VOLUME-8, ISSUE-6, JUNE-2019 • PRINT ISSN No. 2277 - 8160

Original Research Paper

Pulmonary Medicine

A STUDY ON SENSITIVITY OF CARTRIDGE BASED NUCLEIC ACID AMPLIFICATION TEST (CBNAAT) TO DETECT MYCOBACTERIUM TUBERCULOSIS IN PLEURAL FLUID AMONG SUSPECTED CASES OF TUBERCULOUS PLEURAL EFFUSION

Dr. Santanu Ghosh — Professor and head. Department of Pulmonary Medicine. Burdwan Medical College, Burdwan.

Dr. Niranjan Kumar Sit* — Associate Professor. Department of Pulmonary Medicine. Burdwan Medical College, Burdwan. *Corresponding Author

Dr. Soumya Mitra — Senior Resident. Department of Pulmonary Medicine.Nadia District Hospital .

Dr. Sourindra Nath Banerjee — Assistant Professor. Department of Pulmonary Medicine. Burdwan Medical College, Burdwan.

ABSTRACT

Background: The incidence and prevalence of tuberculosis is very high in India. Pleural Effusion is one of the commonest manifestations of extra-pulmonary tuberculosis. Diagnosis of tuberculous pleural effusion is based mostly on indirect evidences as specific methods like smear and culture for the organism from pleural fluid lack sensitivity. Pleural biopsy helps the diagnosis as by showing tubercular granuloma. Practically, blind pleural biopsy is not done many a times and conventional culture is time consuming.

CBNAAT is helpful in diagnosis of pulmonary tuberculosis from sputum samples but its efficacy to detect tuberculous bacilli in pleural fluid samples is not yet studied extensively.

Considering this fact, this study was done to detect diagnostic yield of CBNAAT of pleural fluid to diagnose tuberculous pleural effusion directly in comparison to other conventional methods commonly employed by us.

Method: After the approval of the Institutional Ethics Committee of Burdwan Medical College and Hospital and permission of the West Bengal University of Health Sciences (WBUHS), the present thesis work was carried out under the Department of Pulmonary Medicine, Burdwan Medical College, Burdwan between March 2017 to August 2018.

After obtaining written informed consent, 106 patients were selected for the study on the basis of the predefined inclusion and exclusion criteria. All of them undergone diagnostic thoracocentesis under aseptic precautions.

The aspirated pleural fluid was subjected to pathological, biochemical and microbiological analysis. The patients also had undergone routine blood investigations, sputum for AFB smear examination and relevant radiological investigations.

Diagnosis of tuberculosis was established by the composite reference standard (CRS).And the patients were put on appropriate antitubercular therapy according to the RNTCP guidelines. Those who had tuberculous empyema, were treated with water- seal intercostal tube drainage along with ATT.

All the data were analyzed with the help of SPSS software.

A total of 106 patients studied including 65.1 % men and 34.9% women. Majority of cases of tuberculous pleural effusion occurred in the age group of 21 to 30 years comprising 33% of cases. The mean age was 34.28 years.

In the present study, commonest symptoms were cough in 96(90.6%), fever 91(85.8%), shortness of breath 67(63.2%), chest pain 34 (32.1%), hemoptysis 25 (23.6%), and weight loss 18 (17%).

Diabetes was the most prevalent co-morbid illness which was present in 15 (14.2%) patients.

Most of the cases (97.2%) were unilateral. Among them, 38.7% cases presented as left sided and 58.5% cases were right sided pleural effusion. Only three cases (2.8%) presented as bilateral pleural effusion.

Maximum cases 63 (59.4%) were moderate effusion. There were 27 (25.5%) cases of minimal and 16 (15.1%) cases of massive pleural effusion.

Pleural fluid analysis showed most samples were straw coloured, mean total cell count was 1669, mean value of pleural fluid protein, sugar and ADA were 5.38 gm/dL, 72.03 mg/dL, 94.02 IU/L respectively.

Pleural fluid AFB smear, CBNAAT and mycobacterial culture were positive in 15 (14.2%), 48 (45.3%) and 53 (50%) cases respectively. In the hydropneumothorax subgroup (comprising of 43 cases), pleural fluid CBNAAT was able to detect MTB in 42 cases where mycobacterial culture was positive in all the 43 cases.

