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Original Research Article

Diagnostic utility of cartridge based nucleic acid amplification tests in tubercular pleural effusions

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Abstract

Objective: To assess the utility of Cartridge Based Nucleic Acid Amplification Test in diagnosing tubercular pleural effusions.
Methods: 43 patients who attended Respiratory medicine OPD from year 2019- 2021were diagnosed as cases of tubercular pleural effusion based on clinical suspicion, radiology, biochemical and pathological investigations of pleural fluid and response to anti TB treatment. Pleural fluid sample of all patients was also subjected for CBNAAT test.
Results: Out of the 43 patients, only 4 (9.3%) patients had CBNAAT positive results. None of the CBNAAT positive had Rifampicin resistance.
Conclusion: In this study we concluded that the diagnostic value of Cartridge Based Nucleic Acid Amplification Test in tubercular pleural effusion is limited due to the low bacillary load of the effusion. Hence the initiation of anti-tubercular treatment for such cases should be based mainly on symptoms, clinical signs along with various biochemical, pathological and microbiological tests of pleural fluid.
Keywords: CBNAAT, Tuberculosis, Extrapulmonary TB, Diagnosis, Pleural effusion.

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1. Introduction

Tuberculosis is one of the leading causes of morbidity and mortality in India. Pulmonary tuberculosis accounts for the maximum number of cases followed for tuberculosis of the lymph node and tubercular pleural effusion. About 25% of active tuberculosis cases are due to an extra pulmonary cause. World Health Organization recommends the use of Cartridge Based Nucleic Acid Amplification tests for the rapid diagnosis of tuberculosis. It also forms a major part of the diagnostic protocol proposed by the Revised National Tuberculosis Programme. [1,2] However, little is known about its efficacy as a "routine to do test" in diagnosing extra pulmonary tuberculosis.[3]

Cartridge Based Nucleic Acid Amplification Test (CBNAAT) is fully automated Nucleic Acid Amplification test which satisfies the criteria of "easy", "rapid" and "affordable". It provides results in 2 hours thus making same day diagnosis possible and helps in rapid initiation of treatment.[4] It uses a cartridge based system in which lysis, purification amplification and detection of the DNA occurs by real time polymerase chain reaction. It amplifies the targeted nucleic acid and also identifies the Rpo B gene which is associated with Rifampicin resistance. Another advantage of CBNAAT is there is no cross reaction with other mycobacteria especially non tubercular mycobacteria. This is because the processing of the sample occurs in a closed chamber. The staff training required for the processing and loading of the samples is also less. Thus making CBNAAT an excellent choice for diagnosis of tuberculosis.[5,6]

Indications of CBNAAT:

1) Suspected cases of tuberculosis.
2) Suspected cases of MDR tuberculosis.

3) People living with HIV.
4) Sputum smears negative cases of tuberculosis.

2. Material and Methods

2.1 Study Duration:

The study was conducted from October 2019 to October 2021.

2.2 Source of patient:

43 patients of clinically diagnosed tubercular pleural effusion visiting outpatient department or admitted under Department of Respiratory Medicine at MGM Medical College and Hospital, Aurangabad were included in the present study.

2.3 Selection of participants:

Clinically and radiologically diagnosed cases of tubercular pleural effusion fulfilling inclusion and exclusion criteria were enrolled till the desirable sample size was achieved.

2.3.1 Inclusion criteria:

1) Patients with a history compatible with tubercular pleural effusion such as presence of cough, fever, breathlessness, chest pain, loss of appetite, loss of weight.
2) Chest Radiograph / CT Chest suggestive of pleural effusion.
3) Pleural fluid cytology suggestive of exudative fluid, lymphocytic predominance and absence of malignant cells.

2.3.2 Exclusion criteria:

1) Patients not willing for the study.
2) Patients who did not respond to six months of standard anti tubercular treatment.
3) Transudative pleural effusions.

2.4 Study Procedure:

Informed written consent was obtained from all participants of the study. All participants were explained about the study objectives and procedure in their own language. After enrolling as per inclusion & exclusion criteria a brief history was taken from each patient regarding demographic data like age, clinical data with symptoms, comorbidities.

After clinical examination and taking written and informed consent, patients were subjected to thoracocentesis. Pleural fluid samples were sent for various investigations like Routine microscopy and cytology, Pleural fluid pH, protein and LDH. Serum protein and serum LDH were also done. All patients fulfilling Light's criteria for exudative pleural effusion were further subjected to CBNAAT.

