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Research Article

Adenosine deaminase and interferon-gamma in diagnosis of tubercular pleural effusion

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ABSTRACT

Background: Pleural effusion is a common extrapulmonary manifestation of tuberculosis (TB). The conventional culture suffers from lack of sensitivity. Many pleural fluid markers have been evaluated to diagnose tubercular pleural fluid but none has proved to be an ideal. We have studied Adenosine deaminase (ADA), Interferon gamma (IFN) and their combination for diagnosis of tubercular pleural effusion.
Methods: All consecutive patients with pleural effusion were subjected to thoracentesis and segregated into transudative and exudative using Light`s criteria. Patients with exudative pleural effusion were enrolled and divided into two groups. Group-I comprised of patients with tubercular etiology, Group-II- non-tubercular etiology. 45 patients were selected for each group. ADA and IFN in pleural fluid of these patients were measured. The sensitivity, specificity and predictive values were calculated.
Results: The sensitivity, specificity, positive predictive value, negative predictive value of ADA and IFN were 88.89%, 99.85%, 86.96%, 99.87%; 97.78%, 97.78%, 97.78%, 97.78% respectively. Combination of ADA and IFN didn't improve the sensitivity or specificity compared to IFN alone.
Conclusions: Pleural fluid ADA, IFN were found to be useful in differentiating tubercular from non-tubercular patients. Combination of ADA and IFN doesn't give additional benefit over IFN alone.

Keywords: Extrapulmonary tuberculosis, Pleural disease, Exudate

INTRODUCTION

Pleural effusion is one of the commonest extrapulmonary manifestations of tuberculosis (TB).[1]

Detecting the organism on smear or culture is considered to be gold standard for diagnosis but smear examination suffers from lack of sensitivity and culture has low sensitivity along with long duration to result such that acid fast bacilli (AFB) staining is positive in only 10 to 25% of the cases, while culture for AFB is positive in less than 25% of the cases.[2]

Many pleural fluid markers have been evaluated for diagnosis of tubercular pleural effusion, but none has been found to be ideal as they lack in sensitivity or specificity, difficulty in performing or difficult availability etc. Some of these markers include serum CA 125, pleural alkaline phosphatase, pleural fluid Procalcitonin concentration, pleural fluid Interleukin-1, pleural tumor necrosis factor, pleural fluid PCR, cell count (total and differential count), biochemical tests [Adenosine deaminase (ADA)], and microbiological tests [Ziehl-Neelsen (ZN) stain, culture] and T cell products (Interferon gamma) etc.[3]

We have studied ADA, Interferon gamma (IFN) and their combination for diagnosis of tubercular pleural effusion. Present study is the first of its kind from Indian subcontinent.

METHODS

The present study was an observational study to differentiate between tubercular and non-tubercular pleural effusion using ADA and IFN. The study was conducted after approval from institutional ethical committee.

The study was conducted in the Department of Pulmonary Medicine at a tertiary care teaching institute of North India. All consecutive patients with pleural effusion were subjected to thoracentesis.

Patients were further segregated into transudative and exudative based on Light`s criteria. Patients with exudative pleural effusion were enrolled in study after written and informed consent.

They were divided into two groups, Group-I comprised of patients of tubercular etiology and Group-II comprised of patients of non-tubercular etiology. Forty five patients were taken in each group. The following inclusion and exclusion criteria were used while selecting patients.

Inclusion criteria

- Age >20 years
- All consecutive patients with exudative pleural effusion

Exclusion criteria

Patients with

- Pyothorax
- Hemothorax
- Transudative pleural effusion

Immunocompromised (HIV - positive)

Diagnosis of tubercular pleural effusion (Group-I) was based on at least one of the following criteria:

- Pleural fluid positive for AFB smear/ culture.
- Sputum for AFB positive by smear/ culture.
- Histopathology positive for TB (presence of granulomas or AFB) in the pleural biopsy specimen.
- Patients with clinical features suggestive of TB and favourable response to anti-tubercular treatment were also retrospectively included in the study.