Taking mycobacterial culture as gold standard the sensitivity, specificity, positive predictive value and negative predictive value of CBNAAT in pleural fluid were 90.6%, 100%, 100% and 91.4% respectively. And in the hydropneumothorax population the sensitivity of pleural fluid CBNAAT was 97.7%.

CONCLUSION: In our study, the sensitivity, specificity, positive predictive value and negative predictive value of CBNAAT in pleural fluid were 90.6%, 100%, 100% and 91.4% respectively taking mycobacterial culture as gold standard.

Our findings suggest that CBNAAT should be considered in the routine diagnostic work up in suspected cases of tuberculous pleural effusion as time factor has a very crucial role in its laboratory diagnosis. CBNAAT has also the added advantage of detection of Rifampicin resistance.

KEYWORDS :

INTRODUCTION:

Tuberculosis (TB) is one of the oldest and commonest infectious diseases also known as the master of death. In 1993, the WHO in an unprecedented step declared TB as the global emergency. 1/3rd of the world's population is currently infected with Mycobacterium tuberculosis, causative agent of tuberculosis; it kills approximately 1.1 million persons per year[2]

The rate of incidence is increasing even greater in developing countries, as a result of high rates of endemicity, declining/chronically poor socio-economic conditions and the

increasing number of HIV-infected individuals.[1]

India is the highest tuberculosis burden country with World health Organization (WHO) statistics for 2014 giving an estimated incidence figure of 2.2 million cases of tuberculosis for India out of a global incidence of 9.6 million cases [2]. WHO statistics also show that India is 17th out of the 22 high burden countries in terms of Tuberculosis incidence rate. The estimated tuberculosis prevalence figure for 2014 is given as 2.5 million [3]. 40% of the Indian population is infected with tuberculosis bacteria, the vast majority of whom have latent rather than active tuberculosis [4].

Tuberculosis can potentially involve any system or organ of the body. Pulmonary tuberculosis is most common presentation; extrapulmonary tuberculosis is also an important clinical problem [5]. Pleural effusion is one of the common complications of primary tuberculosis (after tuberculous lymphadenitis) or in conjunction with pulmonary infiltrate typical of post Pulmonary Tuberculosis. Failure to diagnose and treat pleural TB can result in progressive disease with the involvement of other organs in as many as 65% of patients.

The obvious explanation for the development of the tuberculous pleural effusion is that the delayed hypersensitivity reaction increases the permeability of the pleural capillaries to protein, intense inflammatory reaction in the parietal pleura impedes the lymphatic drainage from the pleural space and leads to the accumulation of pleural fluid [6].

The diagnosis of pleural tuberculosis (TB) with confidence still remains a challenge even more than 100 years after the discovery of TB bacilli due to its nonspecific clinical presentation and paucibacillary nature. Conventional methods, such as direct testing for acid fast bacilli (AFB) and culture of pleural fluid, lack sensitivity (less than 5% and 40%, respectively). Pleural fluid culture is more sensitive than direct examination but Mycobacterium tuberculosis requires 4 to 6 weeks to grow. The sensitivity of Blind Pleural biopsy is slightly higher (39-80%) but it is a blind and invasive procedure associated with significant morbidity.

Many studies have demonstrated the diagnostic significance of increased adenosine deaminase (ADA) in tuberculous pleural effusion, other studies have shown that ADA is of limited value[6],as raised levels are also associated with a number of other diseases including malignancies (especially those of hematological origin),bacterial infections (Q-fever, brucellosis), empyemas, and collagen vascular diseases (including SLE and Rheumatoid arthritis).

World Health Organization (WHO) has endorsed cartridge-based nucleic acid amplification test (CBNAAT) as a rapid diagnostic test for the detection of MTB and rifampicin resistance on December 2010. Later, the WHO published a policy update on 2013 emphasizing the role of Xpert MTB/RIF in early diagnosis of extrapulmonary TB[7]. Since then, several studies and meta-analysis reports have been published assessing sensitivity and specificity of CBNAAT in pleural effusion, ranging from 15-55% and 98-100%, respectively [7-12]. This wide variation is partly due to the use of different reference standards adapted for the analysis. Hence this study is aimed to reevaluate whether Pleural fluid CBNAAT can provide an efficient means for diagnosing tuberculous pleural effusion.

