Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2016; 4(8D):3020-3027 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjams.2016.v04i08.055

Original Research Article

A Co-relative Study of ADA and CYFRA 21-1 in Serum and Pleural Effusion Secondary to Tuberculosis and Cancer

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Abstract: Lung cancer and Pulmonary Tuberculosis are two major public health problems associated with significant morbidity and mortality in India. Wrong diagnosis of lung cancer cases as pulmonary tuberculosis delays the onset of anti-cancer chemotherapy and initiation of DOTS thus increases complication in malignancy patients. In this context easy, cost effective diagnostic tool at primary level must be the priority and need of hour. This study was done to evaluate any significance of ADA, CYFRA 21-1in serum and pleural effusion secondary to tuberculosis and lung cancer. Case control study was carried out on 100 cases of tuberculous effusion, 50 cases of malignant effusion and 100 age and gender matched apparently healthy controls. Correlation between ADA and CYFRA 21-1 was evaluated to find any significance between three groups. Blood and pleural fluid samples were collected and analyzed by using Erba Mannheim Chem 5 plus V2 semi autoanalyzer and LISA SCANII Elisa reader. Statistical analysis was done by using ANOVA and student's't' test. P value <0.05 was considered significant. ADA levels in serum and pleural fluid was significantly higher in pulmonary TB group than lung cancer group('p' <0.001) and both are higher than control group('p'<0.001).CYFRA 21-1 in serum and pleural fluid was significantly higher in lung cancer group than TB group('p' < 0.001) but both were higher than control group('p'<0.001). The results suggests early quantization of these parameters can differentiate pulmonary tuberculosis from lung cancer and thus can decrease the mortality rate of lung cancer cases though more extensive study with increased sample size may provide more insights.
Keywords: Differential diagnosis, malignant pleural effusion, tuberculous effusion, ADA, CYFRA 21-1.

INTRODUCTION

The etiological diagnosis of exudative pleural effusion poses a significant dilemma in clinical practice, especially in terms of the differentiation between malignant and benign pleural effusion as there is significant difference in treatment and prognosis [1].

Statistics for India revealed 63,000 new lung cancer cases are reported each year [2] which was once considered to be rare [3] and now responsible for 1.38 million deaths worldwide out of which significant contributor is also India [4].

Tuberculosis is another cause of exudative pleuritis and is most major health problems associated with significant morbidity and mortality in India along with lung cancer. In 2012, India declared tuberculosis to be notifiable disease [5] and it is the highest tuberculosis burden country with WHO statistics revealed 2.2 million cases out of global 8.7 million cases so approximately 40% of Indian population is infected with *Mycobacterium tuberculosis* bacteria, the vast majority of whom have latent than active tuberculosis [6.7]. So, any patient arrives with

predominantly lymphocytic exudative pleural effusion, the suspicion is targeted towards diagnosis of malignant or tuberculous pleuritis primarily. Diagnostic dilemma happens as traditional methods of diagnosis tuberculosis fail to recognise it [8] moreover, microbiological results reported so late that decisions regarding management of the symptoms are already taken and initiated which causes wrong diagnosis and management of lung cancer as pulmonary tuberculosis. Most often to deal with this diagnostic dilemma combination of pleural biopsy culture and histology is done which increases diagnostic chances up to 90% in tuberculous pleuritis [9] but it is an invasive approach and frequently requires more than six samples [10]. Lung cancer accounts to 68% of all cases of malignant pleural effusion [11], the diagnosis is mostly done by cytopathological study of pleural fluid but sensitivity is only 50%. It can be increased to 80% if needle biopsy of pleura is performed [12]. Due to such low sensitivity sometimes the results are inconclusive and thus thoracoscopy is done to identify the type and loci of malignancy [13].

Because diagnosis of these two common causes of pleural effusion which is often having similar biochemical profiles and predominance of lymphocytes with other diagnostic difficulties already discussed can delay or misdiagnose a case of lung cancer as sputum negative pulmonary tuberculosis is very high and often these patients presumptively treated with anti-tubercular drugs not only delays the diagnosis of lung cancer but also causes progression of the disease to stage IIIB or IV by that time they are beyond the scope of curative resection [14.15]. In this context, the objective of our present study were to describe the characteristics and laboratory performances of ADA and CYFRA 21-1 in serum and pleural effusion of patients suffering from tuberculosis and lung cancer as these are non invasive as well as in expensive test and can be performed in primary health care setup.

