

Original Article

Clinical study of Pleural Effusion and correlation of their biochemical, cytological and radiological characteristics

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ABSTRACT:

INTRODUCTION: Pleural effusion is an excess accumulation of fluid in the pleural space. This study aims to provide a practical approach to pleural effusions by correlating the results of clinical examination and biochemical, cytological and radiological investigations. It evaluates the reliability, diagnostic efficacy and correlation of pleural fluid protein, glucose, Lactate Dehydrogenase (LDH), Adenosine Deaminase (ADA), cholesterol and TB PCR.

MATERIAL AND METHODS: In this prospective, observational study, sixty patients with pleural effusion were studied from September 2015-August 2017 with respect to demographic characteristics and clinical, radiological and laboratory features.

RESULTS: The commonest effusion was tubercular (35) followed by malignancy (9), transudative (7), synpneumonic (6) and empyema (3). Cough, breathlessness and fever were commonest presenting complaints. Right sided and moderate effusions were more common, particularly in tuberculosis. Blood counts and ESR were significantly elevated in exudates. Pleural fluid cytology revealed elevated lymphocytes in tubercular and malignant effusions and elevated polymorphs in acute infections. Cytology was diagnostic for malignancy in 6 cases. ADA was significantly raised in tubercular effusions. Exudates had decreased glucose and increased protein, LDH and cholesterol compared to transudates. Pleural biopsy was diagnostic in 58.33%. Pleural fluid cultures were positive in only 2 patients, both with Empyema. TB-PCR was 94.29% sensitive and 100% specific in diagnosing tubercular effusion.

CONCLUSION: Although clinical features give a clue to etiology of effusion, radiological, biochemical and cytological evaluation helps to narrow the diagnosis for better management.

KEYWORDS: Pleural effusion, ADA, Cholesterol, TB-PCR

INTRODUCTION

Pleural effusion, i.e., excessive accumulation of fluid in the pleural space, indicates an imbalance between pleural fluid formation and removal.⁽¹⁾ The cause may be pulmonary or extra-pulmonary in origin.⁽²⁾ In India, the commonest causes include tuberculosis, malignancy, congestive cardiac failure and pneumonia.⁽³⁾ Pleural effusions are classified as transudates or exudates according to Light's criteria. Transudates are ultrafiltrates of plasma occurring due to an imbalance in oncotic and hydrostatic pressures. Exudates are formed by pleural inflammation or decreased lymphatic drainage.^(4,5) Differentiating transudate from exudate is key to further management.^(3,5) Several studies report about 20-25% cases of effusion every year in whom a definite diagnosis cannot be made.^(1,4,6) Thoracoscopy, which is the 'gold standard' can be used for diagnosis but is expensive and not widely available.^(7,8)

One-fifth of patients with no diagnoses continue to have recurrent effusions.⁽⁶⁾ In India, most undiagnosed and recurrent effusions are empirically treated as tubercular, and in most cases, response to treatment clinically confirms the diagnosis. However, recurrent effusions particularly in elderly may be

malignant.⁽³⁾ Other less common causes include chylothorax, rheumatoid arthritis, SLE, HIV infection, Benign asbestosis, drug induced effusion and pulmonary embolism.^(4,7)

AIMS AND OBJECTIVES

1. To study clinical features and etiological factors of pleural effusion.
2. To evaluate reliability, diagnostic efficacy and correlation of biochemical investigations like pleural fluid protein, glucose, Lactate Dehydrogenase (LDH), Adenosine Deaminase (ADA), cholesterol and TB PCR.
3. To evaluate the cytological and radiological profile of pleural effusions.
4. To correlate biochemical, cytological and radiological profiles.

MATERIAL AND METHODS

This prospective, observational study was conducted at Wanless Hospital, Miraj, Maharashtra, which is a 450-bedded tertiary care centre. 60 patients between 18-75 years of age, admitted over two years (September 2015 to August 2017) and with clinical and radiological evidence of pleural effusion were included in this study after taking written, informed consent. Pregnant and lactating patients were excluded.

Demographic data and detailed history with examination was done. Routine investigations like CBC, ESR, Sputum examination, Mantoux test were performed. Serum protein, Serum LDH and Serum cholesterol were done in all patients. Chest X-ray with Ultrasound was done to determine site and volume of effusion. All patients underwent pleural biopsy, either Ultrasound or CT guided. Bronchoscopy and CT Chest were performed if required. Thoracentesis was done with all aseptic precautions, either blindly, or ultrasound guided in minimal effusions. Pleural fluid was analyzed with respect to sugar and protein content, cell count, cell type, presence of malignant cells, presence of organisms by Gram Stain and 10% KOH and Acid Fast Bacilli by Ziehl Neelsen stain. Pleural fluid LDH, ADA and cholesterol levels were measured.