The diagnosis of Tubercular Pleural Effusion was made by clinical examination, radiological presentation and results of pleural fluid. All patients were started on anti-tubercular therapy and were followed up with a chest X-ray to document the response to anti-tubercular therapy at the end of six months.

Presence of all of the following criteria was adopted to label a case as tubercular pleural effusion:

1) Clinical presentation consistent with TB with the exclusion of other clinical considerations
2) Exudative (according to Light's criteria), lymphocytic pleural effusion.
3) Definite clinical and radiological improvement after 6 months of administration of anti-tubercular treatment.

3. Observation and Results

Table 1: Distribution of cases according to pleural fluid CBNAAT report

Sr. No.	Pleural fluid CBNAAT	Number of Cases (N)	Percentage (%)
1	**Positive**	4	9.3 %
2	**Negative**	39	90.7 %
Total		**43**	**100 %**

Table 1 shows distribution of cases according to pleural fluid CBNAAT results. Amongst total 43 (100 %) cases CBNAAT positive results were seen in only 4 (9.3 %) cases. Rest of the patients had a CBNAAT negative result (90.7%)

Graph 1: Distribution of cases according to Pleural fluid CBNAAT report

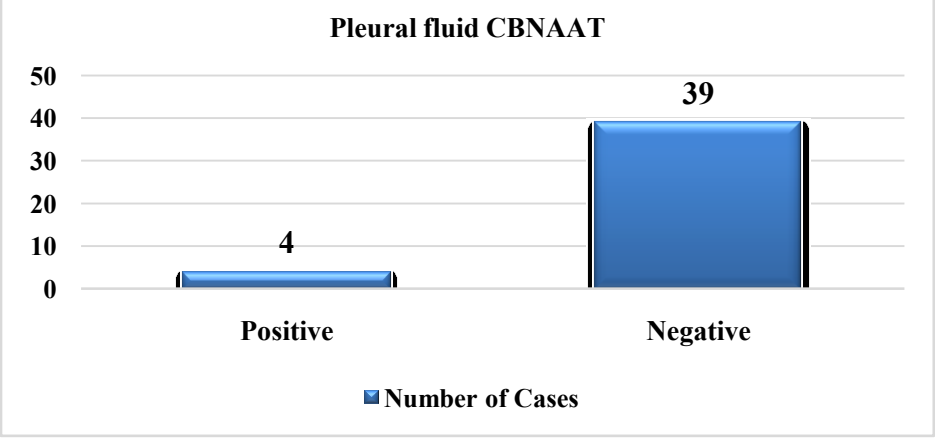


Table 2: Distribution of Cases according to Age

Sr. No.	Age group (Years)	CBNAAT Positive N (%)	CBNAAT Negative N (%)	Total N (%)
1	**< 20**	1 (2.33 %)	6 (13. 95 %)	7 (16.28 %)
2	**21 to 40**	1 (2.33 %)	24 (55.81 %)	25 (58.14 %)
3	**41 to 60**	1 (2.33 %)	5 (11.63 %)	6 (13.95 %)
4	**> 61**	1 (2.33 %)	4 (9.30 %)	5 (11. 63 %)
Total		**4 (9.3 %)**	**39 (90.7 %)**	**43 (100 %)**

Table 2 shows distribution of cases according to age. Amongst CBNAAT positive cases 1 (2.33%) was from <20 years, 21-40 years, 41-60 years and > 61 years age groups each. Amongst CBNAAT negative maximum i.e. 24 (55.81 %) cases were from 21 to 40 years, 6 (13.95 %) were from <20 years, 5 (11. 63 %) were from 41-60 years age group and 4 (9.30 %) were from > 61 years age group. Overall 21 to 40 age group had most frequently developed pleural effusion out of the total 43 patients.