Adenosine deaminase (ADA; EC 3.5.4.4) is an enzyme required for converting adenosine to inosine, a stage in purine catabolism. Since 1978, when ADA activity was found high in tuberculous pleural exudates as well as in serum [16], since then activity of total ADA has been used to diagnose tuberculous pleural effusion but degrees of sensitivity and specificity varies in different study [17 – 21].

CYFRA 21-1 is a cytokeratin-19 fragment; an acid type of cytoplasmic protein having molecular weight of 40 KD is a major component of cytoskeleton intermediate filaments of simple epithelial cells and is over expressed in various carcinomas. Following cell death it is released in the serum in the form of soluble fragments [22 – 26]. CYFRA 21-1 is a potential marker for malignant pleural effusion and is not only found in serum but also present in pleural fluid [27]. So we have included these two parameters to find out its efficacy for differential diagnosis between malignant pleural effusion and tuberculous pleural effusion.

MATERIALS AND METHODS

This case control study was conducted in the Department of Biochemistry from October 2014 till January 2016 in collaboration with Department of Pulmonary Medicine, Rohilkhand medical college and hospital, Bareilly, Uttar Pradesh. Ethical clearance was procured from Institutional Ethical Committee with vide reference no. IEC/64/2014.

We have taken 100 cases of diagnosed tuberculous effusion, 50 cases of lung cancer who were earlier considered as smear negative pulmonary tuberculosis cases and administered DOTS in primary health centre later referred to Department of Pulmonary Medicine as complication started and 100 cases of age and sex matched apparently healthy controls (who appeared for general health check up in study age and sex group).

We have only considered exudative pleural effusion cases as per Light's criteria i.e. (a) Pleural fluid/Serum total protein ratio > 0.5, (b) Pleural fluid/serum LDH ratio > 0.6, (c) Pleural Fluid LDH > 200.0 IU/L[28] and Roth *et al*. i.e. Serum-Pleural Effusion Albumin Gradient of ≤ 1.2 gm/dL suggests exudates and > 1.2 gm/dL suggests transudates [29].

Standardised Diagnostic Criteria for Tuberculosis were:

a) Pleural biopsy demonstrating a granulamatous process.
b) Detection of Mycobacterium tuberculosis in pleural fluid or tissue by Z-N staining.
c) A compatible clinical history and radiological examination, in patients with a lymphocytic exudates and ADA levels higher than 24 IU/L as well as favourable clinical evaluation after specific treatment.

Standardised Diagnostic Criteria for Lung Cancer:

a) Finding of neoplastic cells in pleural fluid or tissues obtained by needle biopsy.
b) CT scan of thorax.
c) In inconclusive cases, diagnosis was established by thoracoscopy guided biopsy or surgery.

The following patients were excluded from our study:

a) Pleural exudates other than tuberculosis and lung cancer.
b) Other cases of cancer.
c) Chronic diseases like DM, hypertension etc.
d) Any liver, renal and muscular disorders.
e) Known HIV positive cases.

The following parameters were evaluated i.e. Adenosine deaminase (ADA) and Cytokeratin fragment CYFRA 21-1 in serum and pleural effusion secondary to tuberculosis and lung cancer. Evaluation of ADA was done in Erba Chem 5 plus V2 semi auto analyzer by enzymatic kinetic ADAZYME method procured from Tulip Diagnostics and CYFRA 21-1 was done by sandwich ELISA method procured from Elabscience Biotechnology and reading was taken by LISA SCANII ELISA reader and automated ELISA washer by Erba Mannheim.

After taking informed consent from patients pleural fluid was collected by thoracocentesis done by Department of pulmonary medicine and 4 mL blood was collected in serum separation tube (SST) by venipuncture under aseptic condition. Serum was separated after allowing the blood to stand for 30 min at room temperature and then centrifuged at 2000 rpm for 5 min. Fresh samples were used for our study.

