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Original Article

Comparative analysis of CBNAAT and Ziehl-Nielsen (ZN) staining test in clinically suspected cases of Tubercular Pleural Effusion

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ABSTRACT

Background: Tuberculous pleural effusion is a common form of extrapulmonary tuberculosis and remains a diagnostic challenge due to the paucibacillary nature of pleural fluid. Ziehl–Neelsen staining is simple, rapid, and inexpensive, but its sensitivity is limited. Cartridge-Based Nucleic Acid Amplification Test is a rapid molecular method for detecting *Mycobacterium tuberculosis*; however, its diagnostic role in pleural fluid samples needs further evaluation.
Aim and Objectives: To compare the diagnostic utility of Cartridge-Based Nucleic Acid Amplification Test and Ziehl–Neelsen staining in clinically suspected cases of tuberculous pleural effusion.
Materials and Methods: This cross-sectional, hospital-based study was conducted in the Department of Pathology, Clinical Pathology Central Laboratory, Sri Venkateswara Ramnarayan Ruia Government General Hospital, Tirupati, Andhra Pradesh, over six months. Ethical approval was obtained from the Institutional Ethics Committee, S.V. Medical College, Tirupati (Ref. No.: 34/2025). A total of 72 pleural fluid samples from clinically suspected tuberculous pleural effusion cases were included. Samples were processed for cytological evaluation, total and differential leukocyte counts, Ziehl–Neelsen staining, and Cartridge-Based Nucleic Acid Amplification Test. Positivity rates were calculated, and agreement between both tests was assessed using Cohen's kappa statistic.
Results: Among 72 patients, 45 (62.5%) were males and 27 (37.5%) were females. The highest number of cases was observed in the 41–50 years age group. Lymphocytic predominance was seen in 55 cases (76.4%). Ziehl–Neelsen staining was positive in 7 cases (9.7%), while CBNAAT detected *M. tuberculosis* in 4 cases (5.6%). The overall agreement was 95.8%, with a kappa value of 0.71, indicating substantial agreement.
Conclusion: CBNAAT showed variable detection in pleural fluid samples. Negative results should be interpreted cautiously, and complementary diagnostic methods remain essential.

Keywords*: Tuberculous pleural effusion; CBNAAT; Ziehl–Neelsen staining; extrapulmonary tuberculosis; pleural fluid; Mycobacterium tuberculosis.*

INTRODUCTION

Extrapulmonary tuberculosis (EPTB) accounts for approximately 15–53% of all tuberculosis cases, with pleural involvement representing a significant proportion of these cases [1, 2]. Nearly 25% of patients with tuberculosis may present with extrapulmonary manifestations such as lymphadenopathy or pleural effusion [3, 4]. Among the various forms of EPTB, tuberculous pleural effusion is considered the second most common presentation after tuberculous lymphadenopathy [5].

Mycobacterium tuberculosis, a pathogenic bacterial species belonging to the family *Mycobacteriaceae*, is the principal causative organism of tuberculosis [6-8]. Although the organism was first identified by Robert Koch in 1882, tuberculosis remains one of the deadliest communicable diseases despite the availability of effective anti-tubercular therapy and the widespread use of *Bacillus Calmette–Guérin* vaccination in many parts of the world [9]. According to the World Health Organization, the global incidence of tuberculosis remains high, with some regions reporting rates as high as 134 cases per 100,000 population [9].

The diagnosis of tuberculous pleural effusion is often challenging because pleural fluid usually contains a low bacillary load, resulting in poor sensitivity of conventional diagnostic methods. Ziehl–Neelsen (ZN) staining is a simple, inexpensive, and widely used technique for the detection of acid-fast bacilli; however, its diagnostic yield in pleural fluid is often limited due to the paucibacillary nature of the disease [10]. Culture, although considered more sensitive, requires prolonged incubation and may delay treatment initiation.