Graph 2: Distribution of cases according to age

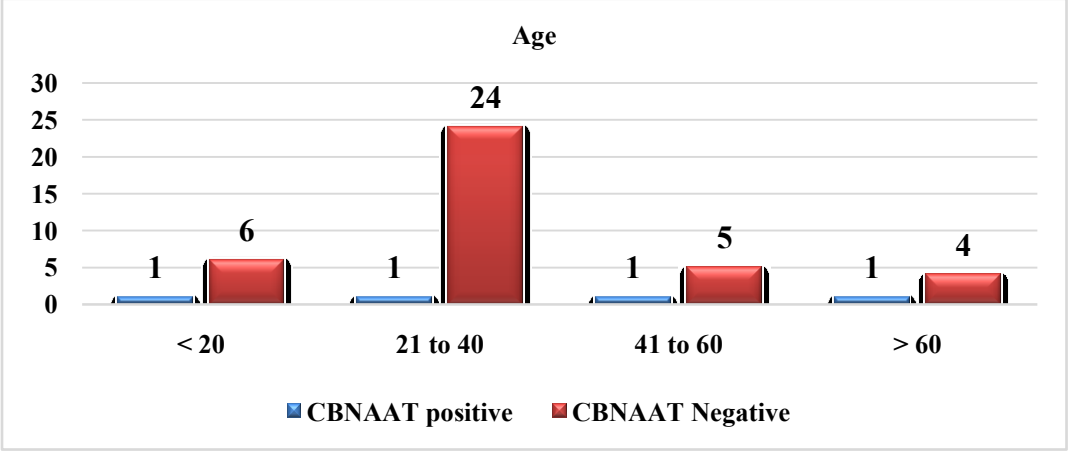


Table 3: Distribution of cases according to sex

Sr. No.	Sex	CBNAAT Positive N (%)	CBNAAT Negative N (%)	Total N (%)
1	**Male**	4 (9 %)	26 (60.46 %)	**30 (69.76 %)**
2	**Female**	0 (0 %)	13 (30.23 %)	**13 (30.23 %)**
Total		**4 (9.3 %)**	**39 (90.7 %)**	**43 (100 %)**

Table 3 shows distribution of cases according to sex. All CBNAAT positive cases were males whereas amongst CBNAAT negative cases 26 (60.46 %) were males and 13 (30.23 %) were females.

Graph 3: Distribution of Cases according to Sex

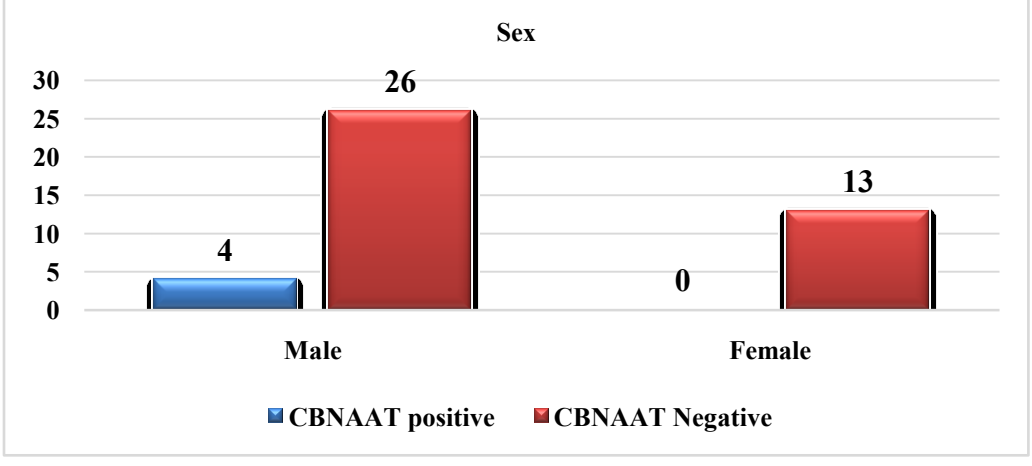


Table 4: Distribution of cases according to symptoms

Sr. No.	Symptoms	CBNAAT Positive N (%)	CBNAAT Negative N (%)	Total N (%)
1	Cough	3 (75%)	30 (76.92%)	33 (76.74%)
2	Expectoration	(0%)	4 (10.26%)	4 (9.3%)
3	Breathlessness	2 (50%)	20 (51.28%)	22 (51.16%)
4	Chest pain	4 (100%)	30 (76.92%)	34 (79.07%)
5	Fever	3 (75%)	38 (97.43%)	41 (95.3%)
6	Loss of weight	2 (50%)	12 (30.77%)	14 (32.56%)
7	Loss of appetite	4 (100%)	25 (64.1%)	29 (67.44%)
8	Other	(0%)	7 (17.95%)	7 (16.28%)

