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Medicine

PLEURAL FLUID LYMPHOCYTE NEUTROPHIL RATIO INTHE DIAGNOSIS OF TUBERCULOUS PLEURAL EFFUSION

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ABSTRACT

Background: Increased pleural fluid adenosine deaminase (ADA) activity is classically associated with tuberculous pleuritis. However, increasedactivity can also occur in a number of other diseases and this maynegatively affect the diagnostic utility of ADA measurements and decreaseits specificity for the diagnosis of tuberculosis (TB). The presence of ADAin pleural fluid reflects the cellular immune response in the pleural cavityand in particular, the activation of T lymphocytes. Different disease entitiesare typically associated with the presence of particular type of leucocytes.

Objectives: To determine whether the combined use of ADA activity andlymphocyte neutrophil ratio would provide a more efficient means fordiagnosing tuberculous pleurisy than the use of ADA levels alone.

Methods: Biochemistry, cytology and microbiology studies wereperformed on 90 consecutive pleural fluids. ADA and differential countswere determined on all exudative effusions.

Results: ADA activity in tuberculous effusions was significantly higherthan in any other diagnostic group. At a level of 50U/L, the sensitivity,specificity, positive predictive value (ppv), negative predictive value (npv),and efficiency for the identification of TB calculated at 61%, 71%, 83%,45%, and 64% respectively. When the additional requirement of alymphocyte neutrophil ratio of 0.75 or greater was included, the sensitivity,specificity, ppv, npv and efficiency for the identification of TB wererecalculated at 100%, 83%, 93%, 100% and 95%, respectively.

Conclusion:ADA when combined with lymphocyte/neutrophil ratioremains a useful test in the diagnosis of tuberculous pleuritis.

KEYWORDS : Adenosine deaminase; Lymphocyte/ neutrophil ratio; tuberculous pleuritis

INTRODUCTION:

Pulmonary tuberculosis is the most frequent cause of death by aninfectious agent worldwide. Among the extra pulmonary presentationsafter tuberculous lymphadenitis, pleural tuberculosis is the second mostfrequent. Failure to diagnose and treat pleural tuberculosis can result inprogressive disease with the involvement of other organs in as many as65% of patients.Conventional methods have proven to be insufficient for diagnosisof pleural tuberculosis. Direct examination of pleural fluid is inefficientbecause sensitivity is about 1%[1]. Pleural fluid culture is more sensitive than direct examination but Mycobacterium tuberculosis requires 4-6weeks to grow[2].Many studies have demonstrated the diagnostic significance ofincreased adenosine deaminase (ADA) in tuberculous pleurisy, otherstudies have shown that ADA is of limited value[3,4], as raised levels arealso associated with a number of other diseases including malignancies(especially those of hematologic origin), bacterial infections (Q fever,brucellosis), emphysemas, and other collagen vascular diseases(includingSLE and rheumatoid arthritis). Lymphocytes predominate inmalignant and tuberculous pleural effusions[5-8].

Hence this study is aimed to determine whether combined use ofpleural fluid lymphocyte/neutrophil ratio and ADA activity would provide amore efficient means for diagnosing tuberculous pleurisy than the use ofADA levels alone.

OBJECTIVES OF THE STUDY:

To determine whether the combined use of ADA activity andlymphocyte/neutrophil ratio would provide a more efficient means fordiagnosing tuberculous pleural effusion than with the use of ADA alone.

MATERIALS AND METHODS:

The present study was conducted at ASRAM Medical College, Eluru during the period between 2014-2016. 120 consecutive pleural fluidspecimens are collected from patients admitted to medical wards.

Inclusion criteria: All exudative pleural effusion cases.

Exclusion criteria:

1. Patients with transudative pleural effusion
2. Patients with immunodeficient states like HIV/AIDS, those on chemotherapy were excluded.
3. Patients having hemothoraces or emphysemas too turbid for analysiswere excluded.

Besides a detailed history and clinical examination, the followingin vestigations were carried out:

a) Blood investigations
b) Urine examination
c) Sputum examination- for acid fast bacilli by Zeihl Neilson (ZN)stain, Gram's stain, culture.
d) Mantoux test
e) Chest radiography – posterolateral and lateral view in selected cases
f) Pleural fluid analysis
g) Pleural biopsy: Pleural biopsy was performed using the Abrahm'sbiopsy needle.

Equipment: Spectrophotometer, spectral-line photometer or simplephotometer (with tungsten lamp and filter) suitable for accuratemeasurements at wavelengths between 620 and 650nm, water bath(37°C).

Reagents and solutions: Purity of reagents- solutions must be preparedwith doubly distilled ammonia-free water. Ammonia can be removed byaddition of a little sulphuric acid and potassium permanganate, and asecond distillation from a glass apparatus. This preparation is necessarywhen the ammonia content of tap water is high.

Preparation of solutions:All solutions are prepared with re-purified water.
(A) Phosphate buffer (50mmol/l, pH=6.5)
(B) Buffered adenosine solution (Adenosine-21mmol/l; phosphate-50mmol/l; pH: 6.5)
(C) Ammonium sulphate stock solution (15mmol/l)
(D) Ammonium sulphate standard solution
(E) Phenol nitroprusside solution
(F) Alkaline hypochlorite solution

Statistical Analysis:Mean and standard deviation for continuous variables andproportions for categorical variables are reported. ADA alone, L/N aloneand ADA values were then combined with various L/N ratios andevaluated at various cutoff levels for ADA and L/N ratios by calculatingsensitivity, specificity, ppv, npv, and efficiency. An interactive dot diagramwas used for cut-off points and plot versus criteria values graph was used.SPSS version 16.0 was used for statistical analysis.

