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DIAGNOSTIC VALUE OF BRONCHOALVEOLAR LAVAGE CB-NAAT IN SPUTUM SMEAR NEGATIVE SUSPECTED PULMONARY TUBERCULOSIS

Pulmonary Medicine

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ABSTRACT

Objective: To study role of bronchoalveolar lavage CBNAAT in sputum smear negative pulmonary tuberculosis patient and to evaluate the diagnostic yield of Bronchoalveolar lavage and Bronchoalveolar lavage CB-NAAT in microbiological confirmation of sputum smear negative clinically and/or radiologically suspected pulmonary TB patients. **Methods:** Total 50 patients of clinically and radiologically suspected pulmonary tuberculosis having negative sputum smear for AFB were taken into study. **Results:** Bal CB-NAAT was positive in all TB patients, Bal AFB smear and CB-NAAT was positive in 20% of TB patients. In Non-TB patients Bal pyogenic culture was positive in 42% of patients. Bal CB-NAAT was diagnostic for TB in 31 patients. Out of these patients, Rifampicin was sensitive in 13 patients with history of TB and in 17 patients with absent past history of TB. Only 1 patient had rifampicin resistance with the history of TB. **Conclusion:** BAL CB-NAAT is useful in diagnosing suspected Pulmonary Tuberculosis patients whose sputum smear is negative on repeated occasions and chest Xray is suggestive of findings suggestive of TB. It also helps in microbiological confirmation of TB, early diagnosis of MDR cases and in initiating early treatment of TB. Bronchoalveolar lavage also helps in ruling out non-TB conditions like bacterial pneumonia, fungal pneumonia, malignancy, etc.

KEYWORDS

Pulmonary Tuberculosis, Bronchoalveolar lavage, Bronchoalveolar lavage CB-NAAT, AFB smear, symptoms.

INTRODUCTION:

Pulmonary tuberculosis [TB], caused by Mycobacterium tuberculosis is a major public concern world-wide. The global burden of tuberculosis is enormous. More than 9 million new mycobacterium tuberculosis (MTB) cases and 1.7 million deaths occur annually worldwide.[1] It is a communicable disease that is a major cause of ill health and one of the leading causes of death worldwide. Until the coronavirus (COVID-19) pandemic, TB was the leading cause of death from a single infectious agent, ranking above HIV/AIDS[2]. Every year, 10 million people fall ill with tuberculosis. Despite being a preventable and curable disease, 1.5 million people die from TB each year – making it the world's top infectious killer[2].

Reduced access to TB diagnosis and treatment has resulted in an increase in TB deaths. Best estimates for 2020 are 1.3 million TB deaths among HIV-negative people (up from 1.2 million in 2019) and an additional 214000 among HIV-positive people (up from 209000 in 2019), with the combined total back to the level of 2017[2]. Actions to mitigate and reverse these impacts are urgently required.

The disease typically affects the lungs (pulmonary TB) but can affect other sites also. TB is curable and preventable. Microbiological diagnosis is the main stay for the effective treatment of Pulmonary TB. The microbiological detection of Pulmonary TB bacilli allows patient to be currently diagnosed and started on the most effective regimen as early as possible. To confirm Pulmonary TB, WHO recommended sputum-smear examination to detect AFB by Acid Fast staining or Zeihl-Neelson staining (ZN stain), which is a highly specific, low cost and appropriate test. To label a properly expectorated sputum sample as positive for acid fast bacilli (AFB), the bacillary population has to be at least 10,000 per milliliter.

Sometimes smear may be negative even in advanced disease due to immunosuppression, poor quality of sample collection, deficient preparation, staining or examination of the sputum-smear. Difficulties arise when a patient who is suspected of active Pulmonary TB both clinically and radiologically, doesn't produce sputum. Published studies show that, about half of the active Pulmonary TB patients may fail to expectorate enough sputum, or when it is available, AFB may be negative. The transmission rate of smear negative Pulmonary TB cases compared to smear positive Pulmonary TB cases is reported as 22%[3]. Repeated sputum-smear negative for AFB has several disadvantages such as failure of therapy in case of multi-drug resistant tuberculosis (MDR-TB) and delay in reaching the correct diagnosis. Previous studies suggest that, more than 50% of smear negative patients would need chemotherapy, if left untreated.