In recent years, Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) has emerged as a rapid molecular diagnostic tool for the detection of *M. tuberculosis* and rifampicin resistance [11, 12]. CBNAAT is well established for sputum-based diagnosis of pulmonary tuberculosis, but its role in extrapulmonary samples, particularly pleural fluid, remains less clearly defined. Because timely and accurate diagnosis is essential for early initiation of therapy and improved clinical outcomes, evaluation of CBNAAT in clinically suspected cases of tuberculous pleural effusion is important.

Therefore, the present study aims to compare the diagnostic utility of CBNAAT and Ziehl–Neelsen staining in clinically suspected cases of tuberculous pleural effusion and to assess the relative performance of these methods in detecting *M. tuberculosis* in pleural fluid samples.

MATERIALS AND METHODS

Study Design and Setting

This was a cross-sectional, hospital-based study conducted in the Department of Pathology, Clinical Pathology Central Laboratory, Sri Venkateswara Ramnarayan Ruia Government General Hospital (SVRRGGH), Tirupati, Andhra Pradesh. The study was carried out over a period of six months after obtaining ethical approval from the Institutional Ethics Committee, S.V. Medical College, Tirupati **(Ref. No: 34/2025).** All procedures were performed in accordance with institutional ethical guidelines, and written informed consent was obtained from all participants.

Study Population

The study included 72 pleural fluid samples from patients clinically suspected to have tuberculous pleural effusion. The samples were received in the cytopathology section of the Department of Pathology, SVRRGGH, Sri Venkateswara Medical College, Tirupati.

Inclusion Criteria

All pleural fluid specimens from clinically suspected cases of tuberculous pleural effusion received in the Department of Pathology were included in the study, provided that written informed consent was obtained from the patients.

Exclusion Criteria

Inadequate samples and all cases diagnosed as malignant effusions were excluded from the study.

Sample Collection and Processing

Fresh pleural fluid samples from clinically suspected cases of tuberculous pleural effusion were collected by thoracocentesis or intercostal drainage under aseptic precautions. The samples were received in sterile containers and divided into three parts for further processing.

The first part of the sample was subjected to routine centrifugation at 3000 rpm for 5 minutes. Smears were prepared from the sediment and stained with routine Hematoxylin and Eosin (H&E) stain and Ziehl–Neelsen (ZN) stain for cytological evaluation and detection of acid-fast bacilli.

The second part of the sample was used for total white blood cell count and differential cell count. Based on cell predominance, the pleural effusion cases were categorized into lymphocyte-predominant and neutrophil-predominant effusions.

The third part of the sample was sent for Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) using the CBNAAT analyser, and the results were recorded.

Cytological Evaluation
Cytological evaluation of the stained smears was performed independently by two cytopathologists. The findings were recorded using pre-designed evaluation forms. The cytological features, cellular predominance, ZN staining results, and CBNAAT results were documented for each case.

Diagnostic Test Comparison
The positivity rates of CBNAAT and ZN staining were calculated. The results obtained by ZN staining were correlated with CBNAAT findings to assess the diagnostic agreement between the two methods in clinically suspected cases of tuberculous pleural effusion.

Statistical Analysis
All results were recorded and tabulated. Data analysis was performed using Microsoft Excel 2010 and R programming language for statistical computing and data visualization, version 4.4.2. Categorical variables were expressed as numbers and percentages. Cohen's kappa statistic was used to assess the level of agreement between CBNAAT and ZN staining.

RESULTS
A total of 72 patients with clinically suspected tuberculous pleural effusion were included in the study. Of these, 45 patients (62.5%) were males and 27 patients (37.5%) were females, showing a male predominance. The age of the study participants ranged from 19 years to more than 81 years. The highest number of cases was observed in the middle-aged population, particularly in the 41–60 years age group.

Among males, the maximum number of cases was seen in the 51–60 years age group, comprising 11 cases (24.4%), followed by the 41–50 years age group with 10 cases (22.2%) and the 61–70 years age group with 9 cases (20.0%). Among females, the highest number of cases was observed in the 41–50 years age group, with 10 cases (37.0%), followed by the 19–30 years and 31–40 years age groups, with 5 cases (18.5%) each. No female cases were observed in the 71–80 years and >81 years age groups. Overall, males predominated across most age groups (Table 1).

