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Research Article

Diagnostic role and estimation of adenosine deaminase in serosal effusions

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Abstract

Objective: The objective of the study was to estimate the minimum value of adenosine deaminase (ADA) in tubercular (TB) and non-tubercularserosal effusions.

Methods: The study was conducted on 336 subjects attending to OPD & IPD of TB chest & respiratory disease, Medicine and Orthopedics departments of Subharti Medical College and its associated hospital, Meerut, U.P. India. Out of these 155 subjects were tubercular (tubercular pleural effusion 45, peritoneal 34, synovial 34 and cerebrospinal fluid 42) and 181 were non tubercular (pleural 42, peritoneal 48, synovial 37 and cerebrospinal fluid 54). ADA levels of different serosal fluids were estimated by Microexpresskit, based on Guisti&Galantimethod. Data was analyzed by one way Anova.

Results: In our study, we found the following cut off values of ADA in different types of serosaleffusions. In tubercular pleural effusion it was 65Unit/Litre (U/L), peritoneal 75U/L, synovial 42U/L and cerebrospinal fluid 13U/L respectively. In non-tubercular pleural effusion it was 6U/L, peritoneal 4U/L, synovial 13U/Land cerebrospinal fluid 2U/L respectively. Sensitivity & specificity of tubercular effusion were 100% and 0% respectively. While for non-tubercular pleural effusion it was 93% & 53%, for peritoneal 90% & 67%, for synovial & CSF it was 0%& 100% respectively.

Conclusion: ADA estimation will be helpful as an alternative diagnostic method for early detection of TB and to differentiate between pulmonary and extra pulmonary tuberculosis.

Keywords: ADA, Pleural effusion, Peritoneal effusion, Synovial effusion, TB

1. Introduction

Tuberculosis is one of the oldest and commonest infections in India affecting not only lungs but also extra pulmonary sites. Diagnosis of TB is confirmed by sputum examination of acid fast bacilli (AFB) and its culture which is positive in only one third of serosal fluid sample and has low sensitivity.[1-3] Other tests are X-ray and tuberculin test which can be negative and non-specific.[4-5] Adenosine deaminase (ADA)is an enzyme (E.C. no 3.5.4.4)for the catabolism of purine bases, capable of catalyzing the deamination of adenosine forming inosine in the process.[6]ADA is encoded by 12 exon, 32 kb gene located on chromosome no 20q13.11. ADA catalyze the replacement of 6-amino group of adenosine (Ado) and 2'-

deoxyadenosine (dAdo) with oxygen producing inosine (Ino) and 2'-deoxyinosine (dIno). These ADA products as well as guanosine and 2'-deoxyguanosine, which are also 6-oxypurines, undergo purine nucleoside phosphorylase catalyzed phosphorylite cleavage to yield component base, hypoxanthine or guanine and ribose or 2'-deoxyribose-1-PO_4. ADA is expressed at high levels in lymphoid cells. Its activity is greatest in cortical thymocytes and decreases with maturation of B-cells. In tissues like activated T-cells ADA is complex of >200 kD bound to cell membrane associated glycoprotein. Its principle biological activity is related to proliferation and differentiation of lymphocytes. The enzyme activity is greater in T-lymphocytes then in B- cells.[7-9]

Tubercular effusion is the result of a cell mediated immune response to the presence of *Mycobacterium tuberculii* and is characterized by the accumulation of activated T-lymphocytes and macrophages. Cellular immune response and in particular activation of T- lymphocytes is reflected by the presence of ADA in pleural fluid. Thus ADA activity, a marker of T cell activation and cell mediated immune response can help differentiate tubercular etiology from non-tubercular. ADA was introduced in 1978 for diagnosing of tubercular effusions. It is simple and inexpensive colorimetric test. Studies have confirmed high sensitivity and specificity of ADA for early diagnosis of extra pulmonary TB.[10-19] As very few studies have considered all serosal effusions (pleural, peritoneal, synovial and cerebrospinal fluid), so we took this study to estimate cut off values of ADA in all serosal effusions and to compare ADA values in different serosal effusions due to tubercular and non-tubercular etiology.

2. Materials and Methods

The research protocol for the present study was approved by the ethical committee of our institution and informed consent was obtained from each subject prior to inclusion in the study. The study was conducted from 2009 to 2012. Subjects were selected from OPD & IPD of TB chest & respiratory disease, medicine and orthopedics department of Subharti Medical College hospital and its associated rural and urban health centre at Sarawani, Mahalwala, Khajoori and Multannagarcoming under District Meerut of India. Only those, who were having serosal effusion due to tubercular or non-tubercular etiology, were selected. Total no of patients included in our study were 336, out of which 155 were tubercular and 181 were non tubercular. There were 186 males and 150 females, ranging from 15-65 years of age. Patients on anti-tubercular therapy were excluded from the study. After taking written consent from the patients, detailed clinical history was taken and investigation like Hb, TLC, DLC, GBP, CBC, AFB culture, sputum for AFB and X-ray were done. Cases of TB were diagnosed by – clinical presentation of TB, AFB staining of sputum and radiological findings.Five ml of serosal fluid was collected in plain vial. After centrifuging it, ADA was estimated in supernatant by colorimetric method using Microexpress readymade kit from Tulip Diagnostic India Pvt Ltd, based on Guisti & Galanti method.[20]Absorbance was read at 580 nm. Data was analyzed by one way Anova.

