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Evaluation of Real-Time PCR in Clinico-Radiologically Suspected Smear-Negative Pulmonary Tuberculosis Patients Attending a Tertiary Care Hospital, Central India

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Abstract-

Introduction- Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, continues to be a major public health burden, especially in developing countries. Smear-negative pulmonary tuberculosis (SNPT) presents a diagnostic challenge due to its low bacillary load and limited sensitivity of conventional microscopy and culture. The present study evaluates the diagnostic performance of Real-Time Polymerase Chain Reaction (RT-PCR) compared with Middlebrook 7H9 broth culture in clinico-radiologically suspected smear-negative pulmonary TB patients.

Material and method -This cross-sectional observational study was conducted at Index Medical College, Hospital & Research Centre, Indore (M.P.), from July 2019 to July 2021. A total of 350 clinically and radiologically suspected but smear-negative patients were enrolled. Samples included sputum, bronchoalveolar lavage (BAL), and gastric aspirates. All specimens underwent Ziehl-Neelsen staining, Middlebrook 7H9 broth culture, and RT-PCR using the Qiagen Care TB PCR kit. Culture results served as the gold standard for evaluating RT-PCR sensitivity and specificity. Data were analyzed using SPSS version 20.

Result- Out of 350 cases, 73 (20.9%) were positive by Middlebrook 7H9 culture, while RT-PCR detected *M. tuberculosis* in 100 (28.6%) cases. Among the sample types, BAL showed the highest positivity (31.4%), followed by gastric aspirates (28.6%) and sputum (27.8%). Males (58.6%) were more affected than females, and the predominant age group was 20–40 years.

Conclusion- RT-PCR demonstrated higher sensitivity than culture for detecting *M. tuberculosis* in smear-negative patients. Its rapid turnaround time and superior detection make it a valuable adjunct to conventional diagnostics, facilitating early diagnosis and improved patient management.

Key words- RT- PCR, BAL, TB, ZN

Introduction

Tuberculosis (TB), caused primarily by *Mycobacterium tuberculosis*, remains one of the oldest and most persistent infectious diseases, posing a major global health challenge despite the availability of effective chemotherapy for decades.¹ According to the World Health Organization (WHO), an estimated 10.6 million people developed TB in 2023, resulting in 1.47 million deaths, making it the second leading infectious killer after COVID-19.² The disease burden remains disproportionately high in low- and middle-income countries due to poverty, undernutrition, HIV co-infection, and limited access to healthcare.³

The emergence of multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) has further complicated global control efforts.⁴ Pulmonary TB is the most common and transmissible form, while extra pulmonary TB contributes significantly to morbidity, particularly among immunocompromised individuals.⁵ Diagnosis of smear-negative pulmonary TB (SNPT) remains a major challenge because of its paucibacillary nature and the limited sensitivity of conventional methods such as Ziehl–Neelsen (ZN) staining and culture, which require high bacillary loads and longer turnaround times.⁶

Molecular diagnostic tools, particularly nucleic acid amplification tests (NAATs) such as real-time polymerase chain reaction (PCR) and the GeneXpert MTB/RIF assay, have revolutionized TB detection.⁷ These techniques enable rapid and accurate identification of *M. tuberculosis* and rifampicin resistance within hours, thereby facilitating early treatment initiation and improved infection control. The WHO now recommends GeneXpert as a first-line diagnostic test, particularly in high-burden settings, as it significantly improves case detection in both smear-positive and smear-negative patients.

Material and method

Study design, clinical specimen -This cross-sectional observational study was conducted from July 2019 to July 2021 at Index Medical College, Hospital & Research Centre, Indore, M.P. A total of 350 clinically suspected but ZN smear-negative patients were enrolled from both outpatient and inpatient departments. Specimens included sputum, bronchoalveolar lavage (BAL), and gastric aspirates.

Sample size was calculated to achieve sufficient power for sensitivity and specificity estimation with 95% confidence, considering an expected sensitivity of 85%, specificity of 90%, precision

of $\pm 7\%$, and a prevalence of confirmed TB among smear-negative patients of 30%, resulting in ~333 patients.

Ethical approval was obtained from the Institutional Ethics Committee. The study utilized specimens collected as part of routine clinical care; therefore, informed consent was obtained where applicable.

Study population- Patients of all ages and genders clinically and radiologically suspected of pulmonary tuberculosis (PTB) but with two negative sputum smears were included. Exclusion criteria comprised patients already diagnosed with PTB and undergoing anti-TB treatment.

Specimen collection- Two specimens were collected per patient: one spot sample and one early morning sample, following RNTCP guidelines. Patients were instructed to cough deeply to provide representative sputum. For patients unable to expectorate, gastric aspirate or BAL was collected under standard sterile procedures. All specimens were labeled, transported promptly in leak-proof containers, and maintained under cold chain conditions to preserve *Mycobacterium tuberculosis* viability.

