PLEURAL EFFUSION, ADENOSINE DEAMINASE (ADA) AND LACTATE DEHYDROGENASE (LDH) ENZYMES LEVEL, CORRELATED WITH CYTOLOGICAL EVALUATION.

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Abstract:

Background: Measurement of pleural fluid adenosine deaminase (ADA) and Lactate dehydrogenase (LDH) enzymes activity has gained increasing popularity as a diagnostic test for tuberculous and non-tuberculous pleuritis, especially in countries where the prevalence of TB is high. It carries a high sensitivity, inexpensive and easy.

Objective: To demonstrate the diagnostic value of increased level of ADA and LDH in pleural effusion correlated with the cytological, biochemical and bacteriological assessment.

Methods: seventy-five patients presented with pleural effusions were studied (53 males and 22 females) their mean age was 43.8 years. In all cases after the clinical assessment, evaluation of the pleural fluid was done and this included cytological exam with biochemical tests (adenosine deaminase "ADA" enzyme, lactate dehydrogenase "LDH", protein and glucose level) and bacteriological tests (Gram stain, and Ziehl-Neelsen stain).

Results: From the clinical data and lab tests, patients were divided into six groups according to the etiology of pleural effusion. Most (32 patients) were tuberculous, malignant effusion13 patients, infection 10 cases, heart failure 8 cases, idiopathic effusion 6 cases and miscellaneous 6 cases. Significant difference was found in ADA level in different effusions ($P<0.005$). Highest value of ADA was in TB effusions (the mean was 76.6 u/l), compared to malignant effusions (the mean was 32.4 u/l) and less values in other effusions. LDH highest value was in malignant and TB effusions (mean 321.1 and 314u/l respectively).

Conclusion: Increased ADA levels in TB effusions can be used to differentiate tuberculous from non-tuberculous effusions. And high LDH levels were useful in confirmation malignant effusions.

Keywords: ADA, Pleural effusion, TB.

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Introduction

Measurement of pleural fluid ADA activity has gained increasing popularity as a diagnostic test for tuberculous pleuritis since 1978, especially in countries where the prevalence of TB is high. It carries a high sensitivity (90-100%), inexpensive and easy to measure[1, 2]. ADA is an enzyme of purine catabolism, which catalyzes deoxyadenosine and adenosine to deoxyinosine and inosine and ammonia. High level of ADA is available in activated CD4+ T-lymphocytes, therefore ADA considered as a marker of cell mediated Immunity and play a role in maturation of monocytes to macrophages [3-5].

It has been reported that TB pleural effusion has significantly higher ADA level than other non-tuberculous effusion, and in the latter is seldom exceeded the diagnostic cut off for TB effusion [6-8]. Moreover no significant correlation between activities of ADA in pleural fluid and serum was observed.[9]. This indicates that ADA is being locally synthesized by cells within the pleural cavity in these diseases (local cell mediated immune response) [9, 10] ADA expresses the sum of two isoenzymes ADA1 and ADA2. ADA1 is ubiquitous in all cells including lymphocytes and monocytes,

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where as ADA2 is found mainly in monocytes[11-13].

ADA in TB pleuritis increases at the expense of ADA2 because it produces by monocytes and that the ADA1/ADA total activity ratio improves performance in terms of sensitivity, specificity, and accuracy. But this procedure is highly elaborate [3-5, 11-13]. Studies on this enzyme show wide range of cut-off values (from 25u/l to 70u/l) [3-5,8,9].

Two possible causes in the variation of cut-off values were suggested. The first is related to the method of ADA activity estimation, which is either colorimetric or spectrophotometric method [8]. The second source of discrepancy is related to the characteristics of the population studied in each case, considering areas with a high incidence of both HIV and TB infection.. Further studies show that ADA is independent of HIV serology [1,2,6,7].