Data was presented as mean ± SD, comparison between cases (TB and Lung cancer group) was done by Unpaired student's 't' test. Significance between three groups (TB, Lung cancer and Control groups) was calculated by using one way ANOVA. P value <0.05 was considered as statistically significant. Statistical analysis and ROC curve analysis was done by using licensed SPSS 16.0 software (SPSS Inc., Chicago, IL, USA) for windows. We have used licensed MS Excel software to present and prepare charts and graphs for our present study data.

RESULT

The demographic distribution of our study population for the pulmonary tuberculosis and lung cancer group is shown in Table-1. Patients with pulmonary tuberculosis were significantly of lower age group (mean ±SD for males 45.46±13.525; for females 40.918±13.781) than lung cancer group (for males 58.186±8.764; for females 62.428±7.656) with 'p' value <0.0001.

The biochemical analysis of serum and pleural fluid ADA in pulmonary tuberculosis and lung cancer is shown in Table-2 which shows concentration of serum and pleural fluid ADA was significantly higher in TB group (mean ± SD of serum ADA 33.731±7.355 IU/L; pleural fluid ADA 107.08±23.09 IU/L) than that of lung cancer group (mean ±SD of serum ADA 16.206±6.356 IU/L; pleural fluid ADA 31.828±11.913 IU/L) when compared by unpaired student's 't' test 'p' value were <0.001 for both serum as well as pleural fluid values but both were significantly higher than control group (mean±SD of serum ADA 5.512±1.862 IU/L) with 'p' value < 0.001 (for both TB and Lung cancer group when compared with control group). ANOVA of serum ADA in TB group, Lung cancer group and Control group was 'p' value 0.00 and F value 647.8327. When plotted in ROC curve in TB vs. Lung cancer the best cut off values for serum ADA was 20.5 IU/L (sensitivity 98%, specificity 86%) shown in Fig-1. For pleural effusion the best cut off values was 59.7 IU/L (sensitivity 99%, specificity 98%) presented as Fig-2.

The biochemical analysis of serum and pleural fluid CYFRA 21-1 is shown in Table-3 which shows serum CYFRA 21-1 was significantly higher in Lung cancer group (mean±SD; 14.004± 10.578 ng/mL) than pulmonary tuberculosis group (mean±SD 1.6951±0.553 ng/mL) with 'p' value < 0.001. But both were higher than serum values in control group (0.976±0.421 ng/mL). ANOVA of serum CYFRA 21-1 in TB group, Lung cancer group and control group was 'p' value 0.00 and F value 143.9277. When plotted in ROC curve, the most probable cut off value of serum CYFRA 21-1 was 2.99 ng/mL (sensitivity 100%, specificity 100%) which is presented as Fig-3..

Pleural fluid CYFRA 21-1 was also significantly higher in lung cancer group (mean±SD; 79.918±34.973) than TB group (11.486±4.798 ng/mL) with 'p' value < 0.001. When plotted in ROC curve the most probable cut off value was 23.15 ng/mL (sensitivity 100% and specificity 100%) which is shown in Fig-4.

Table-1: Demographic Distribution of study population

	Pulmonary Tuberculosis group	**Lung Cancer Group**	**Control Group**
Male	63	43	63
Female	37	7	37
Mean Female age (yrs)	40.918 ±13.781	62.428±7.656	40.287±13.757
Mean Male age (yrs)	45.46±13.525	58.186 ±8.764	45.662±13.635

Table-2: Values of ADA (IU/L) in TB, Lung cancer and Control Group

Tuberculosis Group		**Lung Cancer Group**		**Control Group**
Serum	Pleural Fluid	Serum	Pleural Fluid	Serum
33.731±7.355 IU/L *vs. Lung cancer 'p' <0.001 *vs. Control 'p' <0.001	107.08±23.09 IU/L *vs. Lung cancer 'p' <0.001	16.206±6.356 IU/L *vs. TB group 'p' <0.001 *vs. Control group 'p' < 0.001	31.828±11.913 IU/L *vs. TB group 'p' < 0.001	5.512±1.862 IU/L
ANOVA analysis of serum ADA in TB, Lung Cancer & Control Groups				
'p' Value = 0.00, F value= 647.8327, Fcrit= 3.032361 Between groups; SS= 40282.22366, df=2, MS= 20141.11 Within groups; SS=7679.2277, df=247, MS=31.08999				