In such situations, clinicians have to start Anti- TB treatment especially if clinical and radiological suspicion is high. But the drawback is that, most clinical features of Pulmonary TB and abnormalities on chest x-ray or histology results generally associated with Pulmonary Tuberculosis have low specificity, which may lead to false diagnosis of Pulmonary TB, and hence to people being wrongly enrolled on Anti-TB treatment. These people get unnecessarily exposed to potentially toxic drugs for prolonged duration and economic burden and are often misdiagnosed due to the limitations of conventional diagnostic techniques. Alarming increases in such cases, documented transmission, and rapid morbidity and mortality in these patients have highlighted the urgent need for rapid diagnostic methods.

No single diagnostic test currently satisfies all the demands of "rapid", "affordable", and "easy". The World Health Organization (WHO) has endorsed the use of commercially available liquid culture systems and molecular line probe assays (LPAs) to rapidly detect MDR-TB; however, due to the tests' complexity and cost, and need for sophisticated laboratory infrastructure and trained personnel, uptake has been limited in many resource-constrained settings[1]

The development of the Xpert® MTB/RIF assay for the GeneXpert platform was completed in 2009 and is considered an important breakthrough in the fight against TB. For the first time, a molecular test is simple and robust enough to be introduced and used outside conventional laboratory settings. Xpert MTB/RIF detects M. tuberculosis as well as mutation that confer rifampicin resistance using three specific primers and have unique molecular probes to ensure a high degree of specificity[4].CBNAAT is a Genotypic/Molecular method for the diagnosis of Tuberculosis. CBNAAT or Gene Xpert MTB/RIF is useful for rapid diagnosis and has the potential to replace microscopy as first-line diagnostic test. In additional it allows rapid screening of rifampicin resistance[5,6]. It is a real-time Polymerase Chain Reaction (PCR) assay designed for the rapid and simultaneous detection of Mycobacterium TB and Rifampicin resistance within 2 hours[1,7,8].

The World Health Organization (WHO) recommended the use of Xpert® MTB/RIF and Xpert MTB/RIF Ultra (Ultra) (Cepheid, Sunnyvale, United States of America [USA]) for the detection of TB and rifampicin-resistant TB in 2010 and 2017, respectively[9]. Both tests are widely implemented as initial tests for patients with presumptive TB and are performed on GeneXpert instruments with 6-colour optics. In 2021, WHO recommended the class of low complexity automated nucleic acid amplification tests (NAATs) to detect resistance to amikacin, ethionamide, fluoroquinolones and isoniazid[10].The first-in-class test is the Xpert MTB/XDR (Cepheid, Sunnyvale, USA). In contrast to the Xpert MTB/RIF and Ultra, this test requires an instrument with 10- colour optics and cannot be performed on the existing 6-colour instrument systems.

Thus, diagnosis based on clinical and radiological suspicion without bacteriological confirmation can lead to either under or over diagnosis. Therefore, samples other than sputum can play an important role in clinically and radiologically suspected, sputum-smear negative

Pulmonary Tuberculosis. A number of studies confirm the usefulness of BAL (Broncho Alveolar Lavage) CBNAAT to arrive at an early diagnosis of Pulmonary TB in such patients.

AIM AND OBJECTIVE:

- To study role of bronchoalveolar lavage CBNAAT in sputum smear negative pulmonary tuberculosis patient.
- To evaluate the diagnostic yield of Bronchoalveolar lavage in microbiological confirmation of sputum smear negative clinically and/or radiologically suspected pulmonary TB patients
- To evaluate the diagnostic yield of Bronchoalveolar lavage CB-NAAT in microbiological confirmation of sputum smear negative clinically and/or radiologically suspected pulmonary TB patients
- To study the clinical profile of such patients.

MATERIAL AND METHODS:

The present observational, prospective, cross- sectional study was conducted in department of Respiratory Medicine, GMERS Medical college and hospital, Gotri, Vadodara during period of December 2019 to December 2021. The study was started after approval of the study protocol by institutional review board. Patients were enrolled in the study after taking informed written consent. All patients of clinically and radiologically suspected pulmonary tuberculosis having negative sputum smear for AFB were taken into study with following inclusion and exclusion criteria.

Inclusion Criteria:

- Patients with respiratory symptoms suspicious of having PTB like cough for 2 weeks or more, evening rise of temperature, weight loss, loss of appetite.
- Sputum either not produced or sputum negative for AFB on two samples.
- Chest radiograph showing changes consistent with active PTB.
- Age more than 18 years.
- Patients who are willing to go for Bronchoscopy.