Table 1: Age and Sex Distribution of Study Participants (n = 72)

Age group (years)	Males, n (%)	Females, n (%)
19–30	1 (2.2)	5 (18.5)
31–40	8 (18.0)	5 (18.5)
41–50	10 (22.2)	10 (37.0)
51–60	11 (24.4)	3 (11.0)
61–70	9 (20.0)	4 (15.0)
71–80	4 (9.0)	0 (0.0)
>81	2 (4.4)	0 (0.0)
Total	**45 (62.5)**	**27 (37.5)**

Based on cellular predominance, lymphocytic predominance was observed in 55 cases (76.4%), whereas neutrophilic predominance was noted in 17 cases (23.6%). Thus, lymphocyte-predominant effusion constituted the majority of cases in the present study, supporting the chronic inflammatory nature commonly associated with tuberculous pleural effusion (Table 2).

Table 2: Categorization of Pleural Effusion Cases Based on Cellular Predominance

Cellular predominance	Number of cases	Percentage (%)
Neutrophilic predominance	17	23.6
Lymphocytic predominance	55	76.4
Total	**72**	**100.0**

The positivity rate of CBNAAT was 5.6%, with *Mycobacterium tuberculosis* detected in 4 out of 72 cases. Ziehl–Neelsen staining showed acid-fast bacilli positivity in 7 out of 72 cases, giving a positivity rate of 9.7%. Thus, ZN staining showed a slightly higher positivity rate than CBNAAT in the present study (Table 3).

Table 3: Positivity Rate of CBNAAT and ZN Staining

Diagnostic test	Number of positive cases	Positivity rate
CBNAAT	4	5.6% (4/72)
ZN staining	7	9.7% (7/72)

Out of the 72 pleural fluid samples examined, 7 cases were positive for acid-fast bacilli on ZN staining, while 65 cases were negative. Representative ZN-stained smears showing acid-fast bacilli are illustrated in Figures 1and 2.