ADA level of more than 33u/l considered diagnostic for TB effusion, the sensitivity raised to 100%, the specificity to 95%, and the accuracy to 96% [9]. Others reported that ADA above 70u/l is highly suggestive of tuberculous effusion, whereas level below 40u/l rules out this diagnosis [3-7]. Cytology is an important test for diagnosing malignant cells in pleural effusion with overall accuracy 50-90%, increases by submission of a second specimen and or combined cytology and pleural biopsy [14,15]. Acid fast smears are positive in less than 20% of tuberculous effusions and cultures are positive in 67%, but culture combined with histological examination establish the diagnosis in about 95% of tuberculous pleuritis [6,16]. The aim of this study is to demonstrate the diagnostic value of increased level of ADA in the tuberculous effusion with the application of cytological, biochemical and bacteriological tests. . Ideally the workup of a pleural effusion begins with classification of fluid into either transudate or exudates according to Light et al criteria (1972) [17].

Patients & Methods

This prospective study was carried out during the period from December 2003 to June 2004 in Dept of Pathology and Medical Research Center in College of Medicine Al-Nahrin University, and Al-Kadhemia Teaching Hospital in Baghdad-Iraq. Seventy-five patients with pleural effusion (53 males and 22 females) their age ranged from 6-79 years (mean=43.8 years) were enrolled in this study. Detailed clinical history, physical examination was done.

Pleural fluid specimens were aspirated and submitted for cytological, bacteriological (direct smears and culture) and biochemical exam. Five smears for each case were prepared from the sediment, 3 smears were fixed in 95% alcohol for 20 minutes and stained with H&E for cytological exam and two air dried smears one for gram stain and the second for Ziehl-Neelsen stain. The supernatant of pleural fluid were submitted for biochemical tests (ADA enzyme level measured by colorimetric method (Galanti and Guisti method) [17] and the cutoff value used in this study was 33u/l, LDH activity was measured according to Wroblewski and Ladue method [18] total protein was determined by Biuret method [18] and Glucose was measured by enzyme colorimetric method [18].

Total and differential cell count of pleural fluid was done by dilution of 0.4ml of fluid with 0.4ml of glacial acetic acid using counting chamber for calculation and differentiation. The results were analyzed by appropriate computer soft ware program (SPSS 10.0).

Results

From the clinical data, and lab tests, patients were divided into six groups according to the etiology of pleural effusion. Tuberculous (TB) effusion 32 cases, malignant effusion13 cases, infection 10 cases, heart failure 8 cases, idiopathic effusion (no specific etiology demonstrated) 6 cases and miscellaneous (include uremia, connective tissue disorders, and other rare causes of pleural effusion) 6 cases. All TB effusions, malignant effusions and infection cases were exudates. (Table 1).

Table 1: Levels of Different Parameters in Transudates and Exudates.

Effusion type	ADA U/L mean±SD	LDH U/L mean±SD	Protein gm/L mean±SD	Glucose mol/L mean±SD
Transudate (n =20)	11.9±9.2	174.6±23.9	21.2±7.5	5.3±1.7
Exudate (n = 55)	55.4±45.9	301.5±70.6	43.8±9.6	2.1±1.1

TB effusions (n=32); Form 43% of the cases of idiopathic pleural effusion. Twenty three cases were left sided effusions, and 9 were right sided. The mean ADA value was 76.7u/l, in 30 cases (93.7%) exceeded the cutoff value (33u/l) and only 2 cases (6.3%) were below the cutoff value. Ziehl Neelsen stain was positive in two smears (6.3%). Cytological smears and cell count revealed moderate-severe chronic inflammatory reaction with paucity of mesothelial cells. LDH, mean value was 314.2u/l. (Table-2).

Table 2: Mean Age of Patients and Levels of Different Parameters in the Pleural Fluid of the studied groups

Diagnosis (Cause of pleural effusion)	Age Years	ADA U/L mean±SD	LDH U/L mean±SD	Protein gm/L mean±SD	Glucose mmol/L mean±SD	Cell count/ccm
Idiopathic* (N=6)	57.2	14.9±10.6	177.2±16.2	24.0±7.7	4.5±2.0	816.7±1075.5
Infection** (N=10)	36.7	21.1±14.3 2 cases > 33U/L 8 cases < 33U/L	279.8±42.4	38.5±4.6	2.6±1.2	3650.0±2848.5
TB (N=32)	32.4	76.7±41.1 30 cases > 33U/L 2 cases < 33U/L	314.2±69.7	43.7±8.6	2.1±.9	3218.8±2232.2 Lymphocytes form 98% of the cells.
Heart failure (N=8)	63.6	24.8±17.8	171.9±16.0	27.1±5.8	4.9±1.7	1137.5±1627.2
Malignancy (N=13	60.8	32.4±51.3 3 cases > 33U/L 10 cases < 33U/L	321.1±60.2	50.5±11.3	1.6±.9	2007.7±1651.0
Miscellaneous*** (N=6)	39.5	6.7±5.9	165.3±11.8	16.5±7.8	6.0±1.3	366.7±310.9

