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Original Research Article

Diagnostic Efficacy of Pleural Fluid ADA, ICAM-1 and VCAM-1 to Differentiate Tubercular and Nontubercular Pleural Effusion

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ABSTRACT

Background: Tuberculosis infection can occur in almost every organ in the body but involvement of lungs is most common. In extra pulmonary tuberculosis, tubercular pleural effusion is one of the commonest manifestations. The conventional culture suffers from lack of sensitivity. Various pleural fluid biochemical markers have been evaluated for diagnosis of tubercular pleural effusion but none of them was found to be ideal in terms of sensitivity, specificity and applicability.

Materials and 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. 66 patients with exudative effusion out of 87 patients screened were included. 30 patients were selected for each group as per group characteristics. Differential cell count, ADA, ICAM-1 and VCAM-1 in pleural fluid of these patients were measured. The sensitivity, specificity and predictive values were calculated.

Results: A The sensitivity, specificity, positive predictive value, negative predictive value of Differential cell count, ADA ICAM-1 and VCAM-1 were 90.0%,80.0%,81.8% 88.9%,83.3%,90.0%, 89.3 %, 84.4 %, 80.0 %, 56.67 %, 64.90 %, 73.90%, 83.33 %, 80.0%, 80.6%, 82.8%, respectively. Combination of ADA with ICAM-1 and VCAM-1 improve the sensitivity or specificity compared to ICAM-1and VCAM-1 alone.

Conclusion: Pleural fluid Differential cell count, ADA, ICAM-1 and VCAM-1 were found to be useful in differentiating tubercular from non-tubercular patients. Combination of ADA with ICAM-1 and VCAM-1 significantly improves the sensitivity and specificity.

Keywords: ICAM-1, VCAM-1, Tubercular pleural effusion

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INTRODUCTION

Tuberculosis infection can occur in almost every organ in the body, but lungs are involved more commonly, accounting for more than 80 percent of tuberculosis cases.[1] In extra pulmonary tuberculosis, tubercular pleural effusion is one of the commonest manifestations.[2] Demonstration of tubercular bacilli is the gold standard diagnosing tuberculosis but sensitivity of acid fast bacilli (AFB) staining is 10-25% and culture for AFB positive is only in 25% cases.[3] Various pleural fluid biochemical markers have been evaluated for diagnosis of tubercular pleural effusion, but none of them have been found to be ideal in terms of sensitivity, specificity and applicability.

Our present study focuses on pleural fluid ADA, with two new markers, Intercellular adhesion molecules-1 (ICAM-1) and vascular cell adhesion molecule-1(VCAM-1) to evaluate the efficacy of these markers to diagnose tuberculous pleural effusion.

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MATERIALS AND METHODS

The present study was an observational study to differentiate between tubercular and non-tubercular pleural effusion using Differential cell count, ADA, ICAM-1 and VCAM-1. 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. Thirty patients were taken in each group. The following inclusion and exclusion criteria were used while selecting

patients. Inclusion criteria were patients with age >20 years and exudative pleural effusion. Patients with Pyothorax, Hemothorax, Transudative pleural effusion, and Immunocompromised were excluded from the study.

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 favorable response to anti-tubercular treatment assessed at end of 2month 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 confirmed 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, ICAM-1 and VCAM-1 were measured in 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 370C. ICAM-1 and VCAM-1 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 analysed by using statistical software SPSS version 22, Quantitative data was expressed in term of Mean ± SD. Independent t - test was used to compare mean of two groups. ROC curve was used to assess sensitivity, specificity of ADA, ICAM-1 and VCAM-1. A p< 0.05 was considered as statistical significant

RESULTS

A total of 87 patient was analysed out of which 60 patients was enrolled as per group and I and II mentioned criteria. 30 patient of enrolled group were having tuberculosis as aetiology for pleural effusion confirmed mentioned criteria, remaining 30 was in group II (Non tubercular). 27 of non-tubercular pleural effusion was due to malignancy confirmed by either cytology or histopathology examination of intrathoracic specimen, and 3 patient were having paraneumonic effusion confirmed by clinical feature suggestive of pneumonia and pleural fluid were negative for bacteria.

Mean values of ADA, ICAM-1, and VCAM-1 in tubercular pleural effusion cases were 55.77, 528.64and 547.78 respectively and in pleural effusion due to other causes was 25.62, 428.20, and 229.51 respectively. Thus, the combination of VCAM-1 and ADA has best sensitivity and specificity 69.13% and 97.20% respectively. Specificity was also increased when ICAM-1 and VCAM-1 combined than alone.