SPECIFIC OBJECTIVES:

i) To detect sensitivity of CBNAAT in detection of Mycobacterium tuberculosis in suspected cases of tuberculous pleural effusion
ii) To detect Rifampicin resistance in cases of tuberculous pleural effusion

STUDY AREA: Department of Pulmonary Medicine, Burdwan Medical College, Burdwan.

STUDY PERIOD: 1 year 6 months (March 2017 to August 2018)

STUDY POPULATION:

Patients of age>12 years, having Exudative Pleural Effusion and fulfill the inclusion and exclusion criteria, attending OPD or undergoing In-patient treatment at Pulmonary Medicine Department of Burdwan Medical College Hospital, Burdwan.

INCLUSION CRITERIA:

(i) Medical History and other evidences compatible with tuberculous pleural effusion
(ii) Pleural Effusion with effusion protein/ serum protein >0.5, effusion LDH/ serum LDH > 0.6 and effusion LDH level > 2/3rd of upper limit of laboratory's reference range of serum LDH (Light's criteria)

EXCLUSION CRITERIA:

(i) Age <12 years,(ii) Patient's refusal for Pleural fluid aspiration,(iii) Transudative Pleural Effusion,(iv) Patient with chronic history of Heart Failure, Nephrotic syndrome, Cirrhosis of Liver, Pancreatitis and esophageal diseases, and malignant pleural effusion,(v) Haemothorax following trauma to the chest,(vi) Any contra-indication of thoracocentesis, (vii) Haemodynamically unstable moribund patients

STUDY TECHNIQUE:

The patients attending OPD or indoor with clinical and radiological evidence of pleural effusion were recruited for initial screening. After ruling out probable cardiac, renal or hepatic etiology from history and clinical examination, necessary consent was taken from the patient or near relatives and diagnostic thoracocentesis was performed under sonographic guidance. A minimum of 20-25 ml pleural fluid sample was aspirated and sent for physical analysis, biochemical tests (sugar, protein, LDH and ADA) and microbiological tests (Gram stain, Z-N stain, aerobic and mycobacterial BACTEC MGIT liquid culture) in tube without anticoagulant (brown topped). Pleural fluid cytology and differentials were sent in EDTA- treated vial (purple topped). Finally another 5 ml pleural fluid was sent in falcon tube for CBNAAT.

Cartridge based nucleic acid amplification test

The Xpert MTB/Rif test is a cartridge-based fully automated NAAT (nucleic acid amplification test) currently recommended by WHO 9 and adopted by revised national tuberculosis control programme run by government of India for detection of tuberculosis case and rifampicin resistance.

The test is highly specific and does not give cross reactions with any other bacterial species including a comprehensive panel of mycobacteria thereby excluding non-tubercular mycobacteria. Although molecular amplification is already a proven technology in TB diagnosis, other existing tests are too complex for routine and widespread use in field conditions at peripheral level. GeneXpert, the test device platform, was launched by Cepheid in 2004 and simplifies molecular testing by fully integrating and automating the three processes (sample preparation, amplification and detection) required for real-time PCR-based molecular testing.

Principle: The underlying principle of Xpert assay being detection MTB and rifampicin resistance by polymerase chain reaction based amplification of the 81-bp rpoB gene segment and probing for the mutations that are related to rifampicin resistance. The assay is automated and completes within 2 hours, with minimal hands-on technical time.

Procedure: The Xpert MTB/RIF test uses a cartridge containing all elements necessary for the reaction, including lyophilized reagents, liquid buffers and wash solutions. With observing aseptic technique, pleural fluid sample was collected in a falcon tube. The sample was loaded into cartridge and analyzed for presence of mycobacteria and rifampicin resistance in GX4 System (with 4 modules).