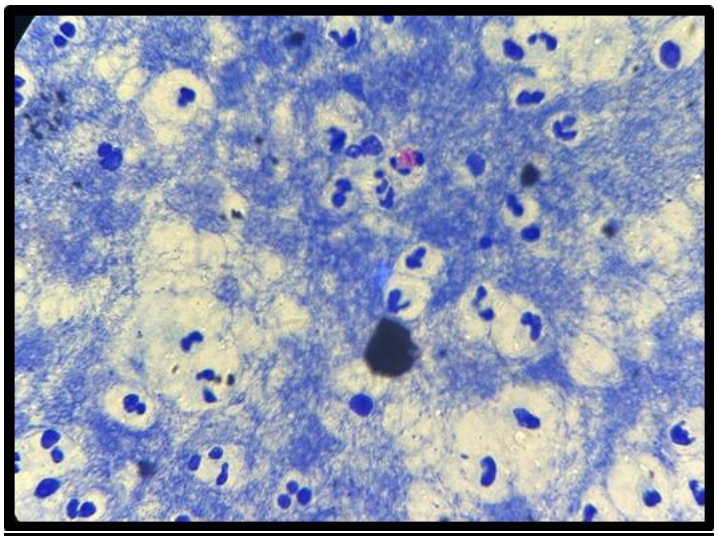

Figure-1: ZN Stain (100x) Shows Neutrophilic population

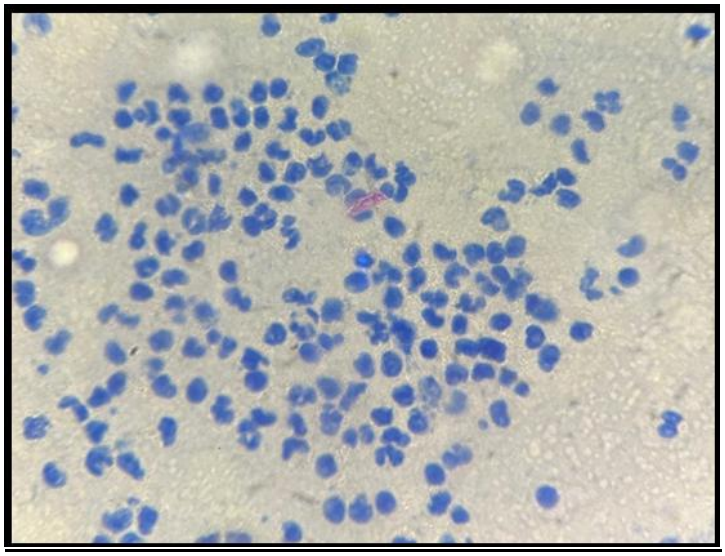

Figure- 2: ZN Stain (100x) Shows mixed cellular population

On comparison of CBNAAT with ZN microscopy, CBNAAT detected *M. tuberculosis* in 4 smear-positive cases, while 3 ZN smear-positive cases were negative by CBNAAT. None of the smear-negative cases showed MTB detection by CBNAAT. Therefore, CBNAAT positivity was observed only among ZN smear-positive cases in the present study (Table 4).

Table 4: Comparison of CBNAAT and ZN Microscopy

ZN microscopy result	CBNAAT: MTB detected	CBNAAT: MTB not detected	Total
Smear positive	4	3	7
Smear negative	0	65	65
Total	4	68	72

The overall agreement between CBNAAT and ZN microscopy was 95.8% [(4 + 65)/72]. Cohen's kappa statistic showed a value of 0.71, indicating substantial agreement between the two diagnostic methods. This suggests that although CBNAAT and ZN staining showed good concordance, ZN staining detected a few additional positive cases that were not detected by CBNAAT in this study population.

DISCUSSION

Tuberculous pleural effusion remains an important manifestation of extrapulmonary tuberculosis and poses a diagnostic challenge because of the paucibacillary nature of pleural fluid. In the present study, a total of 72 clinically suspected cases of tuberculous pleural effusion were evaluated using Ziehl–Neelsen (ZN) staining and Cartridge-Based Nucleic Acid Amplification Test (CBNAAT).

In this study, male predominance was observed, with males accounting for 45 cases (62.5%) and females accounting for 27 cases (37.5%). This finding was comparable to the study conducted by Gupta et al., in which males constituted 70.9% of cases [13]. The higher occurrence among males may be attributed to greater exposure risk, occupational factors, health-seeking behavior, or underlying epidemiological differences in tuberculosis prevalence.

The most commonly affected age group in the present study was 41–50 years, comprising 27.8% of cases. This finding differs from the studies conducted by Gupta et al. and Shrinidhi et al., where most cases were reported in the younger age group of 18–30 years [13, 14]. The difference in age distribution may be due to variations in study population, regional disease burden, referral patterns, nutritional status, immune response, and associated comorbid conditions.

Lymphocytic predominance was observed in 55 cases (76.4%) in the present study, whereas neutrophilic predominance was noted in 17 cases (23.6%). This finding supports the chronic inflammatory nature of tuberculous pleural effusion. However, the lymphocytic predominance observed in the present study was lower than that reported by Gupta et al. and Kate et al., who observed lymphocytic predominance in 92.7% and 90% of cases, respectively [13, 15]. The relatively lower proportion of lymphocytic effusions in the present study may be related to differences in disease stage at presentation, early sampling during the inflammatory phase, or inclusion of clinically suspected cases before complete cytological evolution.

In the present study, the positivity rate of ZN microscopy was 9.7% (7/72). This finding was consistent with the study conducted by Gupta et al., who reported a ZN staining positivity rate of 9.1% [13]. The low yield of ZN microscopy in pleural fluid is expected, as tuberculous pleural effusion is usually paucibacillary. Nevertheless, ZN staining remains a simple, rapid, inexpensive, and widely available diagnostic method, particularly in resource-limited settings.