Table 1: Mean values of ADA, ICAM-1 and VCAM-1 in group-I and group-II (n= 30)

Mean values of ADA, ICAM-1 and VCAM-1 in group-I and group-II				
	Group	**Mean ± Std. Deviation**	**Std. Error Mean**	**P- value**
ADA	**I**	55.77±18.08	3.30	0.0001
(IU/ml)	**II**	25.62±26.82	2.91	
ICAM-1	**I**	528.64±260.41	47.54	
(ng/ml)	**II**	428.20±355.08	64.82	0.0217
VCAM-1	**I**	547.78±313.73	57.27	0.0001
(ng/ml)	**II**	229.51±143.17	26.14	

By Independent t-test

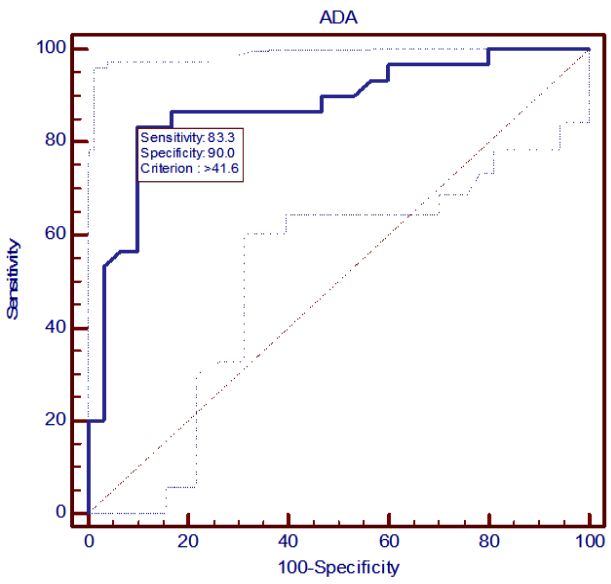

Figure 1- ROC of ADA

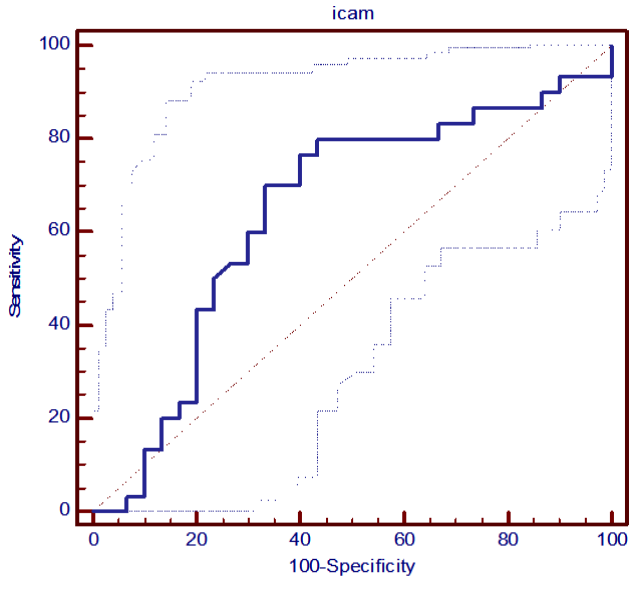

Figure 2- ROC of ICAM-1

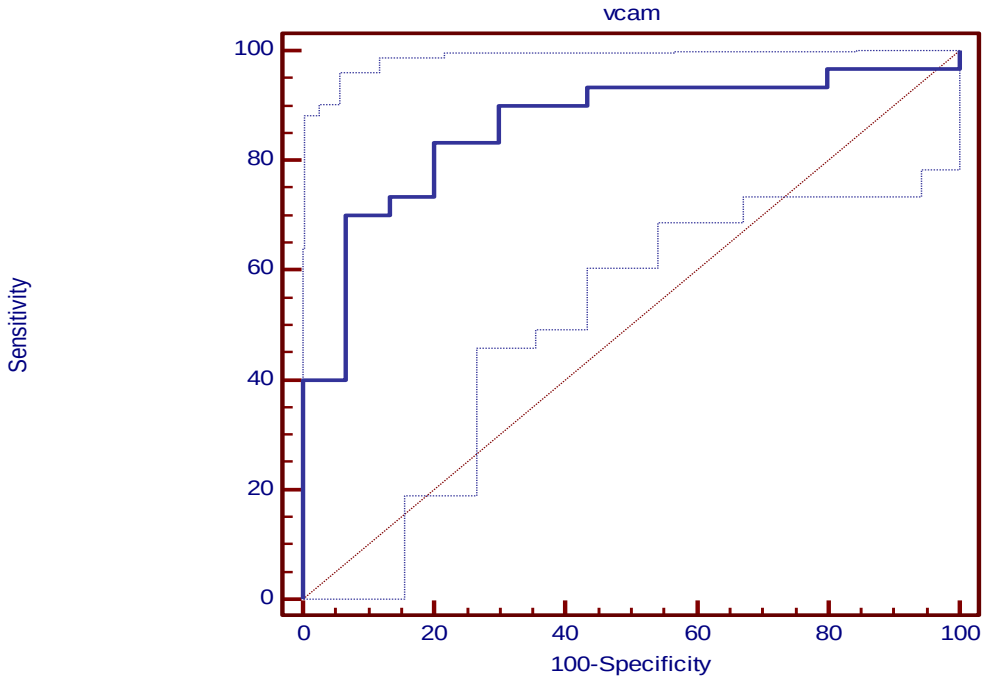

Figure 3- ROC of VCAM-1

Table 2: Sensitivity, Specificity, PPV, NPV of ADA, ICAM-1 and VCAM-1 (n=60)

	Cut of value	Sensitivity	Specificity	PPV	NPV
ADA	>41 IU/L	83.3%	90.0%	89.3%	84.4%
ICAM-1	>310.2ng/ml	80%	56.67%	64.90%	73.90%
VCAM-1	>317 ng/ml	83.33%,	80.0%	80.6%	82.8 %

Table 3: Sensitivity, and Specificity of various combinations of used biomarker (n=60)

	Sensitivity	Specificity
ICAM-1 and VCAM-1	66.4%	91.34%
ICAM-1 and ADA	66.4%	95.67%
VCAM-1 and ADA	69.13%	97.20%

DISCUSSION

Pleural effusions with exudative character are commonly present in clinic but there occur difficulties in making etiological diagnosis. The two most common causes are malignancy and tuberculous effusions.[4] Among the infectious causes tuberculosis is the most common cause of pleural effusion in developing countries but it is notoriously difficult to diagnose due to its paucibacillary nature.[5] Tubercular inflammation is characterized by development of a protective host response with formation of granulomas composed in part of aggregated and fused, apoptotic, infected macrophages.[6]

The laboratory tests on pleural fluid which have been found to be of help in diagnosis of tubercular pleural effusion include cell count (total and differential count), biochemical tests [protein, sugar, lactate dehydrogenase (LDH), adenosine deaminase (ADA)], and microbiological tests Ziehl-Neelsen (ZN) stain, culture, T-cell products (Interferon gamma) etc.[7] But the gold standered remain the conventional method (AFB demonstration by microscopy or culture). ADA estimation in pleural fluid has been shown as a reliable chemical bio-marker to diagnose tubercular etiology.[8] There are several isoforms of ADA, but the prominent ones are ADA1 and ADA2, which are coded by different gene loci.[9] ADA1 isoenzyme is found in all cells, with the highest concentration found in lymphocytes and monocytes, whereas ADA2 isoenzyme appears to be found only in monocytes this would mean ADA2 is more specific for tuberculous pleural effusion.