Diagnosis of Non-tubercular pleural effusion (Group-II) was based on either of the following:

- Malignant pleural effusion by either cytology or histology positive for malignancy
- Parapneumonic effusion by clinical features suggesting of pneumonia and pleural fluid negative for presence of bacteria.
- Any other etiology confirmed by its specific marker e.g. pleural fluid positive for amylase etc.
- No evidence of TB (defined by criteria for group-I)

ADA and IFN were measured of both these groups. ADA was measured by the help of DXC 800 (Beckman Coulter) where One unit of ADA is defined as the amount of ADA that generates one micromole of inosine from adenosine per min at 37^0C.

IFN was detected by ELISA kit. (Gene probe Diaclone) The sensitivity, specificity, Positive predictive value (PPV) and Negative predictive value (NPV) were calculated to differentiate between tubercular and non-tubercular pleural effusion.

Data management and statistical analysis

Data was analyzed by using statistical software SPSS version 22. Quantitative data was expressed in terms of Mean±SD. Mann – Whitney U test was used to compare mean of two groups.

ROC curve was used to calculate cut-off values of all parameters. Sensitivity, specificity, PPV and NPV were calculated at a specific cut off for ADA and IFN. P< 0.05 was considered as statistical significant.

RESULTS

A total of 90 patients were analyzed, of which 67 were males and 23 females. Accordingly to etiological diagnosis, the distribution of patients was as follows: 45 patients with tubercular pleural effusion confirmed as by above mentioned criteria, rest 45 patients with exudative pleural effusion due to malignancy (36) (confirmed by either cytology or histopathology of any intrathoracic specimen), pancreatitis (5) (based on history, investigations {amylase,}), parapneumonic effusion (4) (patients with clinical features suggesting of pneumonia along with pleural effusion, which was negative for presence of bacteria) as shown in Figure 1.

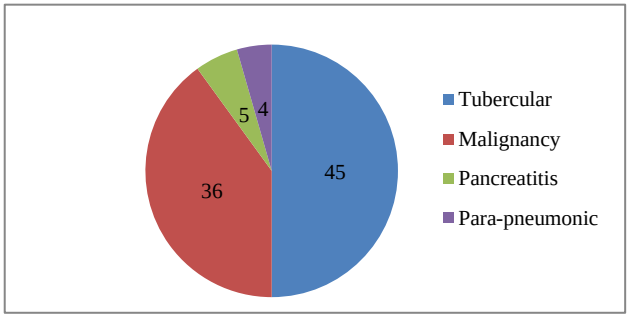


Figure 1: Distribution of cases according to etiology (n=90).

The ADA and IFN were calculated of both the groups and the mean values are mentioned in Table 1.

Mean values of ADA, IFN in tubercular pleural effusion cases was 69.73 and 377.18 respectively and in pleural effusion due to other causes was 25 and 28.60 respectively.

Table 1: Mean values of ADA and IFN in group-I and group-II (n= 45).

	Group	Mean±SD Deviation	P- value
ADA (IU/L)	I	69.73±39.980	0.001*
	II	25.00±16.671	
IFN (PG/ML)	I	377.18±67.656	0.0001*
	II	28.60±62.276	

*Mann-Whitney - U test

Table 2: Sensitivity, specificity, PPV, NPV of ADA and IFN.

Parameter	ADA (using cut off 40IU/L)	IFN (using cut off 200PG/ML)
Sensitivity	88.89%	97.78%
Specificity	99.85 %	97.78 %
PPV	86.96 %	97.78 %
NPV	99.87 %	97.78 %

The intergroup difference of IFN was found to be the most statistically significant among all parameters ($p<0.0001$).

Table 3: Sensitivity, Specificity, PPV, NPV of ADA and IFN combination and comparison with individual parameters (n=90).

Parameter	Sensitivity	Specificity	PPV	NPV
ADA and IFN	97.78 %	84.78%	86.27 %	97.50 %
ADA	88.89 %,	99.85%	86.96%	99.87 %
IFN	97.78%	97.78%	97.78%	97.78%

Table 4: Comparison of sensitivity and specificity of ADA in tubercular pleural fluid at various cut off values with previous major studies.