Exclusion Criteria:

- Patients with uncontrolled Asthma
- Patients with uncontrolled Chronic Obstructive Pulmonary disease
- Patients with Unstable Angina
- Patients already on Anti-tubercular treatment
- Patients not willing to give consent

RESULTS:

Total 50 patients satisfying the required inclusion and exclusion criteria were enrolled in the study. Out of the 50 total patients with suspected Pulmonary Tuberculosis who came to Department of Respiratory Medicine in GMERS Medical College and Hospital, Gotri, Vadodara and enrolled for the study, 41 patients (82%) were male and 09 patients (18%) were female.

Table 1- Distribution of study group by Age and Gender.

Age in years	Male (%)	Female (%)	Total
18-30	12 (24%)	2 (4%)	14 (28%)
31-40	8 (16%)	1 (2%)	9 (18%)
41-50	10 (20%)	2 (4%)	12 (24%)
>50	11 (22%)	4 (8%)	15 (30%)
Total	41 (82%)	9 (18%)	50 (100%)

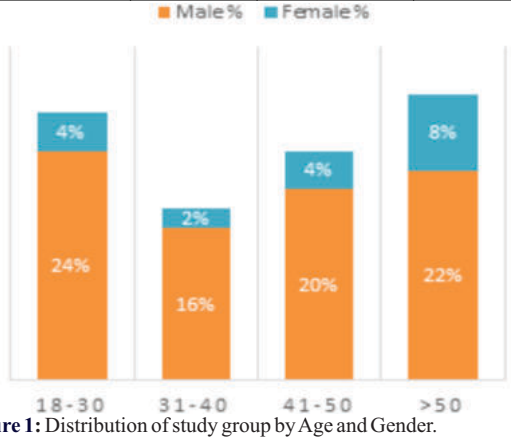

Figure 1: Distribution of study group by Age and Gender.

Table 2: Pulmonary diseases according to age.

Age	TB (n=31)	Non-TB (n=19)	Total
<30	9 (29%)	4 (21%)	13
31-50	15 (48%)	7 (37%)	22
>50	7 (23%)	8 (42%)	15
Total	31	19	50

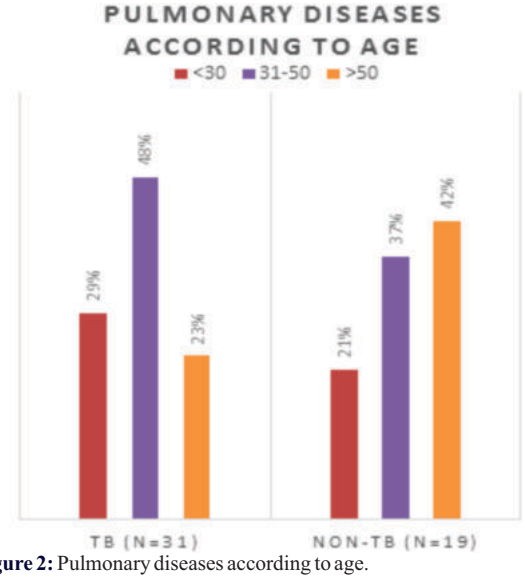

Figure 2: Pulmonary diseases according to age.

Table 3: Distribution of Pulmonary Disease according to Residence.

Residence	TB	Non-TB
Urban (n=31)	22 (71%)	9 (29%)
Rural (n=19)	7 (37%)	12 (63%)

Table 4- Distribution of Pulmonary disease according to Occupation.

Occupation	TB	Non-TB	Total
Labourer (n=14)	11 (79%)	3 (21%)	14
Farmer (n=9)	3 (33%)	6 (67%)	9
Non-worker (n=14)	10 (72%)	4 (28%)	14
Others (n=13)	7 (54%)	6 (46%)	13
Total	31	19	50

Table 5- Past history of Tuberculosis.

History	TB	Non-TB
Present (n=28)	17 (61%)	11 (39%)
Absent (n=22)	14 (63%)	8 (36%)

Table 6- Family History of Tuberculosis.

History	TB	Non-TB
Present (n=25)	14 (56%)	11 (44%)
Absent (n=25)	17 (68%)	8 (32%)

Table 7- Pulmonary disease and Smoking.