[10] Intercellular adhesion molecules (ICAMs) and vascular cell adhesion molecules (VCAMs) are part of the immunoglobulin superfamily. They are important in inflammation, immune responses and in intracellular signaling events.[11] ICAM-1 (CD54) contains five Ig-like domains. It is expressed on leucocytes, endothelial and epithelial cells, and is upregulated in response to bacterial invasion. This protein is a ligand for lymphocyte-function associated (LFA) antigens and also a receptor for CD11a,b/CD18.[12] leukocyte adhesion is accompanied by increases in levels of LFA-1 and its counter receptor (ICAM-1).[13] Chronic inflammatory cytokines IL-18 and IL-12 further up regulate

the level of ICAM-1 of which soluble form is detectable in serum or pleural fluid.[14] Vascular cell adhesion protein 1 also known as vascular cell adhesion molecule 1 (VCAM-1) or cluster of differentiation 106 (CD106) which is encoded by VCAM-1 gene.[15] The majority of T cells recruited to the lung defense express $\alpha 4\beta$ 1 integrin, a ligand for the VCAM-1. Lymphocyte recruited in lung during acute infection initiate inflammation by producing IFN-γ they largely express $\alpha 4\beta 1$. This suggests that increase level of VCAM-1 lead to recruitment of $\alpha 4\beta 1$ T cells, which are then able to limit disease by producing IFN-γ.[16] Hamzaoui A et al[17] in 1996 studied 25 patients with pleural effusion. Based on pleural fluid protein, amylase, pH value & other laboratory tests 15 patients were diagnosed as tubercular pleural effusion whereas 10 patients with neoplastic effusion. They analyzed ICAM-1, VCAM-1 and E-selectin by sandwich ELISA level of soluble adhesion molecules in serum. The mean serum value in 20 healthy individuals (± SD) for VCAM-1& ICAM-1 were 438±140ng/ml & 329±56ng/l respectively Soluble serum ICAM-1 (536±23ng/l) and VCAM-1 (620±56NG/l) exhibited increased levels in patient with tuberculosis compared with healthy controls ($p<0.01$). In patients with neoplastic effusions VCAM-1 (630±180ng/l) and ICAM-1 (430±32ng/ml) were higher than apparently healthy controls ($p<0.001$). In present study we evaluated 2 new markers (ICAM-1 and VCAM-1) in pleural fluid with ADA for diagnostic efficacy. And we found that the combination of ADA and VCAM-1 is having highest sensitivity and specificity 69.13% and 97.2% respectively. VCAM-1 in alone is more sensitive and specific then ICAM-1 (table -2). We also found in our study that ICAM-1 more than 310.2ng/L andVCAM-1 more than 317ng/L has highest sensitivity and specificity. Our study also having similar finding as in Hamazaoui A et al.[17]