For bacterial cultures, pleural fluid samples were inoculated into blood agar, chocolate agar and McConkey's agar. The specimens were concentrated using modified Petroff's method and inoculated in Lowenstein Jensen's medium and BACTEC-12B vials for culturing Mycobacterium. The latter was processed in the BACTEC 460 TB system. Samples were also inoculated on to Sabouraud's dextrose agar (SDA) with antibiotics, SDA without antibiotics in duplicate and/or BHI agar for detection of fungi.

TB PCR was carried out on all pleural fluid samples. Arrow BUGS n BEADS method was used for isolation of Nucleic Acid followed by Real-Time PCR for detection of Mycobacterium tuberculosis. It was found that out of 60 cases, 35 were of tubercular effusion and 25 were non-tubercular. These 60 cases were divided into 5 groups - Tubercular (35), Malignant (9), Transudative (7), Synpneumonic effusion (6) and Empyema (3). Data was entered in Excel Spreadsheet and analyzed. Mean and standard deviations were calculated where required. t test, ANOVA and chi square test were used to compare the means and standard deviations of observations. Sensitivity and specificity of tests were determined by taking standard cut off values. Correlation of radiological and cytological observations with the type of effusion was done using Correlation Coefficient. P value < 0.05 was considered statistically significant.

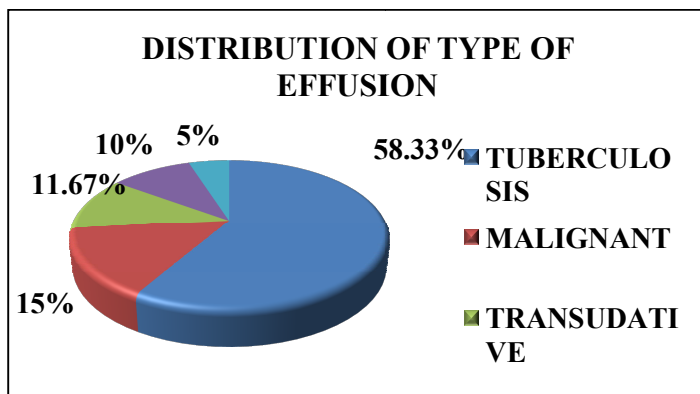
RESULTS

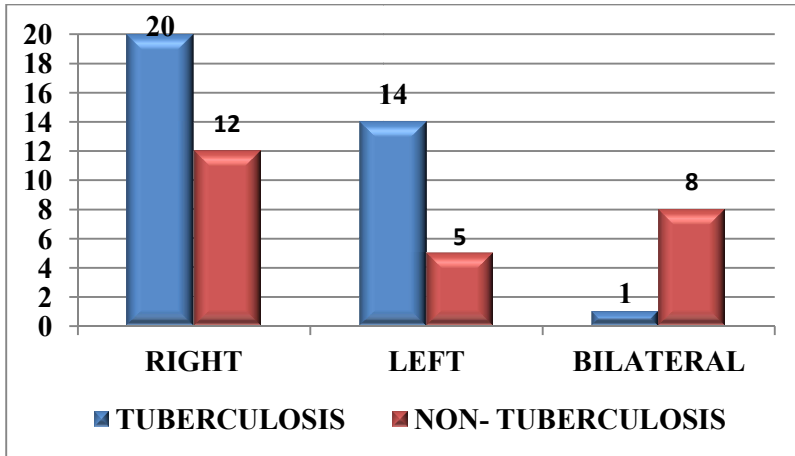
The commonest type of effusion was tubercular (35), followed by malignancy (9). Males were more (66.67%) than females (33.33%), with a male to female ratio of 2:1. Most cases were in the age group of 31 to 60 years (63.33%). Mean age in tubercular group was 41.17 ± 13.21 years.

