 40 U/L to < 60 U/L,
CSF	Normal < 10 U/L, Positive > 10 U/L

Table 1: Reference value (ADA)

Test Procedure

	Blank(ml)	Standard(ml)	Sample blank(ml)	Test(m)
Buffer	0.20	0.20	-	-
Reagent	-	-	0.20	0.20
Adenosine reagent	0.02	-	-	-
Deionised water				
Standard	-	0.02	-	-
Sample	-	-	-	0.02

Table 2

Mix well incubated 37°c for 60 minutes and then adds the following:

Working phenol reagent	1.0	1.0	1.0	1.0
Sample	–	–	0.02	–
WorkingHypochlorite reagent	1.0	1.0	1.0	1.0

Table 3

Mix well incubated 37°c for 15 minutes or at R.T. for 30 minutes.

Measure the absorbance of blank, standard, sample blank and test against distilled water.

Calculations:

$$\text{Total ADA activity In U/L} = \frac{\text{Absorbance of Test - Absorbance of SB}}{\text{Absorbance of S - Absorbance of B}} \times 50$$

Linearity

The Procedure is Linear up to 150 U/L if the values exceeded this limit dilute the sample with deionised water and repeat this assay. Calculate the value using appropriate dilution factor.

Result And Observations

Type of fluid	NO.	ADA activity in U/L		
		normal < 40 U/L	suspect >40 U/L to <60U/L	positive >60 U/L
Pleural fluid	70	17	06	47
Ascitic (Peritoneal fluid)	16	13	01	02
CSF	64	Normal<10 U/L 44	_	Positive>10U/L 20
Total	150	74	07	69

Table 4

Out of total 150 fluid specimens; 70 were pleural fluid, 16 were peritonial fluid and 64 were cerebrospinal fluid. Of 70 pleural fluid tested for ADA activity; 48 showed ADA activity > 60 U/L (positive), 14 showed activity < 40 U/L (normal) and 08 showed ADA activity between 40 -60 U/L (suspect). Of 16 peritoneal fluid tested; 2 showed positive, 13 showed negative & 1 showed suspect result. Of 50 CSF tested; 14 showed ADA activity > 10 U/L (positive) and 50 showed activity < 10 U/L (normal).

Type of fluid	Diagnosis	NO	ADA activity		
			Positive	Suspect	Normal
Pleural Fluid 70	Tuberculosis	51	46 (90.2%)	04(7.8%)	01(2%)
	non TB	19	01 (5.3%)	02 (10.5%)	16 (84.2%)
Peritoneal fluid 16	Tuberculosis	02	02 (100%)	00	00
	Non TB	14	00	01 (7.2%)	13 (92.8%)

Table 5

Type of fluid	Diagnosis	No.	ADA activity	
			Positive	Negative
			>10 U/L	<10U/L
CSF 64	Tuberculosis	21	20 (95.2%)	01 (4.8%)
	Non TB	43	00	43 (100%)

Table 6

On correlating the clinical, radiological, cytological & biochemical findings, out of total 70 pleural effusion patients; 51 were diagnosed as tuberculous effusion of which 46 (90.2%) showed increased ADA activity, 1 (2%) showed normal ADA activity & 4 (7.8%) showed suspect results. From 19 non TB pleural effusions 16 (84.2%) showed normal activity. Of total 16 peritoneal effusion patients; two diagnosed as tuberculosis origin and both (100%) showed increased ADA activity. Of 14 non tuberculosis patients, 13 (92.8%) showed normal ADA activity (<40 U/L). Out of total 64 CSF specimen tested; 21 diagnosed as tuberculous meningitis of which 20 (95.2%) showed increased ADA activity. All 43 patients having non tuberculous etiology showed normal ADA activity (100%) in CSF samples.

So overall 74 patients diagnosed having tuberculosis; 68 (92%) showed increased ADA activity, 2 (2.7%) showed normal ADA activity and 4 showed suspect results. If we take 50 U/L as cut off point, 72 (97.3%) showed increase ADA activity.