ADA in tubercular pleural effusion				
Authors	**N (no. of subjects)**	**Cut Off Value (IU/L)**	**Sensitivity**	**Specificity**
Jayalakshmi et al[8]	50	>40	83.87%	78.94%
Sharma SK et al[10]	75	>35	83.3%	66.6%
Krenke R et al[9]	94	>40.3	100%	93.9%
Maria VV et al[11]	140	>45.5	88.1 %	85.7%
Lesley J et al[12]	303	>50	91%	81%
Present study	90	>40	88.89 %	99.85%

Table 5: Comparison of sensitivity and specificity of IFN in tubercular pleural fluid at various cut off values with previous major studies.

IFN in tubercular pleural effusion				
Authors	**N (no. of subjects)**	**Cut Off Value (pg/ml)**	**Sensitivity**	**Specificity**
Wongtim S et al[14]	66	240	94.9%	96.3%
Krenke R et al[9]	94	75	100%	98.5%
Krenke R et al[15]	90	100	100%	98.5%
Present study	90	200	97.8%	97.8%

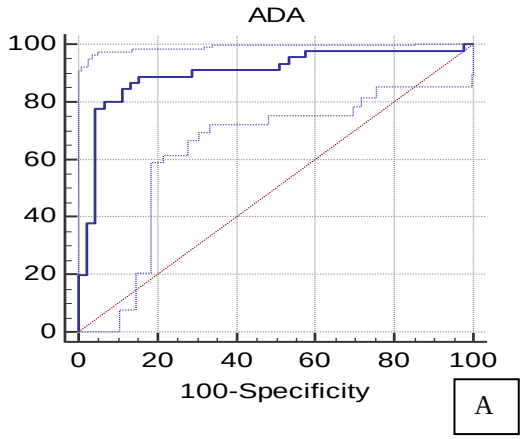
In present study we used a cut off of 40 IU/L for ADA, 200 pg/ml for IFN, based on previous studies and also calculated by using ROC curve as seen in Figure 2.

According to ROC curve, the sensitivity was 88.89%, specificity was 84.44% respectively with value of ADA above 38.36 IU/L, IFN above 115pg/ml, sensitivity was 97.78%, and specificity was 97.78% respectively.

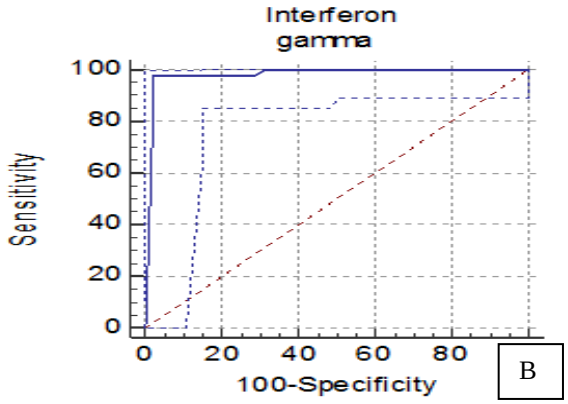
The Sensitivity, Specificity, PPV, NPV of ADA and IFN were calculated and are given in Table-II. The comparison of Sensitivity, Specificity, PPV, NPV of ADA, IFN and its combination is given in Table 3.

Thus, ADA, IFN were found to be useful in differentiating tubercular from non-tubercular patients. ADA being more specific and IFN being sensitive and

Combination of ADA and IFN don't give additional benefit over IFN alone.



Youden index J 0.7333; Associated criterion >38.36; Sensitivity 88.89; Area under the curve 0.901.



Youden index J 0.9556; Associated criterion >115; Sensitivity 97.78; Specificity 97.78; Area under curve 0.98.

Figure 2: ROC curve of ADA and IFN.