Commonest chief complaints were cough (73.33%), breathlessness (71.67%) and fever (71.67%). 28.57% patients with tubercular effusion gave a family history of contact with tuberculosis. 53.33% had right-sided effusion and 31.67% had left-sided effusion. Majority i.e. 38 cases (61.67%) had a moderate effusion. Commonest clinical signs of effusion were stony dull note on percussion, decreased or absent breath sounds, decreased vocal fremitus and vocal resonance and decreased respiratory movements. ESR was significantly elevated in exudative compared to transudative effusions. Total leucocytes were significantly raised in synpneumonic effusions and empyema. Cytology revealed elevated lymphocytes in tubercular and malignant effusions and polymorphs in synpneumonic effusions and empyema. Transudative effusions predominantly showed Lymphocytes, with Monocytes in 1 case. Cytology for malignant cells was positive in 6 out of 9 cases (66.67%) of malignancy.

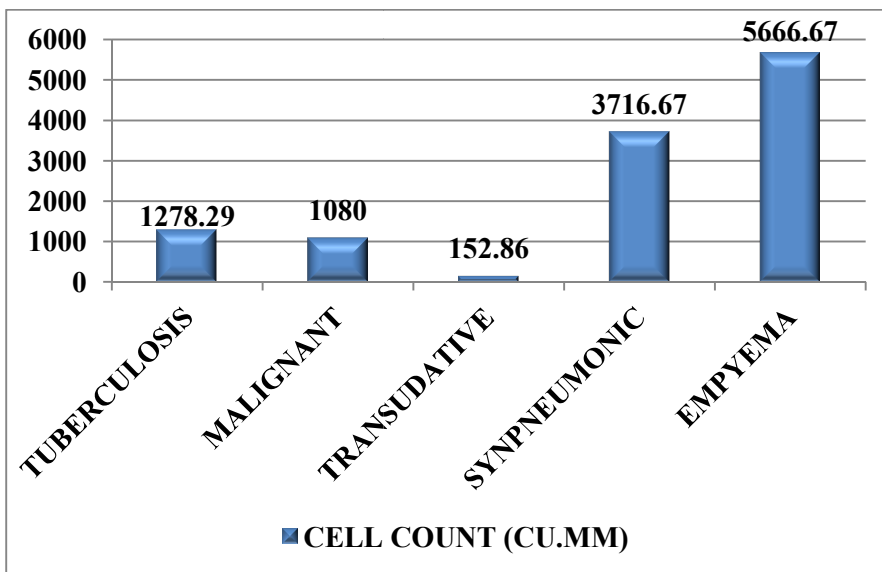
We observed raised pleural fluid protein and cholesterol, high ADA and LDH activity and reduced glucose concentration in tubercular effusions. Malignant effusions also showed increased pleural fluid protein, cholesterol and LDH activity with decreased glucose.

In transudative effusions, pleural fluid glucose was more, whereas protein, cholesterol, ADA and LDH were decreased. Increased pleural fluid protein, cholesterol and LDH activity was seen in synpneumonic effusions and empyema, with significantly decreased glucose. Determination of serum to pleural fluid ratio of protein, cholesterol and LDH was also helpful in reaching a diagnosis. Real-Time TB-PCR had sensitivity and specificity of 94.29% and 100% respectively in diagnosing tubercular effusion. All patients underwent Pleural Biopsy, of which 26 cases showed granulomatous inflammation suggestive of Tuberculosis, 8 cases had acute inflammatory and 10 had chronic inflammatory reaction, 4 cases had Metastatic Carcinoma and 5 cases had Primary Lung Carcinoma. Non-specific inflammation was seen in 7 cases.