Smoking	TB	Non-TB
Present (n=13)	6 (46%)	7 (54%)
Absent (n=37)	25 (68%)	12 (32%)

Table 8- Pulmonary disease and Alcohol.

Alcohol	TB	Non-TB
Present (n=41)	26 (63%)	15 (37%)
Absent (n=9)	6 (67%)	3 (33%)

Table 9- Distribution of disease according to Symptoms.

Symptoms	TB (n=31)		Non-TB (n=19)	
	Pre- sent	Ab- sent	Pre- sent	Ab- sent
Cough	30	1	16	3
Dyspnoea	16	15	12	7
Weakness	24	7	14	5
Anorexia	23	8	10	9
Fever	24	7	10	9
haemoptysis	7	24	6	13
Weight loss	23	8	10	9

Table 10- Chest Xray findings in Pulmonary diseases.

Chest Xray findings	TB	Non-TB
Consolidation (n=26)	18 (69%)	8 (31%)
Cavitation (n=14)	8 (58%)	6 (42%)
Nodules (n=4)	3 (75%)	1 (25%)
Infiltration (n=8)	6 (75%)	2 (25%)

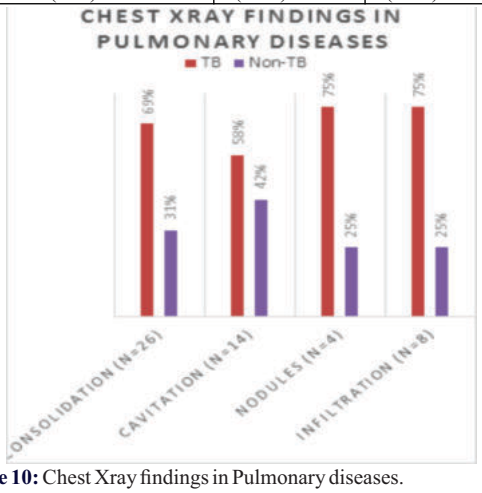

Figure 10: Chest Xray findings in Pulmonary diseases.

Table 11- Site of involvement in Chest Xray.

Site of involvement	TB	Non-TB
UZ (n=13)	12 (92%)	5 (8%)
LZ (n=17)	9 (53%)	8 (47%)
UZ & LZ (n=16)	10 (63%)	6 (37%)
Unilateral (n=30)	17 (57%)	13 (43%)
Bilateral (n=20)	14 (70%)	6 (30%)

Table-12 Extent of disease in Chest Xray.

Zones Involved	TB	Non-TB
< 3 (n=36)	22 (61%)	14 (39%)
≥ 3 (n=14)	9 (64%)	5 (36%)

Table 13- Type of lesion on Chest Xray in TB patients.

Lesion	UZ	LZ	UZ &LZ
Consolidation (n=14)	9 (65%)	3 (21%)	2 (14%)
Cavitation (n=9)	6 (67%)	2 (22%)	1 (11%)
Infiltration (n=6)	1 (17%)	0	5 (84%)
Nodules (n=2)	0	0	2 (100%)

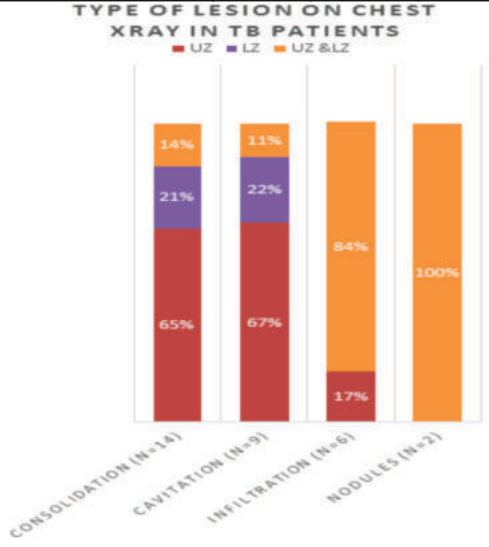

Figure 13: Type of lesion on Chest Xray in TB patients.

Table 14- Type of lesion on Chest Xray in Non-TB patients.