Table 4 shows distribution of cases according to symptoms. Amongst CBNAAT positive cases breathlessness and loss of weight was present in 2 (50%) of cases, fever and cough were present in 3 cases (75%) and loss of appetite and chest pain was present in all 4 (100%) of cases. Expectoration was absent in all 4 (100%) cases. Whereas in CBNAAT negative cases fever was the predominant complaint and was present in 38 (97.43%) cases followed by cough in 30 (76.92%) cases and chest pain 30 (76.92%). Expectoration was present in only 4 (10.26%) CBNAAT negative cases. Breathlessness and loss of appetite were less frequent and present in 20 (51.28%) cases and 25 (64.1%) cases respectively. Other symptoms such as generalized weakness, malaise, etc. were seen in 7 (16.28%) of the total cases. Out of the total 43 cases, fever was the most common presenting symptom present in 41 (95.3%) cases.

Graph 4: Distribution of cases according to symptoms

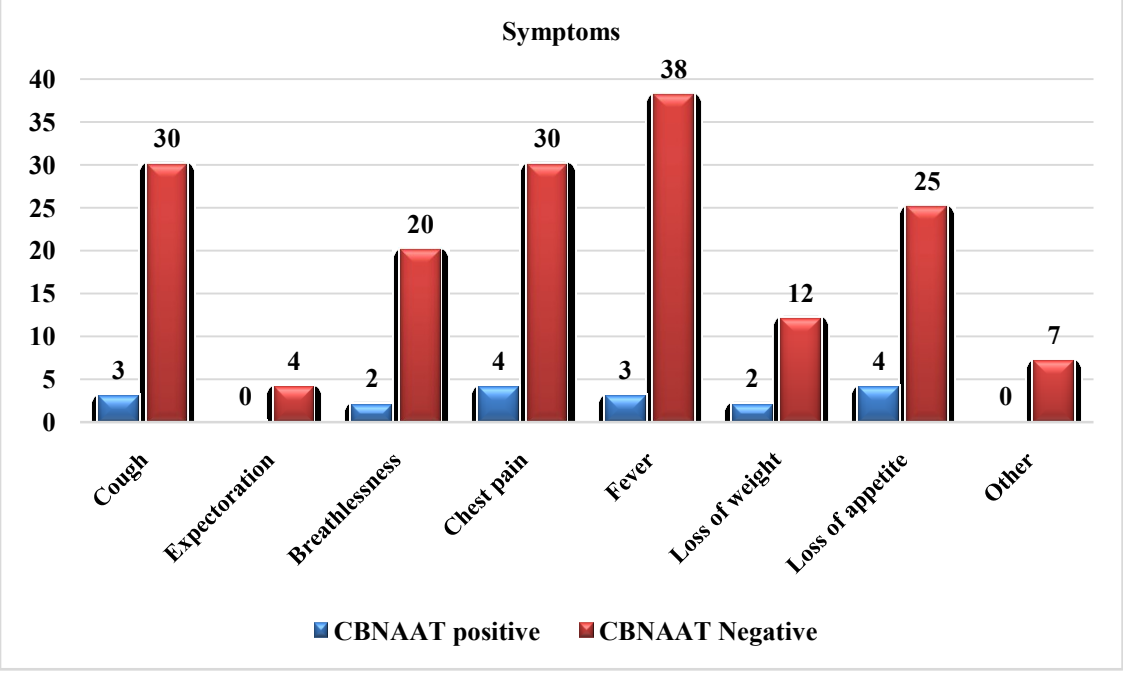


Table 5: Distribution of cases according to pleural fluid parameters

Sr. No.	Pleural fluid parameters	CBNAAT Positive (N=4) Mean ± SD	CBNAAT Negative (N=39) Mean ± SD	P Value
1	Protein (g/dl)	5 ± 0.57	5.3 ± 0.68	0.3994
2	Glucose (mg%)	56.3 ± 13.05	75.8 ± 37.3	0.3070
3	WBC	1192.5 ± 681.83	1253.8 ± 992.95	0.9050
4	Lymphocyte (%)	85 ± 10	88.1 ± 11.78	0.6177
5	Polymorphs (%)	15 ± 10	7.7 ± 7.65	0.0846
6	RBC (/hpf)	11.4 ± 5.94	11.4 ± 8.13	0.9979
7	pH	7.5 ± 0.0	7.6 ± 0.23	0.2279
8	LDH	730 ± 174.93	723.5 ± 482.58	0.9790