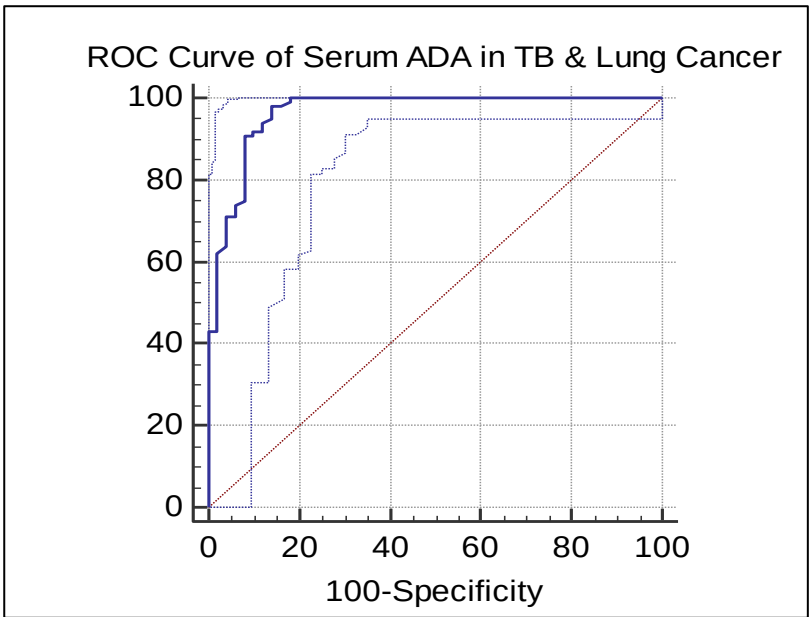

Fig-1:ROC Curve of Serum ADA in TB & Lung Cancer

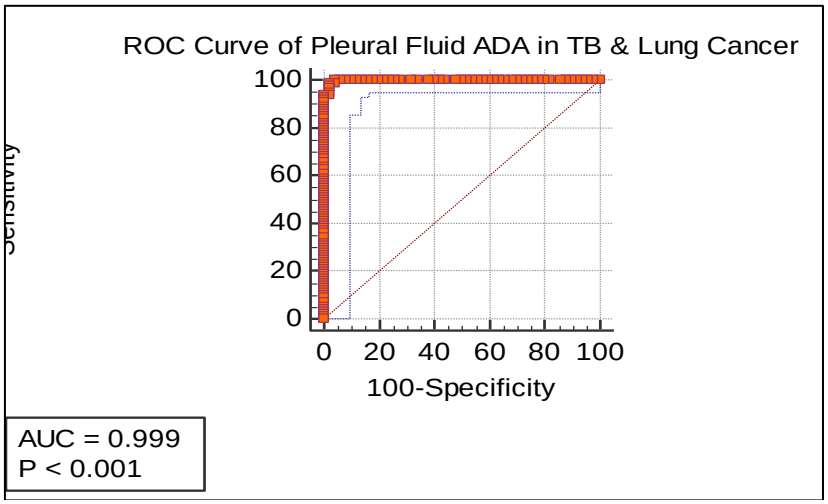

Fig-2:ROC Curve of Pleural fluid ADA in TB & Lung Cancer

Table-3: Values of CYFRA 21-1 (ng/mL) in TB, Lung Cancer and Control Groups

Tuberculosis Group		**Lung Cancer Group**		**Control Group**
Serum	Pleural Fluid	Serum	Pleural Fluid	Serum
1.6951±0.553 ng/mL *vs. Lung cancer 'p' <0.001 *vs. Control 'p' <0.001	11.486±4.798 ng/mL *vs. Lung cancer 'p' <0.001	14.004±10.578 ng/mL *vs. TB group 'p' <0.001 *vs. Control group 'p' < 0.001	79.918±34.973 ng/mL *vs. TB group 'p' < 0.001	0.976±0.421 ng/mL
ANOVA analysis of serum CYFRA 21-1 in TB, Lung Cancer & Control Groups				
'p' Value = 0.00, F value= 143.9277, Fcrit= 3.032361 Between groups; SS= 6445.846, df=2, MS= 3222.923 Within groups; SS=5530.987, df=247, MS=22.39266				