Lesion	UZ	LZ	UZ &LZ
Consolidation (n=8)	4 (50%)	3 (38%)	1 (12%)
Cavitation (n=4)	1 (25%)	3 (75%)	0
Infiltration (n=2)	0	0	2 (100%)
Nodules (n=1)	0	0	1 (100%)

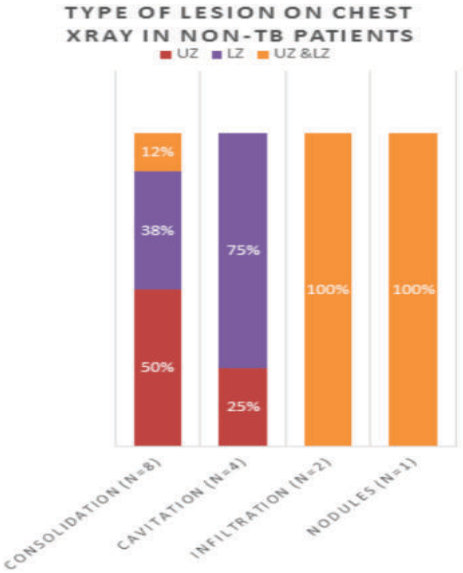

Figure 14: Type of lesion on Chest Xray in Non-TB patients.

Table 15: BAL Investigations for Pulmonary disease .

BAL investigations	TB (n=31)	Non-TB (n=19)
BAL AFB smear	5 (16%)	0
BAL CBNAAT	31(100%)	0
BAL AFB smear and CBNAAT	6 (20%)	0
BAL pyogenic culture	4 (13%)	8 (42%)

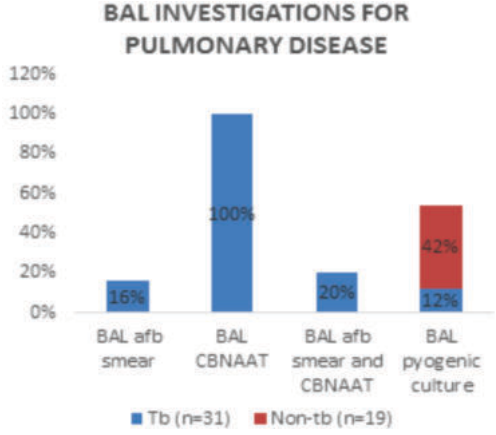

Figure 15: BAL Investigations for Pulmonary disease.

Table- 16 BAL CB-NAAT with Past history of Tuberculosis.

BAL CB-NAAT	History of Tuberculosis	
	Present	Absent
Rif Resistance	1	0
Rif Sensitive	13	17

DISCUSSION:

In present study total 50 patients with suspected Pulmonary Tuberculosis satisfying the required inclusion and exclusion criteria were enrolled in the study.

The most common age group involved in the study was >50 years of age (30%). Age groups 18-30 (28%), 31-40(18%), 41-40 (24%) have almost equal distribution of patients. Youngest patient was 18 years while oldest patient was 70 years of age. There were no paediatric patients in our study.

In present study males constituted the majority of study population out numbering the female, with 41 patients (82%) male patients and 09 patients (18%) female patients, male to female ratio being 4.5:1. High male to female ratio (1: 0.52) was reported by **J.Balakrishna et al (2015)**[18] in pulmonary tuberculosis suspected cases. Male predominance was reported also by **Prashanth Prakash et al (2016)**[19] with a male to female ratio of 2.94 : 1. In the study conducted by **Sharma Shubhakaran et al (2013)**[20], there were 50 males and 7

females with a male to female ratio of 7.14 : 1. Also in study conducted by **Gopathi NR et al (2016)**[15],there were majority of male patients,78 males out of 120 in their study group while females were only 42 and a similar study conducted by **Gowda NC et al. (2018)**[16]**,** also found majority of patients male i.e., 36 patients out of 60 while females were only 24. Thus, studies conducted in India has more male preponderance than female. The male predominance for TB in the present study is consistent with data from available studies.

In low-income countries, women often have a reduced access to economic resources and fewer educational opportunities as compared to men. As a result, many women are unable to locate and reach appropriate health services. The decision regarding woman's treatment is also made by the husband or senior members of family. Furthermore, stigma attached to a positive diagnosis leads many women to forego seeking necessary medical attention. In low-income countries like India, women tend to self-medicate or seek out traditional healers instead of accessing public health facility because they are afraid of being recognised as a TB patient by members of community. These may be cause of sex difference in our study.