Table 6 shows distribution of cases according to pleural fluid parameters. Protein (g/dl) Mean ± SD amongst CBNAAT positive cases was 5 ± 0.57 and amongst CBNAAT negative was 5.3 ± 0.68. Glucose (mg%) Mean ± SD amongst CBNAAT positive cases was 56.3 ± 13.05 and amongst CBNAAT negative was 75.8 ± 37.3. WBC Mean ± SD amongst CBNAAT positive cases was 1192.5 ± 681.83 and amongst CBNAAT negative was 1253.8 ± 992.95. Lymphocyte (%) Mean ± SD amongst CBNAAT positive cases was 85 ± 10 and amongst CBNAAT negative was 88.1 ± 11.78. Polymorphs (%) Mean ± SD amongst CBNAAT positive cases was 15 ± 10 and amongst CBNAAT negative was 7.7 ± 7.65. RBC (/hpf) Mean ± SD amongst CBNAAT positive cases was 11.4 ± 5.94 and amongst CBNAAT negative was 11.4 ± 8.13. pH Mean ± SD amongst CBNAAT positive cases was 7.5 ± 0.0 and amongst CBNAAT negative was 7.6 ± 0.23. LDH Mean ± SD amongst CBNAAT positive cases was 730 ± 174.93 and amongst CBNAAT negative was 723.5 ± 482.58.

Graph 5: Distribution of cases according to pleural fluid parameters

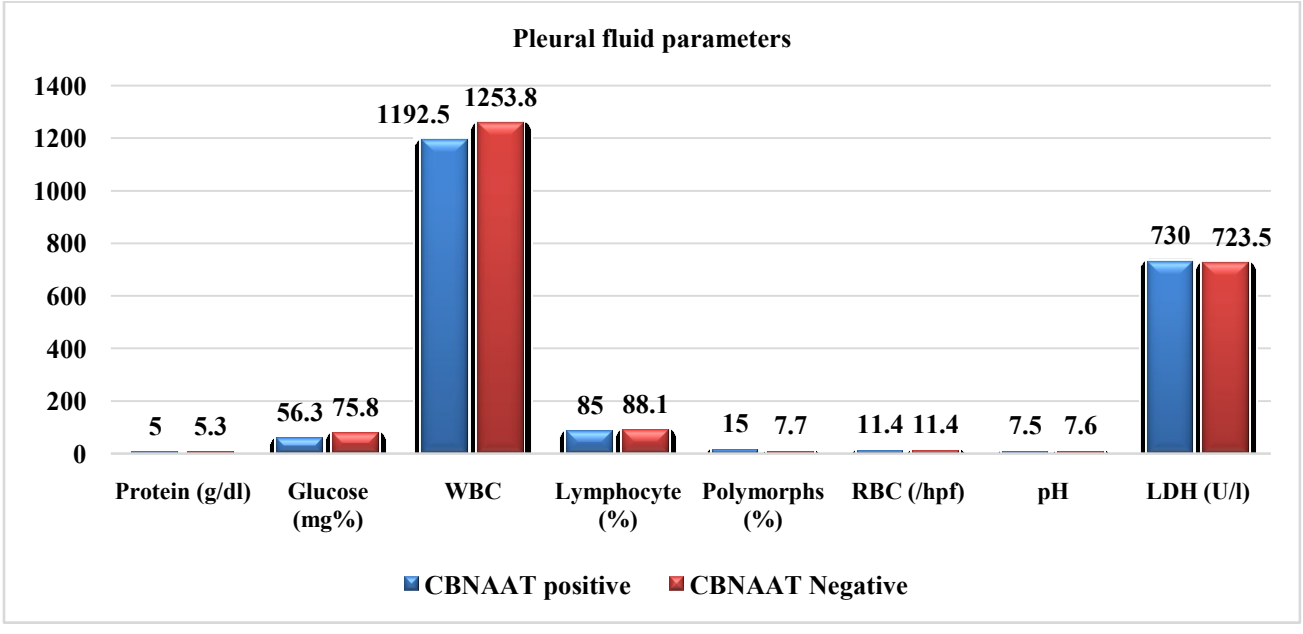


Table 6: Distribution of cases according to Rifampicin resistance

Sr. No.	Rifampicin resistance	Number of Cases (N)	Percentage (%)
1	Positive	0	0 %
2	Negative	4	100 %
Total		4	100 %

Table 8 shows distribution of cases according to Rifampicin resistance. Amongst the 4 CBNAAT positive cases all were negative for Rifampicin resistance.