In the present study, the diagnosis of tubercular etiology was established by a composite reference standard (CRS) which was defined as any of the following.

(1) Demonstration of tubercle bacilli in the pleural fluid or any specimen collected from other body sites (including sputum) by Z-N stain, fluorescent stain, CBNAAT, line probe assay or culture. (2) Exudative (according to Light's criteria) lymphocytic pleural effusion with ADA>40 IU/L and other probable causes of pleural effusion excluded with reasonable certainty. The demonstration of either acid- fast bacilli in culture or smear was considered as a gold standard in the diagnosis of tubercular etiology.

Those who were diagnosed with tuberculous pleural effusion were started ATTs according to the RNTCP guidelines. Those who had tuberculous empyema or hydropneumothorax were treated with water- seal intercostal tube drainage along with ATTs.

The study was conducted after obtaining permission from the Institutional Ethics committee and informed consent from the patients.

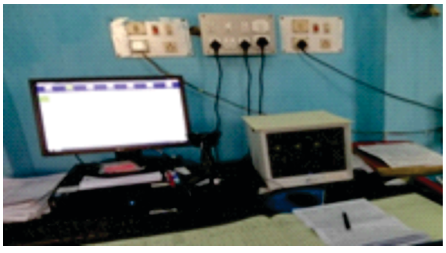
METHODS OF DATA COLLECTION

Data was collected using a pretested proforma meeting the objectives of the study. Detailed history, physical examination and necessary investigations as described were undertaken .All data were put in the excel sheet of Microsoft Windows for analysis.

PLAN FOR DATA ANALYSIS

Standard statistical method for data compilation and analysis, tables, charts, graphs and text .Software package like SPSS version 20.0 applied for statistical analysis.

RESULT



CBNAAT MACHINE

Table- 1 Age group and sex wise distribution of the study population (n=106)

Age (years)	Sex		Male		Total	
	Female					
	No. of cases	%	No. of cases	%	No. of cases	%
≤ 20	10	27.0%	8	11.6%	18	17.0%
21 – 30	10	27.0%	25	36.2%	35	33.0%
31 – 40	8	21.6%	12	17.4%	20	18.9%
41 – 50	5	13.5%	15	21.7%	20	18.9%
51 – 60	2	5.4%	6	8.7%	8	7.5%
> 60	2	5.4%	3	4.3%	5	4.7%
Total	37	100.0%	69	100.0%	106	100.0%

Table – 2 Clinical features of the study population (n=106)

Symptoms	No. of cases	Percent
Cough	96	90.6
Fever	91	85.8
SOB	67	63.2
Chest pain	34	32.1
Weight loss	18	17.0
Hemoptysis	25	23.6

Table- 3 Sputum for AFB smear examination (n=106)

Sputum for AFB	No. of cases	Percent
Negative	88	83.0
NEG--> POS	2	1.9
Not given	3	2.8
Positive	13	12.3
Total	106	100.0

Table – 4 ADA level of pleural fluid (n=106)

Pleural fluid ADA (IU/L)	No. of cases	Percent
< 40	7	6.6
40 – 70	43	40.6
> 70	56	52.8
Total	106	100.0

Table – 5 Acid fast stain results of pleural fluid (n=106)

Pleural fluid for ZN stain	No. of cases	Percent
Positive (AFB+)	15	14.2
Negative	91	85.8
Total	106	100.0

Table – 6 CBNAAT results of pleural fluid (n=106)

Pleural fluid for CBNAAT	No. of cases	Percent
MTB not detected	58	54.7
MTB detected, RIF sensitive	45	42.5
MTB detected, RIF resistant	3	2.8
Total	106	100.0

Table – 7Mycobacterial culture results of pleural fluid (n=106)

Pleural fluid for Mycobacterial culture	No. of cases	Percent
Negative	53	50.0
Positive	53	50.0
Total	106	100.0

Table – 8 Diagnostic accuracy of CBNAAT taking Mycobacterial culture as gold standard (n=106)

Sensitivity	**90.6%**
Specificity	**100.0%**
PPV	**100.0%**
NPV	**91.4%**
Area under the curve	**0.95**

DISCUSSION:

Demonstration of tubercle bacilli as well as caseating granuloma is the gold standard for the diagnosis of tuberculosis. In the parenchymal disease of pulmonary tuberculosis, simple microscopy of expectorated sputum sample can easily establish the diagnosis. On the other hand, the diagnosis of tuberculous pleural effusion often produces great difficulty as mycobacterium tuberculosis bacilli cannot be demonstrated in the pleural fluid easily. Inspite of all the efforts, it is negative in many cases of tuberculous pleural effusion due to its paucibacillary nature and etiology of pleural fluid remains undiagnosed or misdiagnosed. Clinicians usually depend on indirect evidence of tuberculosis such as, finding of lymphocytic Exudative pleural effusion with high Adenosine deaminase level for the diagnosis of tuberculous pleural effusion. Though this approach is often enough for the treatment point of view, but it is not always diagnostic, similar picture may be present in some other

diseases thus, creating diagnostic confusion. So, sometimes further confirmation of the diagnosis by direct evidence becomes a necessity.

Globally the use of GeneXpert assay has resulted in an increase in the number of positive results and this increase has been more important for the extra-pulmonary specimens especially the body fluids.

This study was planned for evaluating the role of CBNAAT in the diagnosis of tuberculous pleural effusion as not many Indian studies are available in the literature.

The mean age of the study population in our present study is 34.28 (SD=+14.16) years, which is consistent with the literature as nowadays, adults are found to be more affected by the disease [16, 17]. The mean age was 37.1 in the study by Biswas et al. [18]

Male patients were more than female in this study (Male- 65.1%, Female- 34.9%). Biswas et al. [18] found a similar pattern (Male- 66.3%, Female- 33.7%)

In this study, 14.2% cases were known diabetic patients, which corresponds with the study of Biswas et al[18] in which 13 out of 105 patients were diabetic (12.4%).

Common presenting symptoms of the study population were cough (90.6%), fever (85.8%), shortness of breath (63.2%) and chest pain (32.1%). In the study by Goyal V et al[19], fever, chest pain, cough and dyspnoea were found in 92%, 72%, 68%, 52% patients respectively.

In our study most of the cases (97.2%) had unilateral pleural effusion. In the study by Rosso F et al [11], 95.9% patients had unilateral pleural effusion.

In our study the extent of pleural effusion was moderate in maximum cases (59.4%). Rosso F et al [11], in their study found moderate pleural effusion in 60.8% cases.

In the current study, parenchymal lesion was seen in 18.9% cases in chest X-ray. In the study by Rosso F et al [11] parenchymal alteration was seen in 33% cases.

In this study, Pleural fluid analysis revealed mean cell count 1669.1. This finding is consistent with the findings of Valdes et al [20], Yam L T et al [21]

In this study, mean pleural fluid ADA was 94.02 IU/L, and most of the patients had ADA value > 70 IU/L. In the study by Patil S et al [22], mean ADA was 70.69 IU/L. Basu et al [23], reported a mean ADA 100.05 IU/L.

In our study, Pleural fluid was positive for AFB in smear examination in 15 cases (14.2%). Biswas et al [18] in their study found that 8.6% cases were AFB smear positive.

CBNAAT detected presence of Mycobacterium tuberculosis bacilli in pleural fluid in 48 out of 106 cases (45.3%). Among them 45 cases (42.5%) were Rifampicin sensitive and in three cases (2.8%), Rifampicin resistance was detected. In the results of the study by Patil S et al, 74% cases were DNA PCR positive. However in the study by Biswas et al [18], CBNAAT detected MTB in pleural fluid in 16 out of 105 cases (15.23%). In the study by Goyal V et al [19], 21 out of 146 cases (14.4%) were positive in the Xpert MTB assay.

Mycobacterial culture was taken as gold standard for assessment of diagnostic accuracy of CBNAAT in our study. According to this reference, the sensitivity and specificity of CBNAAT in pleural fluid came 90.6% and 100.0% respectively.

The comparison of the sensitivity and specificity of CBNAAT in the diagnosis of tuberculous pleural effusion is shown in Table- 8. The results vary widely depending upon the reference standard adapted for analysis.