In present study, TB was found more common in middle age patients with 48% in 31-50 years of age. While non-TB was more common in old age patients with 42% in age group more than 50 years of age. In age less than 30 years there was preponderance of TB patients (29%). This is comparable to study conducted by **Echazarreta A et al (2015)**, having median age of 37 years and more susceptible gender, male for Pulmonary TB[21]. In a similar study conducted by **Dimple Kumar Bhaglani et al (2016)**[22], the mean age of TB patients was found to be 41.18 years. Also, in the study conducted by **Sharma Shubhakaran et al (2013)**[20], the mean age of TB patients was found to be 43.07 ± 14.22 years.

In present study out of 50 patients, 62% belong to urban population while 38% were belong to rural area. Majority (71%) patients from urban area had TB while majority (63%) of patients from rural area had non-TB. This finding was not comparable with the studies conducted by **Chadha VK et al, (2012)**[23] and **Aggarwal AN et al, (2015)**[24] on Prevalence of pulmonary tuberculosis in India that showed TB is more prevalent in rural population.

In present study 79% of Labourers and 72% of Non-workers were affected with TB while 67% of farmers were affected with non-TB. This finding is comparable to study conducted by **Gupta S et al**, **(2011)**[25] showed that out of the 207 patients with pulmonary TB, 74% were labourers. Prevalence of PTB was significantly more common among labourers and white-collar workers. Statistically, labourers >61 years are most vulnerable to pulmonary TB.

In present study out of 50 patients enrolled, 56% had past history of TB which is not comparable to study conducted by **Gowda NC et al. (2018)**[16]**,** in which out of 60 patients 11(18%) had past history of TB.

In current study with past history of TB, 61% were of TB and 39% were of non-TB while 44% of patients had no past history of TB out of which 63% were of TB and 36% were of non-TB. Thus, we conclude that past history of TB is not a risk factor for Pulmonary Tuberculosis.

In present study, 26% were smokers and 74% were non-smokers. In Smokers non-TB (54%) was more common than TB (46%). This is comparable with study conducted by **Brito GMX et al (2021)**[17]**,** out of 22 culture positive TB patients, 16 were non-smoker and 6 were smoker. A similar study from South India by **Shetty et al (2006)**[26] has shown history of smoking and alcohol consumption not a significant risk factor for pulmonary tuberculosis.

In present study 41 patients (82%) were consuming alcohol and 9 (18%) were not. Out of 41 patients 26 (63%) were having TB and 15 patients (37%) were of non-TB. This was comparable with study conducted by **Veerakumar AM et al, (2015)**[27] on Alcohol use disorders among pulmonary tuberculosis patients which showed that alcohol use among PTB patients at the time of diagnosis was 59% and during treatment was 31.5%.

In present study the most common symptoms observed in TB was cough (98%) followed by weakness (77%), fever (75%), anorexia (74%), weight loss (74%), dyspnoea (52%) and haemoptysis were present in only 23%.

Gowda NC et al. (2018)[16] reported that cough was present in 100%, Haemoptysis in 11%, Shortness in breath in 27%, Fever in 78.3% and Significant weight loss in 46%.

Dimple Kumar Bhaglani et al, (2016)[22] reported that, in their study also, cough was the most common symptom (93.93%), followed by dyspnoea (86.36%), chest pain (50%), fever (45.45%), haemoptysis (28.78%). Predominant symptom as cough (86%) was seen in the study conducted by **Prashanth Prakash et al (2016)**[19] too. Other symptoms were fever (60%), haemoptysis (32 %) and chest pain (24%). **Gopathi NR et al (2016)**[15] concluded that Cough was present in 95%, Fever in 80%, Loss of appetite 75%, Dyspnoea in 63%, haemoptysis in 15 %. **Brito GMX et al (2021)**[17]**,** also concluded that the most common symptoms being weight loss in 59.3%, cough in 57.1%, and dyspnoea in 53.6%. A similar study by **Shin et al**[28] included 126 patients, revealed cough as the most common symptom in 70 (55.6%) patients, sputum in 42 (33.3%) patients, haemoptysis in 31 (24.6%) patients, fever in 27 (21.4%) patients, chest pain in 22 (17.4%) patients and weight loss in 4 (3.2%) patients. **Purohit et al (1983)**[11] carried out FOB in 50 patients of SSN-PTB, also showed similar results of cough as most common presenting symptom. **Kulpati and Hira (1986)**[12] also showed similar results with cough as most common presenting symptom in patients suspected of having pulmonary tuberculosis.