ETHICAL APPROVAL

Approved by institute ethics committee.

REFERENCES

1. National Tuberculosis and Leprosy Control Programme .Workers Manual. Federal Ministry of Health of Nigeria, Department of Public Health 2004:4; p.10-19.

2. Fishman Alfred P, Jack A.Elias, Jay A. Fishman, Grippi A. Michael, SeniorM Robert, Allan I.Pack. Nonmalignant pleural effusion. In: Fishman Alfred P editor. Fishmans pulmonary diseases and disorders: 4th edition. New Delhi: Mc Graw Hill;2008.p.1487-9.

3. Scharer L, McClement JH. Isolation of tubercle bacilli from needle biopsy specimens of parietal pleura. Am Rev Respir Dis. ,McClement JH. Isolation of tubercle bacilli from needle biopsy specimens of parietal pleura.Am Rev RespirDis.1968;97(3):466-8.

4. Jimenez Castro D, Diaz Nuevo G, Perez-Rodriguez E, Light RW. Diagnostic value of adenosine deaminase in nontuberculous lymphocytic pleural effusions. EurRespir J. 2003; 21(2):220-4.

5. Porcel JM. Pearls and Myths in Pleural Fluid Analysis Respirology. 2011:16; 44-52.

6. J. Alastair Gracie, Susan E. Robertson.IL 18. Journal of Leukocyte Biology.2003;73no.2:213-24.

7. Crompton GK, McHardy GF. Diseases of the respiratory system. In: Edwards CR, Boucher IA. Davidson's Principles and Practice of Medicine. 16 th edition. Edinburgh: ELBS; 1992. p. 410-2.

8. Gakis C, Calia GM, Naitana AGV, Ortu AR, Contu A. Serum and pleural adenosine deaminase activity: correct interpretation of the findings. Chest. 1991; 99: 1555–6.

9. Hirschhorn R, Ratech H. Isozymes of adenosine deaminase. In: Rattazzi MC, Scandalios JG, Whitt GS, eds. Isozymes: current topics in biological and medical research (vol 4). New York, NY: Liss, 1980; p. 131–57.

10. Kataria YP, Khurshid I. Adenosine deaminase in the diagnosis of tuberculous pleural effusion. Chest. 2001; 120(2):334-6.

11. Gahmberg CG, Tolvanen M. Leukocyte adhesion--structure and function of human leukocyte beta2-integrins and their cellular ligands. European Journal of Biochemistry. 1997; 245(2):215-2.

12. Stanley P, McDowall A. The second domain of intercellular adhesion molecule-1 (ICAM-1) maintains the structural integrity of the leucocyte function-associated antigen-1 (LFA-1) ligand-binding site in the first domain. The Biochemical Journal. 2000; 351(Pt 1):79-86.

13. DesJardin LE, Kaufman TM. Mycobacterium tuberculosis-infected human macrophages exhibit enhanced cellular adhesion with increased expression of LFA-1 and ICAM-1 and reduced expression and/or function of complement receptors, FcgammaRII and the mannose receptor.Microbiology.2002 ;148(Pt10):3161-71.

14. J. Alastair Gracie, Susan E. Robertson.IL 18. Journal of Leukocyte Biology.2003;73no.2:213-24.

15. Cybulsky M, Fries J W. The human VCAM1 gene is assigned to chromosome 1 p31- p32. Cytogenet. Cell Genet. 1991; 58:1852.

16. Bernadette M Saunders.Restraining mycobacteria: Role of granulomas in mycobacterial infections.Immunology and Cell Biology. 2000;78:334-41.

17. Hamzaoui A, Hamzaoui K. Levels of soluble VCAM-1, soluble ICAM-1, and soluble E-selectin in patients with tuberculous pleuritis. Mediators Inflamm.1996;5(4):276-9.

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