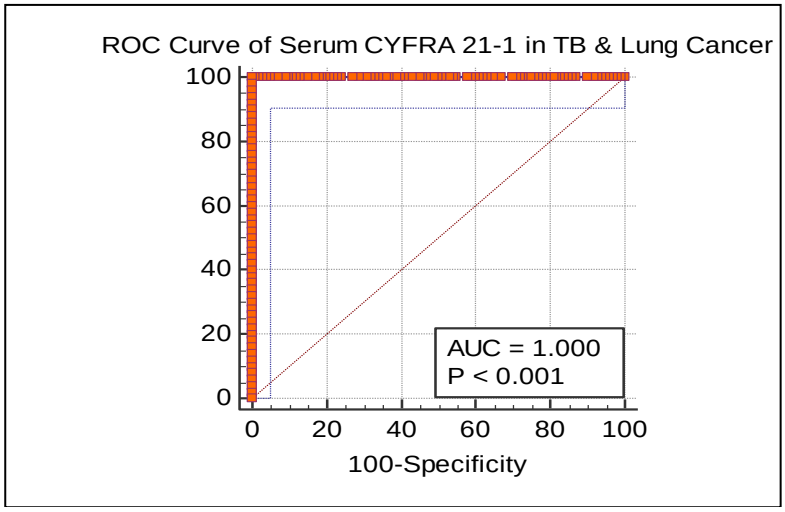

Fig-3: ROC Curve of Serum CYFRA 21-1 in TB & Lung Cancer

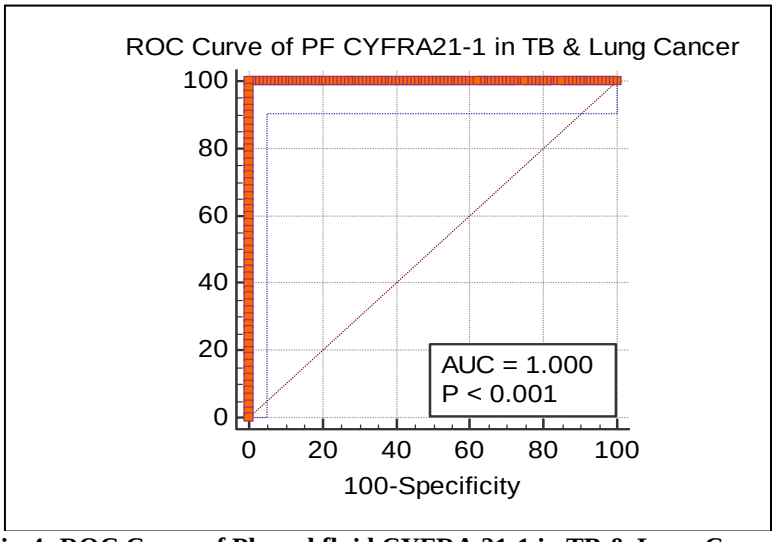

Fig-4: ROC Curve of Pleural fluid CYFRA 21-1 in TB & Lung Cancer

DISCUSSION

Pleural effusions are common complications observed in wide variety of diseases including pulmonary tuberculosis and lung cancer. Thoracoscopy is considered as gold diagnostic standard and analysis of removed fluid is the fastest and easiest way of assessment of causes [30]. Cytological, biochemical and microbiological analysis of pleural fluid is fundamental

for adequate screening although 20% of the cases remain inconclusive [31] to differentiate benign pleural effusion from malignant pleural effusion thus more expensive examination is required which may not be bearded by our low economic group of population in both rural as well as urban areas thus increases the morbidity and mortality of the patients.