DISCUSSION:

Age distribution:The present study comprised of patients aged from 18 years to 78 years. The mean age in case of tuberculous effusion was 36.48± 19.4years consistent with Luis Valdes et al (34 years)9and S.K.Sharma et al(33 years)11and Subhakar.k et al (31 years)12. Earlier studies done inUnited States by Epstein et al50 and Aho K et al 14 showed a mean age of54 and 28 years respectively.

Sex Distribution: There were a greater number of male patients than female patientsin this study with 68.9% males and 31.1 % females with a ratio of 2.21:4which was consistent Quorian et al10– of 201 cases 145 were males(72%)and 56 females(27.9%) with a ratio of 2.58:1. In comparison, the sexdistributions in some of the previous studies are: Luis Valdes et al9-62.5% males and 37.5% females with a ratio of 1.6:1.

Socioeconomic status: Most of the patients in this study belonged to the lower Socioeconomic class. This is consistent with the fact that tuberculosis is adisease more commonly seen among people living in crowded, unhygienic conditions of lower socioeconomic class.It is poverty related disease.

Etiology: Out of the 90 cases of pleural effusion, 62 cases were oftuberculous effusion (60%). This was similar to the observation in anotherstudy from India by Maldhure et al53 where they showed that the tubercularreffusions constitute 66% of the effusions. General prevalence of TB is highin India and Southeast Asian countries than in the West. In Indiatubercular effusion is the commonest cause of all exudative effusions. This observation is different from the Western studies, where the incidence ofparapneumonic effusion and malignant effusion are much highercompared to tubercular effusion. Symptomatology:

The most common symptom encountered by TB patients were dry cough, followed by fever,breathlessness and chest pain in comparison with the study done earlier by Arun Gopi et al16in which mostcommon symptom were chest pain and dry cough.Patients with malignant effusion had cough and dyspnea as predomina ntsymptoms followed by chest pain which was similar to a study by ChernovB et al,17where breathlessness and cough are predominant symptomsfollowed by chest pain.

Pleural fluid cell type and cell count :The majority of effusions had total leukocyte count less than 1000 cells/mm3 of which Tuberculosis constitutes 50%.All patients of emphysema hadcell count greater than 5,000 mm3 (100%) followed by parapneumonic effusions (36.4%), consistent with Light's observation et al[18].The low cellcounts in tuberculous pleural effusion compared to emphysema andparapneumonic effusion may be due to cell mediated immunity,lymphocytepredominance in tuberculous effusion where as antibody mediatedimmunity and neutrophil predominance in emphysema and parapneumoniceffusion. 83.3% of TB effusions and 100% of malignant effusions hadlymphocyte predominance. In comparison to other studies: Valdes L etal9where they have encountered neutrophil predominant tuberculouseffusion in only 6.7% of patients and only one malignant effusion hadneutrophil predominant effusion(3%). Follander19demonstrated predomin ance of lymphocytes and scarcity of mesothelial cells intubercular effusion; Light RW18– large number of neutrophil indicate thepresence of bacterial pneumonia. Lymphocytes predominant in tubercularpleural effusion.

Adenosine deaminase:In tubercular pleural effusion pleural fluid Adenosine deaminase level(ADA) has got a good diagnostic index after excluding other causes ofraised ADA levels.Although a pleural fluid ADA above 70IU/L is diagnosticof tuberculosis18, it has to be considered if the pleural fluid ADA is between40 IU/L and 70 IU/L. An ADA level less than 40IU/L very much unlikely ofpleural tuberculosis. But different authors have used different cut off levelsfor pleural fluid ADA ranging between 33 IU/L to 50 IU/L20-23.In our study pleural fluid ADA >50U/L was taken as diagnostic cut off fortuberculous effusion and it yielded 61% sensitivity,71% specificity,83%positive predictive value,45%negative predictive value and pvalue<0.0001. In our study who were diagnosed as tubercular effusionADA < 50 of 24 are of tuberculous effusion and 20 non tuberculouseffusion.The general prevalence of tuberculosis is high in India,so tuberculosis asetiological cause for pleural effusion is expected to be high.Hence bytaking ADA >40 U/L in our study is reasonable in developing countries likeIndia,such that missing of tuberculous pleural effusion cases will beminimal.Our results show that, at a cutoff level of 50U/L, ADA has asensitivity, specificity, ppv, npv, and efficiency of 61%, 71%, 83%, 45%,and 64% respectively. However, it must be noted that only exudates havebeen included in this study. All transudates, which are characterized bylow ADA levels (<20U/L)15, have been excluded.When the L/N ratio (≥0.75) was considered together with ADAactivity (≥50U/L), the results improved considerably for the diagnosis oftuberculous pleuritis. The sensitivity, specificity, ppv, npv, and efficiencywere 100%, 83%, 93%, 100% and 95% respectively.
In the present study, ADA activity was highest among thetuberculous group. Para-infective conditions were also seen to beassociated with high ADA activities. The relative cell count or L/N ratiocould be used to distinguish between these two entities5-8. In the cases of tuberculous pleurisy, a predominant lymphocyte count was usually found,resulting in a L/N ratio of 0.75 or greater, whereas in the case of parainfectiveeffusions, a predominant neutrophil count was usually found (L/Nratio <0.75).Use of ADA level especially in conjunction with theL/N ratio, is therefore a valuable diagnostic tool in this regard, as itprovides a rapid and accurate means of detecting TB pleurisy.

CONCLUSION:

In conclusion, it is suggested that the combined use of adenosine deaminase activity along with lymphocyte neutrophil ratio would provide amore efficient means for diagnosing tuberculous pleuritis than the use ofADA alone.

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