Sehgal *et al.* published a meta-analysis report compiling the list of sensitivity and specificity of CBNAAT of pleural fluid considering culture and CRS as reference standard from different studies since year 2010.[8] The pooled sensitivity was found to be more when culture positivity was used as benchmark compared to CRS (51.4% in culture sub group and 22.7% in CRS subgroup), whereas specificity was almost same (98.6% in culture group and 99.8% in CRS subgroup).5 The definition of CRS was very different in different studies [11, 23,21,26].

Although the sensitivity and specificity of CBNAAT are 90.6% and 100% in the present study, it has to be kept in mind that the calculation was made with mycobacterial culture taken as gold standard. The overall positivity of CBNAAT in pleural fluid is 45.3% among the cases diagnosed as tuberculous pleural effusion on the basis of the CRS. So, in patients with negative CBNAAT test, tuberculous pleural effusion cannot be ruled out with a high degree of certainty. Conversely, the satisfactory PPV of this test substantiates the immediate start of appropriate antitubercular therapy in cases of positive testing, without any delay.

However in the hydropneumothorax population, pleural fluid CBNAAT was able to detect MTB in 42 out of 43 cases (97.7%). So, CBNAAT can be considered as a rapid and confirmatory diagnostic tool in case of suspected tubercular hydropneumothorax when compared with conventional culture and drug sensitivity tests. However, the sample size was too small, so the result needs to be validated in a larger patient population.

LIMITATIONS:

- Hospital based study –so, the study was limited to the patients who came to the hospital
- Duration of study was only one and half year
- Being a tertiary care hospital many cases were already been initiated treatment before coming to the hospital
- Number of study subjects were only which could have been better validated in a larger patient population.
- Pleural biopsy and culture of the biopsy materials could not be considered due to logistic reasons which might have improved the diagnostic yield.

CONCLUSION:

Although CBNAAT is considered a breakthrough in the diagnosis of TB and EPTB, one of the major limitations of this technique is that it cannot distinguish between viable and non-viable micro-organisms while detecting MTB DNA and should not be used to monitor patients or efficacy of the treatment.

In this study, the sensitivity, specificity, positive predictive value and negative predictive value of CBNAAT in pleural fluid were 90.6%, 100%, 100% and 91.4% respectively considering mycobacterial culture as the gold standard.

The study findings suggested that CBNAAT should be considered in the routine diagnostic work up in suspected cases of tuberculous pleural effusion as time factor has a very crucial role in its laboratory diagnosis. CBNAAT had also the added advantage of detection of Rifampicin resistance.

REFERENCES:

1. Koneman's colour atlas and text book of Diagnostic Microbiology 6th edition pg:1066.
2. www.who.int/tuberculosis/publications/global_report (http://www.who.int/tuberculosis/publications/global_report)
3. Tuberculosis India 2016 Revised National Tuberculosis Control Programme