***Undiagnosed conditions inspite of all possible clinical and lab tests. **Non-specific infection. *** Include nephrotic syndrome, celiac disease, liver cirrhosis, hypothyroidism & connective tissue disorders.**

Malignant effusions (n=13); Form 17% of the cases. ADA was below the cutoff value in (77%) 10 cases and only in 3 cases (23%) were above the cutoff value (Table 2). Five were right sided, 6 were left and 2 were bilateral effusions. Cytological smears were positive in 7 cases (53.8%) and negative in 6 (46.2%). Nine cases were metastatic adenocarcinoma, 3 were squamous cell carcinoma, and one small cell lung carcinoma. LDH, mean values were 321.1u/l. (Table-2). Details of other tests and other effusions are listed in table 2.

Discussion

Evaluation of pleural effusion usually includes complete clinical assessment, radiographic studies lab tests of pleural fluid and pleural biopsy. However following these procedures approximately 20% of patients still has undiagnosed conditions [19]. Current study shows marginal significant correlation between final diagnosis and age of the patients, but not with the side of effusion. Highest level of ADA activity in this study was measured in tuberculous effusions.

Cutoff value of ADA was 33u/l gave 93.7% sensitivity, 86.1% specificity and 89.3% accuracy. These results were comparable with other studies [20,21]. The relationship between ADA and final diagnosis was significant (P<0.005). Only two TB effusions (out of 32) showed ADA below the cutoff value and 3 malignant effusions (out of 13) showed ADA level above the cutoff value. The high ADA level correspond to an increase in CD4+ T-lymphocytes as in TB effusion, while its low level correlated with a higher percentage of CD8+ T lymphocytes and a fall in the CD4+ T lymphocytes as neoplastic effusions [20] *Talib Z. et.al. 2001,* showed sensitivity and specificity of 83% and 70% respectively [21]**.** Determination of individual ADA isoenzymes ADA1 and ADA2 could help in distinguishing various causes of increased ADA activity [4, 5].

High LDH associated with increased lactic acid production from polymorph leukocytes and activated lymphocytes[22] Pleural fluid LDH activity has been used to discriminate malignant from non-malignant effusions[21, 22]. In this study the exudative effusions have relatively higher level of LDH than transudate, which is in agreement with other studies [23, 24]. And it was characteristically high in malignant effusions and nearly all-benign effusions have low LDH values.

The cytological examination and evaluation of cells in effusions can be difficult, as in interpretation of long standing transudate effusions characterized by accumulation of few enlarged mesothelial cells, an erroneous false positive diagnosis of cancer can be made [14,15]. While in tuberculous effusions, the differential diagnosis from lymphoma and leukemia depending on the high proportion of mature lymphocytes with paucity of mesothelial cells, the latter is attributed to deposition of fibrin on the pleural surface, either sealing off or destroying it[6, 7]. A further difficulty was in evaluating the accuracy of neoplastic effusions cytology. It is obvious that no single cellular structural changes are diagnostic by itself, a combination of several abnormalities is necessary for accurate diagnosis. In the current study no false positive results was recorded. The sensitivity, specificity and accuracy of cytological diagnosis was 53%, 100% and 72% respectively. Other workers 14, 19, also obtained similar accuracy rate.

Acid-fast bacilli detection by Ziehl Nelseen stain was positive in only two smears of TB cases, similar percentages reported by other studies. TB effusion is usually the result of delayed hypersensitivity reaction to the protein of mycobacterium and the actual bacterial load in the pleural space is low [6, 7, 16].

In conclusion increased ADA levels in TB effusions may reflect highly local cell mediated immune activity in these patients and can be used to differentiate tuberculous

and non-tuberculous effusions. The LDH, protein and glucose level were useful in separation of exudative and Transudate pleural effusions.

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