In present study we also studied non-TB infections and found that cough (84%) was most common symptom followed by weakness (75%), dyspnoea (63%) and fever, anorexia, weight loss was present in 53% was present in infection other than TB.

In present study the most commonly observed abnormality in Chest X-ray was Consolidation (52%), followed by Cavitation (28%), Infiltration (16%) and Nodules (8%).

In TB patients Consolidation was seen in 69%, Cavitation in 58%, Nodules and Infiltration was observed more in 75% than in non-TB patients. Ground-glass opacity was observed in one single TB patient. While in non-TB patients' consolidation was observed in 31%, cavitation in 42%, nodules and infiltration were present in 25% of patients. Cystic bronchiectasis and fibrosis were observed more in non-TB patients.

Similar findings were observed in study conducted by **Gopathi et al, (2016)**[15] most common findings on chest X-ray were cavitation in 53% cases followed by fluffy or nodular infiltrations in 22% patients. **Gowda NC et al. (2018)**[16]**,** also found consolidation in 56%, Nodules in 60% and Cavitation in 60% of suspected Pulmonary Tuberculosis patients. From above available data we conclude that consolidation and cavitary lesions are more often associated with Pulmonary Tuberculosis. Study by **J.Balakrishna et al, (2015)**[18] also concluded that cavitary lesions were present in 40% of patients.

In present study upper zone involvement in Chest Xray was more common in TB patients i.e., 92% than in non-TB i.e., 8%. Lower zone involvement was almost same in both TB and Non-TB patients. Upper and lower zone involvement was more common in TB patients i.e., 63% than in non-tb patients i.e., 37%. And Extent of disease is more in TB patients (64%) as compared to non-TB patients (36%). Similar findings were observed in study conducted by **Gopathi et al, (2016)**[15] with Upper zone involvement in 70 %, Multi lobar in 25 % and Lower zone in 5%.

In present study Bilateral involvement of lungs was more in TB patients i.e., 70% while in non-TB it was only 30%. A study by **Bachh et al, (2010)**[13] observed that bilateral lesions were present in 9.3% of cases and unilateral in 90.6%, this study was not comparable with our findings.

In present study in TB patients, Consolidation (65%) and Cavitation (67%) is more commonly seen in upper zone. Infiltration (84%) and Nodules (100%) is more commonly seen in both upper and lower zone. And in Non-TB patients, there is no obvious difference in distribution of consolidation in upper zone (50%) and lower zone (38%). Cavitation is more commonly seen in lower zone (75%). And Infiltration (100%) and Nodules (100%) is present in both upper and lower zone. we have not found studies correlating with our data.

In present study Bal CB-NAAT was positive in all TB patients (100%),

Bal AFB smear and CB-NAAT was positive in 20% of TB patients. In Non-TB patients Bal pyogenic culture was positive in 42% of patients. Bal CB-NAAT was diagnostic for TB in 31 patients. Out of these patients, Rifampicin was sensitive in 13 patients with history of TB and in 17 patients with absent past history of TB. Only 1 patient had rifampicin resistance with the history of TB.

Similar study conducted by **Kanwal Fatima Khalil and Tariq Butt et al (2015)**[14] out of the 93 patients who undergone FOB, 81 patients (87.09%) gave positive results in BAL CBNAAT. Also, in study conducted by **Liam C K et al (2007)**[29], positive result was given by BAL gene Xpert in 55 out of 68 patients (80.9%). Our result is also comparable with the results found in the studies conducted by **Sanjay Avashia et al (2016)**[30] among these 69 BAL samples, mycobacterium tuberculosis was detected in 34 BAL samples; i.e., 47.22 % cases were found to be positive when CBNAAT was done on BAL samples. Among these 34 BAL samples, 31 BAL samples were sensitive to rifampicin while 3 were resistance.

CONCLUSION:

The study concludes that BAL CB-NAAT is useful in diagnosing suspected Pulmonary Tuberculosis patients whose sputum smear is negative on repeated occasions and chest Xray is suggestive of findings suggestive of TB. It also helps in microbiological confirmation of TB, early diagnosis of MDR cases and in initiating early treatment of TB.

Bronchoalveolar lavage also helps in ruling out non-TB conditions like bacterial pneumonia, fungal pneumonia, malignancy, etc.

The study also concludes that cough is most common symptom in TB and anorexia, weight loss and fever is common in TB patients as compared to non-TB patients

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Ethical approval: Approved

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