3. Results

Total no of patients in our study were 336, out of which 155 were tubercular (pleural 45, peritoneal 34, synovial 34 and CSF 42) and 181 were non tubercular (pleural 42, peritoneal 48, synovial 37 and CSF 54).Following were the results of our study – the range of ADA value in tubercular pleural effusion was 65-162U/L with mean ± standard deviation (SD) of 109.6±28.9, peritoneal was 75-176U/L with mean ± SD of 119.3 ± 36.4, synovial was 42-92U/L with mean ± SD of 56.6 ± 8.6 and CSF was 13-112U/L with mean ± SD of 32.8 ± 23.4 respectively (Table 1).

The range of ADA value in non-tubercular pleural effusion was 6-60U/L with mean ± SD of 37.3 ± 21.7, peritoneal was 4-38U/L with mean ± SD of 20.3 ± 14.8, synovial was 13-39U/L with mean ± SD of 20.5 ± 5.5 and CSF was 2-22 U/L with mean ± SD of 11.5 ± 7.2 respectively (Table I).From our observation, we found the following minimum (cut off) value of ADA in different types of effusion. In the tubercular pleural effusion it was 65U/L, peritoneal 75U/L, synovial 42U/L and CSF was 13U/L respectively. In the non-tubercular pleural effusion it was 6U/L, peritoneal 4U/L, synovial 13U/L and CSF it was 2U/L respectively. The minimum values of ADA in effusion due to tubercular etiology were higher as compared to non-tubercular ones.

Sensitivity and specificity of tubercular effusion (pleural, peritoneal, synovial & CSF) were 100 percent and 0 percent respectively. While for non-tubercular pleural effusion it was 93% and 53%, for peritoneal it was 90% and 67%, for synovial and CSF both, it was 0% and 100% respectively (Table II).

Table I– Comparison of ADA values in Tubercular and Non Tubercular serosaleffusions.

Variables		Pleural effusion	Peritoneal effusion	Synovial effusion	CSF
Tubercular	Range(U/L)	65-162	75-176	42-92	13-112
	Cut off value	65	75	42	13
	Mean ± SD	109.6 ± 28.9	119.3 ± 36.4	56.6 ± 8.6	32.8 ± 23.4
Non tubercular	Range (U/L)	6-60	4-38	13-39	2-22
	Cut off value	6	4	13	2
	Mean ± SD	37.3 ± 21.7	20.3 ± 14.8	20.5 ± 5.5	11.5 ± 7.2

Table II – Sensitivity and Specificity of Tubercular and Non Tubercular serosaleffusions.

Variables		No of true positive	sensitivity	specificity
Pleural	Tubercular n=45	45	100%	0%
	Non tubercular n=42	39 *(TN 5, FN 3, FP 4)	93%	56%
Peritoneal	Tubercular n=34	34	100%	0%
	Non tubercular n=48	43 (TN 2, FN 5, FP 1)	90%	67%
Synovial	Tubercular n=34	34	100%	0%
	Non tubercular n=37	(FP 37)	0%	100%
CSF	Tubercular n=42	42	100%	0%
	Non tubercular n=54	(FP 54)	0%	100%

*TN – true negative, FN – false negative, FP – false positive

4. Discussion

TB is common cause of serosal effusions. In our study the cut off values of ADA were in agreement with following other studies. Piras *et al*[11] reported high ADA value in tubercular effusion (more than 40U/L). Ocana *et al*[12] in 1983 & Valdes *et al*[15] in 1993 reported 100 percent sensitivity & specificity, positive predictive value and negative predictive value in larger sample size study. Meta-analysis of studies[21] between 1966 & 1999 concluded that the test performance was reasonably good (sensitivity range 47.1-100 percent and specificity range 0-100 percent) in diagnosing etiology in pleural effusion. Voight *et al*[22] studied 41 cases with bacteriologically confirmed tuberculosis and 41 cases with other cause and found that mean ADA level for tubercular etiology were 99.8U/L with sensitivity of 95 percent and specificity of 98 percent. Shrish *et al*[23] reported value of ADA as 12.2±3.13U/L for TB meningitis group and it was significantly higher (*P* value > 0.001) than the partially treated pyogenic meningitis (5.39±2.7U/L) and aseptic meningitis (1.92±0.56U/L). Burgess *et al*[24] showed ADA activity in tuberculous effusion to be higher than in any other diagnostic group. At a level of 50U/L the sensitivity and the specificity for the identification of tuberculosis was 98 and 89 percent respectively. Mathur P.C. *et al*[25] found in their study that ADA level in the tubercular pleural effusion ranged from 45-160U/L with a mean level of 100U/L, while in non-tubercular group it ranged from 5-33U/L with the mean of 18U/L (*P* value < 0.001, highly significant). ADA level in tubercular ascites was 13-135U/L with a mean level of 92U/L while in the non-tubercular group it was 1-28U/L with a mean of 12U/L. (*P* value< 0.001, highly significant). Different researchers have found different minimum values of ADA in serosal effusions, so more studies are required to come to a final minimum value which can be set as standard for diagnosing tuberculosis in tubercular and non-tubercularserosal effusions.

5. Conclusion

The method of ADA estimation is easy, simple and does not require expensive equipments. It can be estimated by simple colorimeter. It is economical and less time consuming (takes only two hours). This test may find a place in routine investigation for early detection of TB in coming days and for differentiating tubercular from non-tubercular etiology in pulmonary and extra pulmonary TB.

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