Specimen	Diagnosis as TB	AFB positive
Pleural fluid	51	03 (5.9%)
Peritoneal fluid	02	00 (0%)
CSF	21	01(4.8%)
Total	74	04 (5.4%)

Table 7

AFB positivity by ZNCF technique as per the RNTCP guideline in specimens suggests very low sensitivity of direct smear examination for AFB in diagnosis of tuberculous effusion.

Age	No. of patients having TB etiology			Total
	Pleural fluid	Peritoneal fluid	CSF	
00-10 Years	08	00	03	11
11-20 Years	05	00	03	08
21-30 Years	14	01	03	18
31-40 Years	13	01	02	16
41-50 Years	04	00	02	06
51-60 Years	02	00	02	04
>60 years	05	00	00	05
Total	51	02	15	68

Table 8

In this study it is found that tuberculous effusion predominantly occurs in adult patients: 20-40 years age group and tuberculous meningitis predominantly occur in pediatric and young patients.

ADA, a product of T lymphocytes has been reviewed as an excellent marker for the diagnosis of tuberculous pleural effusion. The raised ADA activity under antigenic stimulation is found in infections such as tuberculosis where cell mediated immunity (CMI) is stimulated [(11)].

Almost all research workers have shown sensitivity & specificity of 90% to 100% for the value ADA in pleural fluids using different cuts off levels.

Gupta D.K. [(12)] studied 53 cases of pleural effusion out of which 36 were of TB etiology. The mean ADA level I tuberculosis was 50.75 U/L while in malignant and Para pneumonic effusion it was 14.47 U/L and 28.65 U/L respectively. The sensitivity & specificity for diagnosis tuberculosis were 100% & 94% respectively.

Burgess L.J. [13] showed ADA activity in tuberculous effusion was higher than in any other diagnostic group. At a level pf 50 U/L the sensitivity and specificity for the identification of tuberculosis was 90% and 89% respectively.

Value of activity in pleural effusion was studied by Shibagaki T et al [14]. He concluded that tuberculous pleural effusion had a much higher ADA activity than cancer effusion and total ADA activity in tuberculous pleural effusion decreases after anti tubercular therapy.

Using cut off level of 25 U/L in serum and 45 U/L in pleural fluid ADA had sensitivity of 72.4% and 76.10% with specificities of 81.53% and 100% respectively, in tuberculous pleural effusion patients. It has been reported that with an ADA activity cut off value of 54 U/L, the sensitivity is 82% and specificity is 97% for the diagnosis of tuberculosis [15]. (Baganha et al, 1990).

Reported cut off value for ADA (total) vary from 47 to 60 U/L (valeles et al 1993[16], perez-Rodriguez et al, 1999[17], Roth et al, 1999[18].

Several studies have suggested that an elevated pleural fluid ADA level predict tuberculous pleuritis with a sensitivity of 90-100% and specificity of 89-100% where the guisti method (colorimetric) is used [19].

Conclusion

Pleural and peritoneal effusions occur in a large variety of pathological condition. Different laboratory tests on fluid have been advocated but they all have shown either less sensitivity or time consuming.

This study shows very encouraging results with 98.5% sensitivity & 98.57% specificity of ADA test for the diagnosis of tuberculosis infection from pleural, peritoneal & Cerebrospinal fluid.

Many sensitive tests involving molecular diagnosis is available for rapid diagnosis of TB, but they are expensive, require advanced infrastructure and expertise skill, which is not available in developing countries like India. The colorimetric method for the measurement of total adenosine deaminase (ADA) activity has advantages over other methods because of its lower cost, simplicity of technique and rapid turn- around time. The estimation of ADA activity in body fluids such as pleural, peritoneal & CSF there for serves as a reasonable tool in the diagnosis of Tuberculous pleural and peritoneal effusion & TB meningitis when other clinical laboratory tests are negative.

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