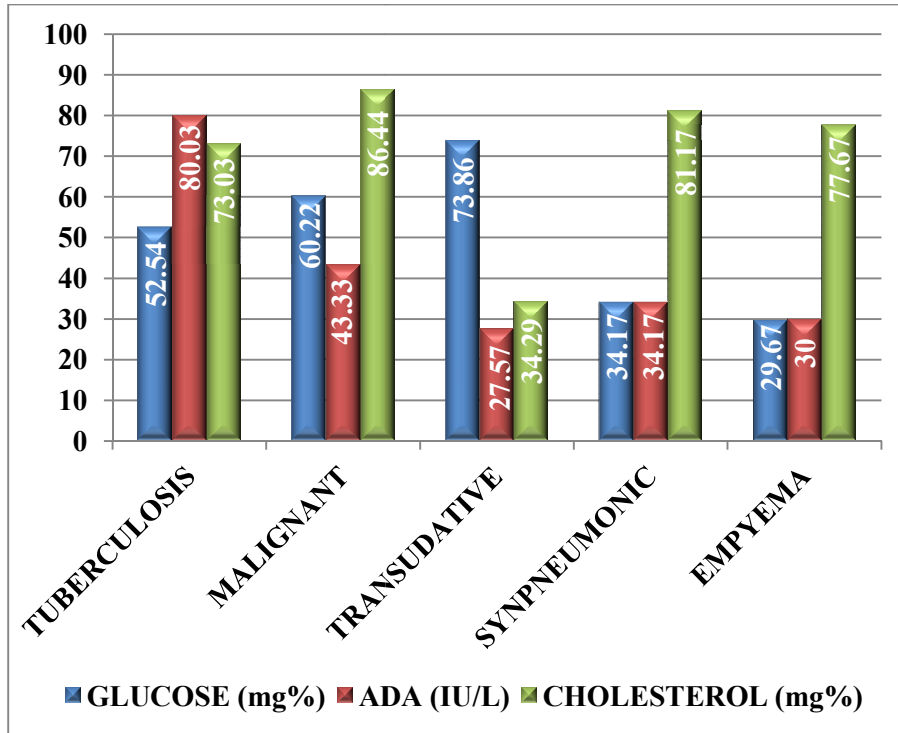




DISTRIBUTION OF SITE OF PLEURAL EFFUSION IN TB AND NON-TB



MEAN PLEURAL FLUID CELL COUNT



ESTIMATED MEAN OF PLEURAL FLUID GLUCOSE, ADA AND CHOLESTEROL

ESTIMATED MEAN PLEURAL FLUID / SERUM RATIO OF PROTEIN, LDH, AND CHOLESTEROL

SR. NO.	TYPE OF PLEURAL EFFUSION	NO. OF PATIENTS	MEAN PLEURAL FLUID / SERUM RATIO		
			PROTEIN	LDH	CHOLESTEROL
1.	TUBERCULOSIS	35	0.65 (>0.5)	0.71 (>0.6)	0.48 (>0.3)
2.	MALIGNANT	9	0.75 (>0.5)	0.80 (>0.6)	0.46 (>0.3)
3.	TRANSUDATIVE	7	0.26 (<0.5)	0.41 (<0.6)	0.18 (<0.3)
4.	SYNPNEUMONIC	6	0.74 (>0.5)	0.99 (>0.6)	0.53 (>0.3)
5.	EMPHYEMA	3	0.72 (>0.5)	2.97 (>0.6)	0.39 (>0.3)

PLEURAL BIOPSY FINDINGS IN DIFFERENT EFFUSIONS			
SR. NO.	PLEURAL BIOPSY FINDINGS	NO. OF PATIENTS	TYPE OF EFFUSION
1.	Granulomatous inflammation s/o TB	26	Tuberculous
2.	Malignancy	9	Malignant
3.	Acute Inflammatory reaction	6	Synpneumonic
		2	Tuberculous
4.	Chronic inflammatory reaction	7	Tuberculous
		3	Empyema
5.	Non-specific inflammation	7	Transudative
TOTAL		60	



FIG: 1 MODERATE PLEURAL EFFUSION (RT.SIDE) IN TUBERCULOSIS.

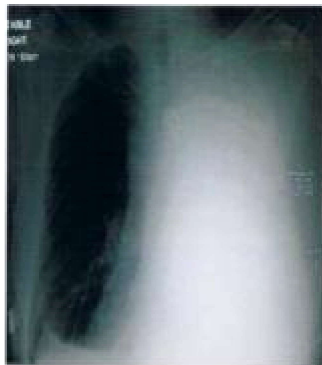


FIG:2 MASSIVE PLEURAL EFFUSION (LEFT SIDE) IN CASE OF LUNG CARCINOMA

DISCUSSION

In this study, 35 out of 60 cases were of tubercular effusion. This reflects the high prevalence of tuberculosis in the study area, which is consistent with studies done in developing countries.^(3,9,10,11) Western studies, such as those done by Luis Valdes and Storey et al, show malignancy as the major cause of pleural effusion^(12,13). This is probably due to low prevalence of infectious diseases in western populations.

88.33% effusions were exudative and 11.67% were transudative. Hamal et al⁽¹⁴⁾ also showed that 69.4% of pleural effusions were exudative and 30.6% were transudative. Thus exudative effusions are more common. Out of 53 exudative effusions, tubercular effusion was the commonest (35), followed by malignant (9)

and synpneumonic effusion (6), and empyema (3). Prabhu desai⁽¹⁵⁾ observed that malignancy was the commonest cause of exudative effusion. Among the 7 cases with Transudative Effusion, there were 3 with Congestive Cardiac failure, 2 with Chronic renal failure, 1 case of Nephrotic Syndrome, and 1 case of Liver cirrhosis. This is similar to most studies where Congestive Cardiac failure and chronic liver and renal disease were major causes of transudative effusion.^(3,11,16-19)