Graph 6: Distribution of cases according to Rifampicin resistance

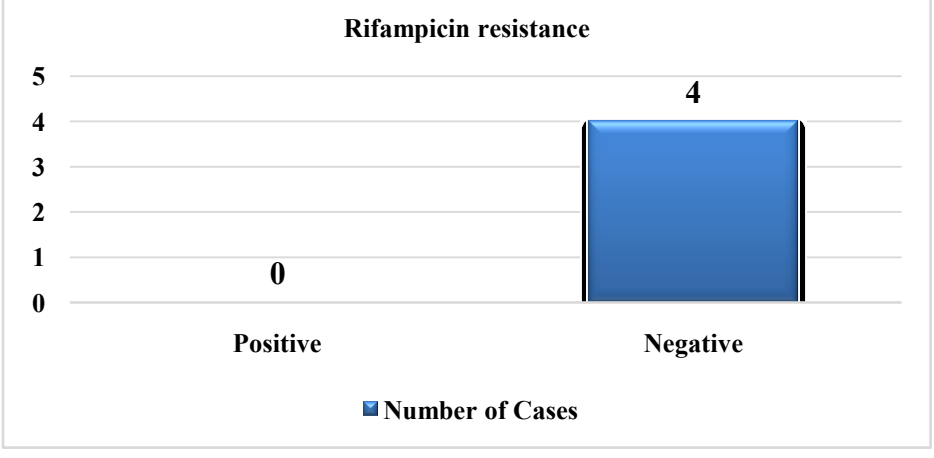
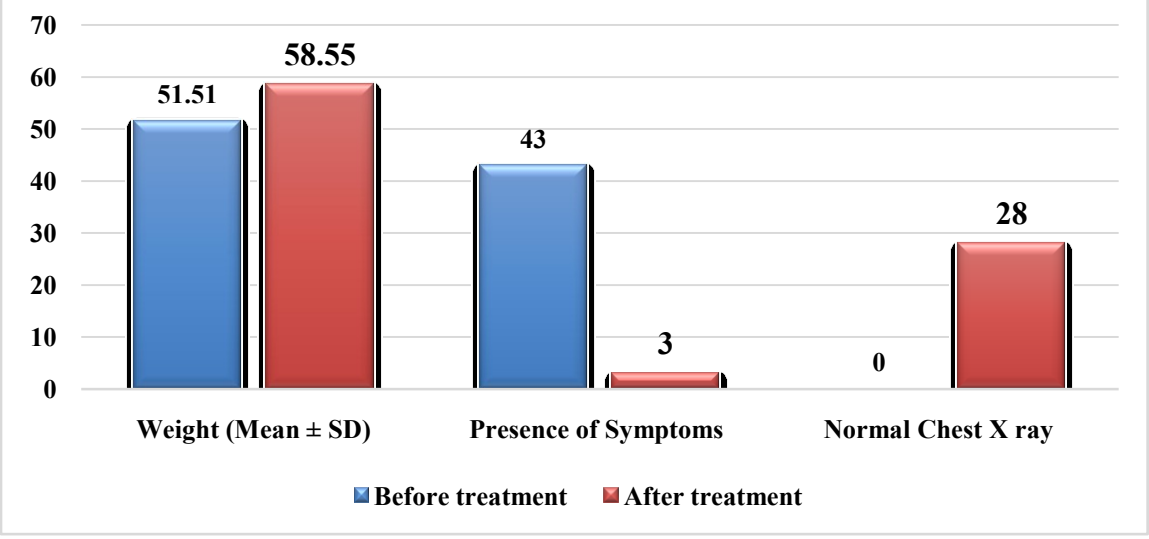


Table 7: Distribution of cases according to treatment outcome

Sr. No.	Parameters	At start of treatment	After 6 months of treatment
1	Weight (Mean ± SD)	51.5 ± 7.9	58.5 ± 7.06
2	Presence of Symptoms N (%)	43 (100 %)	3 (7 %)
3	Normal Chest X ray N (%)	0 (0 %)	28 (65.1 %)
Total		43 (100 %)	43 (100 %)

Table 9 shows distribution of Cases according to treatment outcome. Weight Mean ± SD at start of treatment was 51.5 ± 7.9 and after 6 months of treatment was 58.5 ± 7.06. Symptoms improvement was present in 40 (93 %) cases whereas 3 (7 %) cases still had mild and non-specific symptoms like vague chest pain, weakness, etc. At the end of treatment chest X ray turned normal in 28 (65.1 %) whereas 15 (34.88 %) still showed residual changes like minimal pleural thickening, rib crowding, CP angle blunting, etc.