The CBNAAT positivity rate in the present study was 5.6% (4/72), which was lower than the 23.7% positivity rate reported by Gupta et al. [13]. Although CBNAAT is a rapid molecular test with the added advantage of detecting rifampicin resistance, its sensitivity in pleural fluid may be variable. The lower CBNAAT positivity observed in the present study may be explained by the paucibacillary nature of tuberculous pleural effusion, low mycobacterial DNA load, small sample volume, differences in sample processing, storage conditions, presence of PCR inhibitors, or variation in the stage of disease at the time of sample collection.

On comparison between the two diagnostic methods, CBNAAT detected *Mycobacterium tuberculosis* in 4 ZN-positive cases, while 3 ZN-positive cases were negative by CBNAAT. None of the ZN-negative cases showed MTB detection by CBNAAT. The overall agreement between CBNAAT and ZN microscopy was 95.8%, and Cohen's kappa value was 0.71, indicating substantial agreement between the two tests. However, the finding that CBNAAT did not detect additional cases among smear-negative samples suggests that a negative CBNAAT result should not be used alone to exclude tuberculous pleural effusion.

The diagnostic positivity rates observed in the present study were compared with those reported by Gupta et al. [13]. In the study by Gupta et al., CBNAAT showed a positivity rate of 23.7%, whereas ZN staining showed a positivity rate of 9.1%. In contrast, the present study showed a lower CBNAAT positivity rate of 5.6% (4/72), while the ZN staining positivity rate was 9.7% (7/72). Thus, the positivity rate of ZN staining in the present study was comparable to that reported by Gupta et al.; however, CBNAAT positivity was markedly lower in the present study. This difference may be attributed to factors such as the paucibacillary nature of pleural fluid, variation in sample volume, differences in sample processing methods, storage and transport conditions, presence of PCR inhibitors, and differences in disease stage at the time of sample collection.

Overall, the present study showed a comparable ZN microscopy positivity rate to previous studies but a lower CBNAAT positivity rate. These findings highlight the diagnostic limitations of relying on a single test for tuberculous pleural effusion. A combined diagnostic approach, including clinical evaluation, cytology, biochemical parameters, ZN staining, CBNAAT, culture, and other supportive investigations, remains essential for accurate diagnosis.

LIMITATIONS

The present study had certain limitations. First, the sample size was relatively small, which may limit the generalizability of the findings. Second, correlation with *Mycobacterium tuberculosis* culture, which is considered an important microbiological reference method, could not be performed on pleural fluid samples. Therefore, definitive microbiological confirmation and comparative assessment of diagnostic accuracy could not be established. Third, other supportive diagnostic parameters such as adenosine deaminase levels, pleural biopsy findings, and treatment response were not included in the present analysis.

Despite these limitations, the study provides useful insight into the real-world diagnostic performance of CBNAAT and ZN microscopy in clinically suspected cases of tuberculous pleural effusion.

IMPLICATIONS

The findings of the present study emphasize the need to further evaluate the causes of lower CBNAAT positivity in pleural fluid samples. Particular attention should be given to pre-analytical factors such as sample volume, method of collection, storage conditions, transport time, and the possible presence of PCR inhibitors. Optimization of sample processing protocols, including concentration techniques and improved DNA extraction methods, may help enhance the diagnostic yield of CBNAAT in tuberculous pleural effusion.

The study also suggests that CBNAAT should be interpreted as a complementary diagnostic tool rather than a standalone test in suspected tuberculous pleural effusion. Negative CBNAAT results should be correlated with clinical, cytological, biochemical, radiological, and microbiological findings before excluding the diagnosis.

CONCLUSION

In the present study, ZN staining showed a positivity rate of 9.7%, while CBNAAT showed a positivity rate of 5.6% in clinically suspected cases of tuberculous pleural effusion. Substantial agreement was observed between the two diagnostic methods, with a Cohen's kappa value of 0.71.

CBNAAT showed variable sensitivity in the diagnosis of tuberculous pleural effusion, and negative results should be interpreted with caution. Due to the paucibacillary nature of pleural fluid, no single diagnostic method is sufficient to rule out tuberculous pleural effusion. Therefore, complementary diagnostic approaches remain essential for accurate and timely diagnosis.

Conflict of Interest

The authors declare no conflict of interest.

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