Decontamination of specimens- Specimens were decontaminated using 4% NaOH (2–3× specimen volume) at 37°C for 30 min. Samples were centrifuged at 13,000 rpm for 10 min, washed with 1 mL sterile saline, and re-centrifuged. The pellet was used for smear, culture, and PCR assays. All procedures followed BSL-2 biosafety protocols.

Acid fast bacilli (AFB) smear and identification -Direct smears were prepared from specimens and stained using the Ziehl–Neelsen method. Slides were examined under oil immersion microscopy (100×). Positive smears showed red, slender rods resistant to acid-alcohol decolorization. Smear grading followed RNTCP guidelines: scanty, 1+, 2+, 3+. Patients with two negative smears were classified as smear-negative and eligible for PCR testing.

Culture method- The decontaminated pellet was inoculated into Middlebrook 7H9 broth supplemented with OADC enrichment and PANTA antibiotics, and incubated at 37°C. Cultures were monitored visually twice weekly for up to six weeks. Positive growth was confirmed by Ziehl–Neelsen staining for AFB. Culture results served as the gold standard for evaluating PCR performance.

Real-Time PCR for MTB detection- Molecular detection was performed using the Qiagen Care TB PCR Kit. DNA was extracted from the decontaminated pellet by lysis, heat treatment, and centrifugation. PCR reactions were prepared according to the manufacturer's instructions and run on a stratagene M×3005P real-time PCR cycler.

Controls included:

- Negative control (CT = 40/0/no value depending on instrument)
- High positive control (CT < 25)
- Critical positive control (CT < 35)

Result interpretation:

Category	CT Value / Criteria	Interpretation
Negative	40 (ABI), 0 (iCycler), No value (Light Cycler)	Negative
Positive	≤ 37	Positive
Indeterminate / Retest	> 37	Retest: if re-test CT $\leq 37 \rightarrow$ Positive; else Negative

Statistical analysis- Data were analyzed using SPSS. Continuous variables were expressed as mean $\pm$ SD or median (IQR), and categorical variables as frequencies and percentages. Diagnostic accuracy of RT-PCR was assessed by sensitivity, specificity, PPV, NPV, and overall accuracy with 95% confidence intervals. Agreement with the gold standard was measured using Kappa statistics. Subgroup and ROC curve analyses were performed where applicable, with $p < 0.05$ considered statistically significant.

Result

This study was conducted at Index Medical College, Hospital & Research Centre, Indore (M.P.). This two-year study adopted both MTB culture and rt-PCR for *Mycobacterium tuberculosis*. 350 suspected TB patients were enrolled in various Index Medical College & Research Centre clinical samples were found Positive for Middlebrook7H9 Broth Culture, and were positive for RT-PCR . A total of 350 clinically and radiologically suspected but smear-negative pulmonary tuberculosis patients were included in the present study. Among the respiratory specimens collected, sputum samples constituted the majority (70%, $n=245$), followed by bronchoalveolar lavage (BAL) specimens (20%, $n=70$) and gastric aspirates (10%, $n=35$). Of the study population, 58.6% ($n=205$) were males and 41.4% ($n=145$) were females. The most commonly affected age group was 20–40 years (40%, $n=140$), followed by 41–60 years (34.3%, $n=120$), >60 years (17.1%, $n=60$), and <20 years (8.6%, $n=30$). The most frequent clinical feature observed was cough lasting more than two weeks, present in 82.8% ($n=290$) of patients, followed by fever in 68.6% ($n=240$), weight loss in 51.4% ($n=180$), and night sweats in 31.4% ($n=110$). Hemoptysis was noted in 15.7% ($n=55$) of cases. Additionally, 20% ($n=70$) of patients reported a past history or contact with tuberculosis, and 10% ($n=35$) had associated comorbidities such as diabetes or HIV infection. Diagnostic evaluation revealed that Middlebrook 7H9 broth culture detected *Mycobacterium tuberculosis* in 73 cases, yielding a prevalence of 20.9%, whereas real-time PCR (RT-PCR) identified 100 positive cases, corresponding to a higher prevalence of 28.6%, indicating that RT-PCR was more sensitive than conventional culture in detecting tuberculosis among smear-negative patients.