Graph 7: Distribution of Cases according to treatment



4. Discussion

Pleural effusion indicates pathology caused due to the imbalance between formation and reabsorption of pleural fluid. It is one of the most commonly encountered diseases for physicians worldwide According to the global TB report about 10.0 million people were infected with the disease in 2018 out of which India had a very high incidence. Tuberculosis can be seen in almost any organ of the body but is most commonly found as pulmonary tuberculosis. This is followed by lymph node tuberculosis and tubercular pleural effusion which predominate the causes of Extra Pulmonary Tuberculosis (EPTB). Major diagnostic problem of EPTB is the presence of diverse presentation and the inability of standardized laboratory methods to detect it. Due to this delayed detection of Tuberculosis, problems arise in its management especially when associated with drug resistance. In a country like India where the burden of tuberculosis is high, the most likely cause of exudative lymphocyte-predominant effusions may be Tuberculosis. CBNAAT seems to be a simple and rapid laboratory test especially after its easy and free availability under the RNTCP programme. It may thus help to diagnose tuberculosis as a cause of pleural effusion quickly and with ease. With this objective present study was undertaken in tubercular pleural effusion patients and pleural fluid CBNAAT was performed to look for its efficacy as a single diagnostic test.

Results obtained in this study are summarized as

- Amongst a total of 43 cases, Pleural fluid CBNAAT result was positive in only 4 (9.3 %) cases.
- Maximum were having right sided pleural effusion i.e. 27 (62.79 %) followed by left 12 (27.90 %) and bilateral 4 (9 %)
- Mean ± SD for age was 34.88 ± 15.57 and male: female ratio was 2.30.
- The most common complaint at the time of presentation was fever (95.3%) followed by chest pain (79.07%). This was followed by cough (76.74%) which was predominantly dry, loss of appetite (67.44%), breathlessness (51.16%) and loss of weight (32.56) in that order.
- Mean ± SD for Protein (g/dl), Glucose (mg%), WBC, Lymphocyte (%), Polymorphs (%), RBC (/hpf), pH and LDH (U/l) didn't find statistically significant association with CBNAAT result.
- Mean ± SD for serum protein (g/dl) and LDH (U/l), also didn't find statistically significant association with CBNAAT result.
- Amongst CBNAAT Positive cases i.e. 4 (9 %) all were negative for Rifampicin resistance.

- Weight Mean ± SD At start of treatment was 51.5 ± 7.9 and after 6 months of anti-tubercular treatment was 58.5 ± 7.06.
- Symptoms improvement was present in 40 (93 %) cases whereas 3 (7 %) cases still had mild and non-specific symptoms like vague chest pain, weakness, etc.
- At the end of treatment chest X ray turned normal in 28 (65.1 %) whereas 15 (34.88 %) still showed residual changes like minimal pleural thickening, rib crowding, CP angle blunting, etc.

5. Conclusion

Cartridge Based Nucleic Acid Amplification Test (CBNAAT) is a very rapid, fairly specific diagnostic test for pulmonary tuberculosis. It is recommended by World Health Organization as the diagnostic test for pulmonary tuberculosis. However we concluded in our study that its role in the diagnosis of tubercular pleural effusion is limited. This is mainly due to the pauci-bacillary nature of the disease. Only advantage in CBNAAT detected tubercular pleural effusions is that of detecting Rifampicin resistance. Low and variable sensitivity of pleural fluid CBNAAT should probably make us reconsider about the decision to routinely include it as a part of diagnostic protocol. No test available at present can be used alone as a single diagnostic test for tubercular pleural effusions. Thus clinical and radiological suspicion of tubercular pleural effusions combined with various biochemicals, microbiological and pathological tests can be used to diagnose tubercular pleural effusions and initiate treatment.

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