There were 66.67% males and 33.33% females, with male: female ratio being 2:1. Majority of similar studies show male preponderance. Mean age of all patients was 45.25 ± 15.51 years. Mean age in tubercular effusions was 41.17 ± 13.21 years. The mean ages in malignant, transudative, synpneumonic effusions and empyema were 59.22 ± 11.58 years, 56.43 ± 17.54 years, 37.33 ± 17.81 and 40.67 ± 7.51 years respectively. In comparison, Harish GM et al⁽²⁰⁾ observed that tubercular effusion was found in lower age group (33.90 ± 9.49 years), while malignant (62.40 ± 7.01 years) and transudative (46.6 ± 8.23 years) effusions were seen in older age groups.

32 out of 60 cases (53.33%) had right sided effusion, of which 20 were tubercular. 19 (31.67%) had left sided effusion and 9 (15%) had bilateral. Right sided effusions were significantly more common in Tuberculosis. Al Quarain⁽⁹⁾ found that Pleural effusion was more common on right side (56%) than on the left (32%). 81.67% patients had a stony dull note on percussion, which is the most important sign of effusion. Breath sounds were diminished or absent in 81.67% patients. Vocal fremitus and vocal resonance were decreased in 81.67%. Reduced respiratory movements were noted in 81.67% and mediastinal shift in 18.33%. Crepitations were present in 51.67% and 20% had a pleural rub. Other associated findings were seen in transudative effusions like ascites (6%), hepatomegaly (6%) and elevated JVP (6%). Harish GM et al⁽²⁰⁾ also found that the commonest clinical sign of pleural effusion was a stony dull note on percussion.

The mean E.S.R. was $48.5 \text{ mm/1}^{\text{st}} \text{ hr}$. ESR was significantly elevated in exudative as compared to transudative effusions. Harish GM et al⁽²⁰⁾ found that ESR was significantly elevated in tubercular effusions. Out of 35 patients with tubercular effusion, Mantoux test was positive in 11 (induration $>10 \text{ mm}$). 12 patients had indurations measuring 5-7 mm and 2 patients were non-reactive. Those with tubercular effusion and Mantoux negative were malnourished and emaciated, which probably produced a false negative response. None of the patients with non-tubercular effusion had a positive test. Anne E. Asuquo et al⁽²²⁾ compared Mantoux test results of healthy individuals with smear positive TB patients and found that around 25% of healthy individuals had indurations $>10 \text{ mm}$ compared to 95% of TB patients. 4.5% of TB patients had indurations $<10 \text{ mm}$. Thus at least 25% healthy subjects are at risk of progressing to active disease under conditions of lowered immunity.

All patients in this study underwent Pleural Biopsy. 43.33% had granulomatous inflammation showing tuberculosis, 15% had malignancy, 13.33% had acute inflammatory reaction, and 16.67% had chronic inflammatory reaction. 11.67% showed non-specific inflammation. 7 of the 10 patients showing chronic inflammation had tubercular effusion while 3 were of Empyema. Out of 8 cases showing Acute inflammation, 6 were Synpneumonic effusions and 2 were tubercular. Thus tubercular effusions showed predominantly granulomatous as well as acute and chronic inflammatory changes on biopsy. Pleural biopsy was diagnostic in 58.33%.

Asad-ur-Rahman⁽²³⁾ observed that on pleural biopsy, 44.06% had granulomatous inflammation most likely tuberculosis, 16.10% had malignancy, 11.86% had chronic empyema and 27.96% had non-specific

inflammation. Tokgoz F et al⁽¹⁹⁾ found that closed pleural biopsy was diagnostic at the rate of 57% in malignancy and 79% in tuberculosis.

Of the 35 cases of tubercular effusion, AFB were demonstrated in sputum by ZN staining in 12 cases (34.29%), of which 8 had associated lung lesions. Subhakar. K et al⁽²⁴⁾ found that 11% of tubercular effusions showed sputum positivity for AFB. Chest X-ray showed presence of fluid in all patients. 26 patients(43.33%) had associated pulmonary lesions. On ultrasound estimation of fluid volume, 80% cases of tubercular effusion had moderate effusion. 88.89% cases of malignant and 8.57% of tubercular effusion had massive collection, while 71.43% of transudative effusions were minimal. Synpneumonic effusions showed mild-moderate fluid collection. All 3 patients of Empyema had moderate effusion. Bowen⁽²⁵⁾ divided effusions into mild (250-600 ml) and massive (>1500 ml). Majority of malignant effusions were massive, while synpneumonic effusions and empyema showed minimal collection.