DISCUSSION

Tubercular pleural effusion is a common manifestation of extrapulmonary TB.[4] It occurs due to rupture of sub-pleural focus exposing tubercular antigen to helper T cells resulting in delayed type hypersensitivity.[5] This mechanism is seen in both primary and reactivation cases of TB. As clearly evident, there will be either no bacteria or less bacteria present in the pleural fluid.[6]

Conventional diagnostic tests for pleural TB include microscopic examination of the pleural fluid for AFB, mycobacterial culture of pleural fluid, sputum or pleural tissue, and histopathological examination of pleural tissue for granulomatous inflammation.

As discussed above, pleural TB being a paucibacillary disease will have a low sensitivity in cases of direct microscopy (0-5%) and mycobacterial culture (25-35%) which is the "gold standard" tests for diagnosing effusion. Only pleural biopsy done via thoracoscopy has high sensitivity.[3] Various alternative novel tests have been evaluated to differentiate between tubercular and non-tubercular pleural effusion. They include pleural fluid ADA, serum CA 125, pleural IFN, pleural alkaline phosphatase, pleural fluid Procalcitonin concentration, pleural fluid Interleukin-1, pleural Tumor necrosis factor, pleural fluid PCR etc. The evaluation of efficacy of alternative novel tests is usually done against gold standard that is culture which has low sensitivity as discussed above.

The results from these tests should be interpreted cautiously taking the complete clinical and other supportive laboratory parameters and should not be followed blindly.

We, in present study, planned to evaluate the efficacy of ADA and IFN individually and in combination, to diagnose tubercular pleural effusion. Elevated levels of ADA, IFN in tuberculous pleural effusion have been noted by several authors but the combination of these markers have not been adequately evaluated in past.

ADA is released by activated lymphocytes, macrophages and neutrophils, is a nonspecific marker of inflammation. It can be raised in both tubercular and non-tubercular pleural effusion. The ADA2 isoenzyme released from monocytes and macrophages is the predominant contributor to total ADA activity.[7] With a cut –off of 40 U/l, in our study, ADA was found to have sensitivity and specificity of around 89% and 99.8% respectively which is quiet similar to various other studies as shown in table-IV

IFN is also released from lymphocytes which are stimulated by the tuberculoprotein causing delayed type hypersensitivity. Hence its value is also highly raised in lymphocytic predominant tubercular effusions and not in non-tubercular effusions.[13] It was found to be more specific and sensitive than interleukin (IL)-12p40, IL-18, immunosuppressive acidic protein or soluble IL-2 receptor

Various studies have used different cut- off values for IFN which could be due to different techniques of processing and measuring IFN.[13] In present study, we used cut off of 200 pg/ml and found that measurement of IFN is highly sensitive and specific. Comparison of our results of IFN with various studies is tabulated in table V.

Therefore, the challenge of diagnostic efficiency in different circumstances of prevalence may be addressed using combinations of these rapid methods on pleural fluid. ADA and IFN measurement are simple; we believe that the results of this study demonstrate that individually these methods can offer a means of obtaining diagnostic efficiency and differentiate between tubercular and non-tubercular pleural effusion, but on combining these test the sensitivity didn't increase in comparison to IFN alone.

Maria V.V did a study on evaluation of PCR, ADA, and IFN in pleural fluid for the differential diagnosis of pleural tuberculosis in 140 cases of pleural effusion using a cut off >45.5 U/L for ADA and sensitivity of IFN assay detection kit was 0.8 U/mL IFN levels in combination with ADA activity presented the specificity of 83.8% and sensitivity of 89.7%, whereas in present study the combination of ADA and IFN has specificity of 84.78% and sensitivity of 97.78.[11]

Present study had the limitation of having small sample size. More number of subjects should be recruited to such study in a larger multicenter study to assess the sensitivity, specificity of these tests. Another limiting factor of our study was presence of a heterogenous group of non-tubercular etiology. Our control group had patients of malignant effusions, pancreatic effusions, parapneumonic effusion. Actual picture of each etiology could be gathered if each etiology is addressed individually.

CONCLUSION

In present study ADA and IFN were found to help in differentiating tubercular pleural effusion from pleural effusion due to other causes but on combining these tests the sensitivity didn't increase in comparison to IFN alone. IFN was found to be most sensitive and ADA the most specific investigation to diagnose tubercular pleural effusion.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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