Annual Status Report, New Delhi.
4. Gopi A, Madhavan SM, Sharma SK, Sahn SA (2007) Diagnosis and treatment of tuberculous pleural effusion in 2006. Chest 131: 880889.
5. Sinzobahamvya N, Bhakta HP (1989) Pleural exudate in a tropical hospital. Eur Respir J 2: 145148.
6. Maartens G, Bateman ED. Tuberculous pleural effusions: increased culture yield with bedside inoculation of pleural fluid and poor diagnostic value of adenosine deaminase.Thorax 1991;46:96-9.
7. Xpert MTB/RIF Implementation Manual: Technical and Operational 'How-To'; Practical Considerations. WHO Guidelines Approved by the Guidelines Review Committee. Geneva: World Health Organization; 2014.
8. Sehgal IS, Dhooria S, Aggarwal AN, Behera D, Agarwal R. Diagnostic performance of Xpert MTB/RIF in tuberculous pleural effusion: Systematic review and meta-analysis. J Clin Microbiol 2016;54:1133-6.
9. James P, Gupta R, Christopher DJ, Balamugesh T. Evaluation of the diagnostic yield and safety of closed pleural biopsy in the diagnosis of pleural effusion. Indian J Tuberc 2010;57:19-24.
10. Solooki M, Miri M. Approach to undiagnosed exudative pleural effusion: The diagnostic yield of blind pleural biopsy. Caspian J Intern Med 2013;4:642-7.
11. Rosso F, Michelon CT, Sperhacke RD, Verza M, Olival L, Conde MB, et al. Evaluation of real-time PCR of patient pleural effusion for diagnosis of tuberculosis. BMC Res Notes 2011;4:279.
12. Rufai SB, Singh A, Kumar P, Singh J, Singh S. Performance of Xpert MTB/RIF Assay in diagnosis of pleural tuberculosis by use of pleural fluid samples. J Clin Microbiol 2015;53:3636-8.
13. Light RW. Update on tuberculous pleural effusion. Respirology 2010;15:451-8. 10.1111/j.1440-1843.2010.01723.x [PubMed] [Cross Ref]
14. Wang XJ, Yang Y, Wang Z, et al. Efficacy and safety of diagnostic thoracoscopy in undiagnosed pleural effusions. Respiration 2015;90:251-5. 10.1159/000435962 [PubMed] [Cross Ref]
15. Prabhu VG, Narasimhan R. The role of pleuroscopy in undiagnosed exudative pleural effusion. Lung India 2012;29:128-30. 10.4103/0970-2113.95304 [PMC free article] [PubMed] [Cross Ref]
16. Moudgil H, Sridhar G, Leitch AG. Reactivation disease: the commonest form of tuberculous pleural effusion in Edinburgh, 1980ae1991. Respir Med 1994; 88:301-304
17. Epstein DM, Kline LR, Albelda SM, Miller WT. Tuberculous pleural effusion. Chest 1987; 91:106
18. Biswas D, Mukherjee S, Begum S, Paul A, Ghosh P, Sarkar S. Role of Cartridge-based Nucleic Acid Amplification Test in Diagnosis of Tuberculous Pleural Effusion Compared to Tuberculous Empyema in HIV-seronegative Patients. International Journal of Scientific Study | September 2017 | Vol 5 | Issue 6: 125-129
19. Goyal V, Agrawal Y, Tank R, Singh A, Mondal A Role of Gene Xpert in Diagnosis of Tuberculous Pleural Effusion, Scholars Journal of Applied Medical Sciences (SJAMS) 2017; 5(11C):4467-4471
20. Valdes L, Alvarez D, Son Jose E et al. Tuberculous pleurisy: a study of 254 patients. Arch Intern Med 1998; 158:2017-2021
21. Yam LT. Diagnostic significance of lymphocytes in pleural effusions. Ann Intern Med 1967; 66:972-982
21. Patil S, Gajanan H, Rujuta A. Role of Nucleic Acid Amplification Tests (NAATs) in Tuberculous Pleural Effusion: Where It Fits In Routine Diagnostic Workup?, Journal of Cell Science & Therapy 2016
22. Basu A, Chakrabarti I, Ghosh N, Chakraborty S (2012) A clinicopathological study of tuberculous pleural effusion in a tertiary care hospital. Ann Trop Med Public Health 5:168¬172.
23. Porcel JM, Palma R, Valdés L, Bielsa S, San-José E, Esquerda A. Xpert® MTB/RIF in pleural fluid for the diagnosis of tuberculosis. Int J Tuberc Lung Dis 2013;17:1217-9.
24. Du J, Huang Z, Luo Q, Xiong G, Xu X, Li W, et al. Rapid diagnosis of pleural tuberculosis by Xpert MTB/RIF assay using pleural biopsy and pleural fluid specimens. J Res Med Sci 2015;20:26-31.
25. Rufai SB, Singh A, Kumar P, Singh J, Singh S. Performance of Xpert MTB/RIF Assay in diagnosis of pleural tuberculosis by use of pleural fluid samples. J Clin Microbiol 2015;53:3636-8.
26. Vadwai V, Boehme C, Nabeta P, Shetty A, Alland D, Rodrigues C. Xpert MTB/RIF: A new pillar in dia gnosis of extrapulmonary tuberculosis? J Clin Microbiol 2011;49:2540-5.