Similarly, Nance KV⁽²⁶⁾ showed that Pleural fluid cytology was diagnostic for malignancy in 71%. PA Devi et al⁽¹⁸⁾ demonstrated that cell counts >5000 cells/ mm³ were predominantly seen in Empyema, and in parapneumonic effusions counts were between 1000-5000. Tubercular and malignant effusions were lymphocyte predominant while parapneumonic effusion and empyema were neutrophil predominant.

Mean pleural fluid protein in tubercular effusion was 4 ± 1.02 gm/dl, in malignant effusion 4.62 ± 0.84 gm/dl, in transudates 1.34 ± 0.44 gm/dl, in synpneumonic effusion 3.63 ± 0.66 gm/dl, and in empyema it was 4.20 ± 0.56 gm/dl. Pleural fluid to serum protein ratio was >0.5 in tubercular, malignant, synpneumonic effusions and empyema and <0.5 in transudative effusions. These findings were similar to those seen by Follader⁽²¹⁾, Lakhotia⁽²⁷⁾ and Light⁽²⁸⁾. Anthony Seaton⁽²⁹⁾ and Richard W. Light⁽³⁰⁾ showed that pleural fluid protein was more than 3gm% in exudates, which is consistent with this study.

Lakhotia⁽²⁷⁾ found that Pleural fluid LDH showed a sensitivity of 95.5%, while pleural fluid to serum LDH ratio showed a sensitivity of 92.75% and specificity of 100%. Richard W. Light⁽³³⁾ also classified transudates and exudates with same criteria.

Average pleural fluid cholesterol in tubercular effusion was 73.03 ± 10.66 mg/dl, in malignant effusions it was 86.44 ± 8.79 mg/dl and in transudative effusions it was 34.29 ± 6.85 mg/dl. Synpneumonic effusions and empyema showed mean pleural fluid cholesterol of 81.17 ± 9.37 and 77.67 ± 2.31 mg/dl respectively. Ratio of pleural fluid to serum cholesterol > 0.3 was used to classify exudates. The mean pleural fluid cholesterol in exudates was significantly higher than transudates. By taking pleural fluid cholesterol < 55mg% as cut-off, the test exhibited 100% sensitivity and 94.34% specificity for diagnosis of transudative effusions.

Valdes et al⁽¹²⁾ found that all transudates had pleural fluid cholesterol lower than 55 mg/dl (100% specificity) with a sensitivity of 84%. The pleural fluid to serum cholesterol ratio was less than 0.3 in transudates, and it was 91.3% sensitive and 93.1% specific. Both parameters combined showed a sensitivity and specificity of 80.3% and 100% respectively.

Pleural fluid cultures were positive in only 2 patients (3.33%), both with Empyema. None of the Tubercular effusions had positive cultures. Pleural fluid staining by Gram and ZN stain was negative in all cases. C.H. Chan, M. Arnold, C.Y. Chan³⁴ found that pleural fluid was smear negative for tubercle bacilli in all the cases, but culture positive in 23%. Follader⁽²¹⁾ showed that pleural fluid culture was positive for tuberculosis in only 22.5% while culture of biopsied pleural tissue was positive in 75%.

In this study, pleural fluid TB-PCR was positive in 33 out of 35 cases with tubercular effusion. Thus, Real Time TB-PCR showed 94.29% sensitivity and 100% specificity. Evaluation of commercially available nested PCR (Amplicor) and Real Time TB-PCR for M. tuberculosis was done in different studies.⁽³⁵⁻³⁹⁾ These studies have shown high specificity of TB-PCR which is consistent with this study. However this study also shows a high sensitivity while previous studies show sensitivity between 50-70%.

CONCLUSION

The commonest cause of pleural effusion is tuberculosis. Suspected cases of effusion should undergo chest ultrasound with routine x-ray. Fluid cytology should be done to confirm tuberculosis or rule out malignancy. All pleural fluid samples should be analyzed for ADA / Cholesterol along with conventional biochemical analysis to differentiate Exudative from Transudative effusions. CT Chest is useful and sometimes essential in minimal effusions where conventional X-ray has its limitations. USG guided pleural tapping is useful especially in minimal and loculated effusions. Real-time TB PCR is a useful and non-invasive additional assay for rapid diagnosis of pleural tuberculosis and can be utilized when routine parameters do not give diagnosis.

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