In our study with respect to gender we have found men are more predisposed to both tuberculosis and lung cancer (63% of TB cases and 86% of lung cancer cases). Tuberculosis usually predominates among younger age group (mean ± SD; males 45.46±13.525; females 40.918±13.781) than lung cancer group (mean±SD; males 58.186±8.764; females 62.428±7.656) which is very similar to other global studies [32-34] although incidence of lung cancer is increasing alarmingly in females who were never smokers [35]. In our study we have got 7 such cases who were never smokers in which 4 cases are having adenocarcinoma, 2 cases of squamous cell carcinoma and 1 case of large cell carcinoma which is following similar pattern according to incidence rate as studied by Noronha V *et al*.[36].In the present study as a rule first we have analyzed and segregated exudates arising from pulmonary tuberculosis and lung cancer by Light's criteria and Roth *et al*. followed by confirmation by our diagnostic criteria and then subjected to further analysis. Serum and pleural fluid activity has been proved to be a valuable biochemical marker that has high sensitivity and specificity for TB diagnosis [37].Serum and pleural fluid ADA activity were significantly higher in TB group than lung cancer group ('p' value <0.001) but both were significantly higher than control group (mean±SD 5.512±1.862). The cut off value for serum ADA was 20.5 IU/L for TB group with 98% sensitivity and 86% specificity with area under ROC curve 0.965 and standard error 0.0158; for pleural fluid ADA the best cut off value was 59.7 IU/L with 99% sensitivity and 98% specificity with area under ROC curve 0.999 and standard error 0.00143 was a significant predictor for pulmonary tuberculosis cases and can differentiate TB cases from malignancy cases significantly which is in accordance to study conducted by Mo-Lung Chen *et al*. [38].Our findings contradict the study conducted by Light *et al*. [30] and Sharma *et al*.[39] who has suggested that lower ADA levels with lesser sensitivities and specificities among Asians in comparison to their European and Caucasian compatriots might compromise its usefulness in TB detection in these population. Our sensitivity and specificity were remarkably high due to improvement in diagnostic methodology as well as quality of the reagent as we have used enzymatic kinetic method instead of Giusti's method or NADH linked method. Exclusion of other increased cell mediated immune response to pathological causes like empyema; liver diseases etc. may also causes significant improvement in achieving greater diagnostic accuracy which was reflected in the area under ROC curve and increase in sensitivity and specificity of the method.

We have evaluated the diagnostic performance of CYFRA 21-1 in serum and pleural fluid in tuberculosis and malignancies in differentiation between these two. There are wide ranges of markers for the detection of malignant pleural effusion but it lacks sufficient diagnostic accuracy in discriminating lung cancer cases from TB cases in early stage. One of the promising tumour markers is CYFRA 21-1 and it can be detected by sandwich ELISA method from both pleural fluid as well as serum [40].

Serum and pleural fluid CYFRA 21-1 is significantly higher in lung cancer group than tuberculosis group ('p' value < 0.001) but both were significantly higher than control group (mean±SD, CYFRA 21-1 0.976±0.421). When ROC curve analysis was done it shows cut off values of CYFRA 21-1 in serum is 2.99 ng/mL with 100% sensitivity and 100% specificity, area under ROC curve was 1.00 and standard error was 0.00. Up on ROC curve analysis of pleural fluid CYFRA 21-1 the most probable cut off value is 23.15 ng/mL with100% sensitivity and 100% specificity. The values are in accordance with study conducted by David *et al*.[41], Li *et al*.[42], Liang *et al*.[43], Huang *et al*.[44] and Dalia H Farag *et al*.[45]. This finding could be attributed to increased Cytokeratin fragment solubility due to modification at the amino and carboxyl terminals of keratin by phosphorylation, glycosylation and transglutamination which mainly occurs during transformation of normal cells to malignant cells. Higher values with high sensitivity and specificity of CYFRA 21-1 in lung cancer cases is further caused due to proteolytic degradation of keratin during cell lysis, abnormal mitosis and tumour necrosis[44].Thus quantisation of serum and pleural fluid CYFRA 21-1 is an excellent discriminator between pulmonary tuberculous effusion and malignant pleural effusion.

CONCLUSION:

Biochemical analytes like Adenosine deaminase and Cytokeratin fragment CYFRA 21-1 levels in serum and pleural fluid is a useful and efficient tool to differentiate between two common exudative causes i.e. tuberculosis and lung cancer along with other specific test as they possess higher sensitivity and specificity. As these tests are easy, inexpensive and thus can help us in early segregation of lung cancer cases from pulmonary tuberculosis in primary health care setup and decrease mortality and morbidity significantly. The limitation of our study is limited sample size and study was conducted in a single region. Larger sample size and multi-centric studies could be done to obtain wider insights.

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