Table 1- Evaluation of RT-PCR in Clinico-Radiologically Suspected Smear-Negative Pulmonary Tuberculosis patients -

Diagnostic Method	Total Cases (n)	Positive Cases (n)	Prevalence (%)
Middlebrook 7H9 Broth Culture	350	73	20.9%
Real-Time PCR (RT-PCR)	350	100	28.6%



Fig1- Evaluation of RT-PCR in Clinico-Radiologically Suspected Smear-Negative Pulmonary Tuberculosis patients -

Table 2- Distribution of Clinical Samples (N = 350)

Sample Type	Number (n)	Percentage (%)
Sputum	245	70.0%
Bronchoalveolar Lavage	70	20.0%
Gastric Aspirate	35	10.0%
Total	350	100

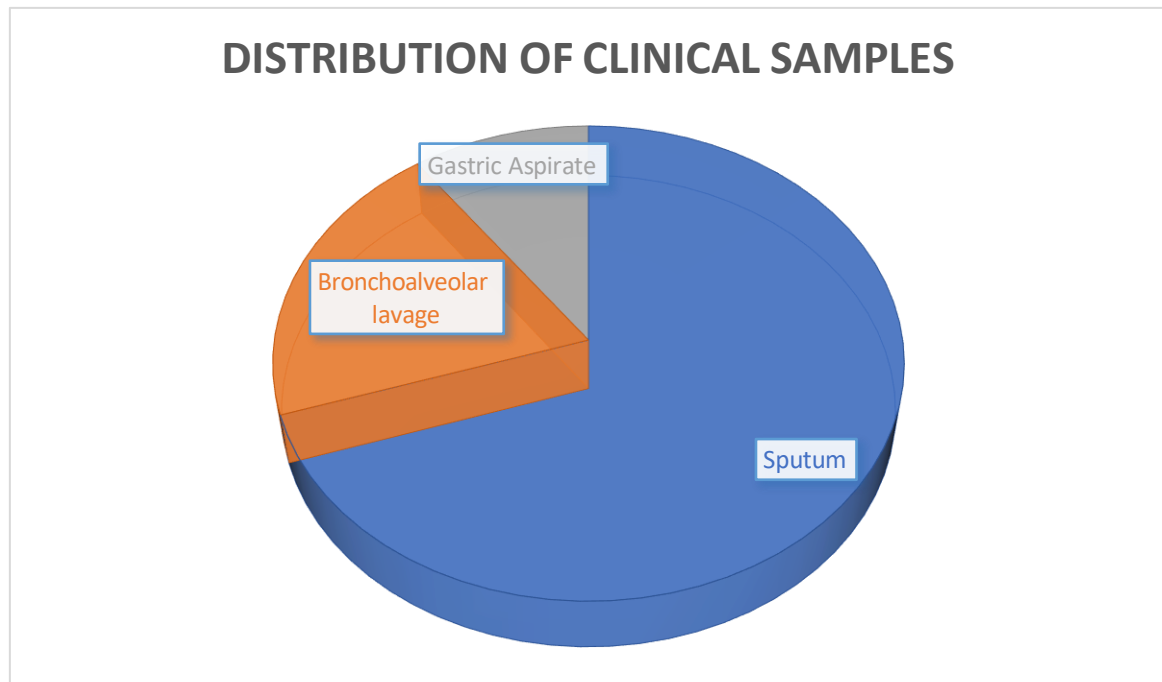


fig 2- Distribution of Clinical Samples (N = 350)

Table -3 Demographic Profile of Patients (N = 350)

Variable	Category	Number (n)	Percentage (%)
Gender	Male	205	58.6%
	Female	145	41.4%
Age Group	<20 years	30	8.6%
	20–40 years	140	40.0%
	41–60 years	120	34.3%
	>60 years	60	17.1%

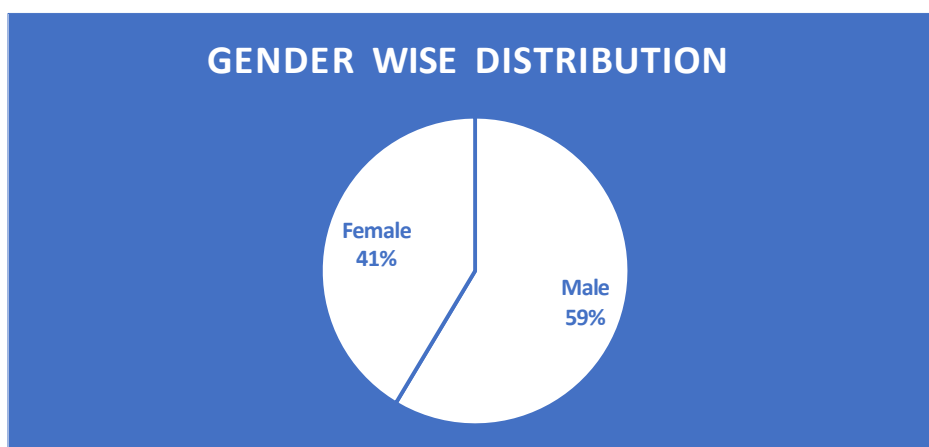


fig-3 Demographic Profile of Patients (N = 350)

Table-4 Clinical Features Among Suspected Cases (N = 350)

Clinical Feature	Number (n)	Percentage (%)
Cough >2 weeks	290	82.8%
Fever	240	68.6%
Weight loss	180	51.4%
Night sweats	110	31.4%
Hemoptysis	55	15.7%
Past TB / Contact history	70	20.0%
Comorbidities (DM/HIV)	35	10.0%

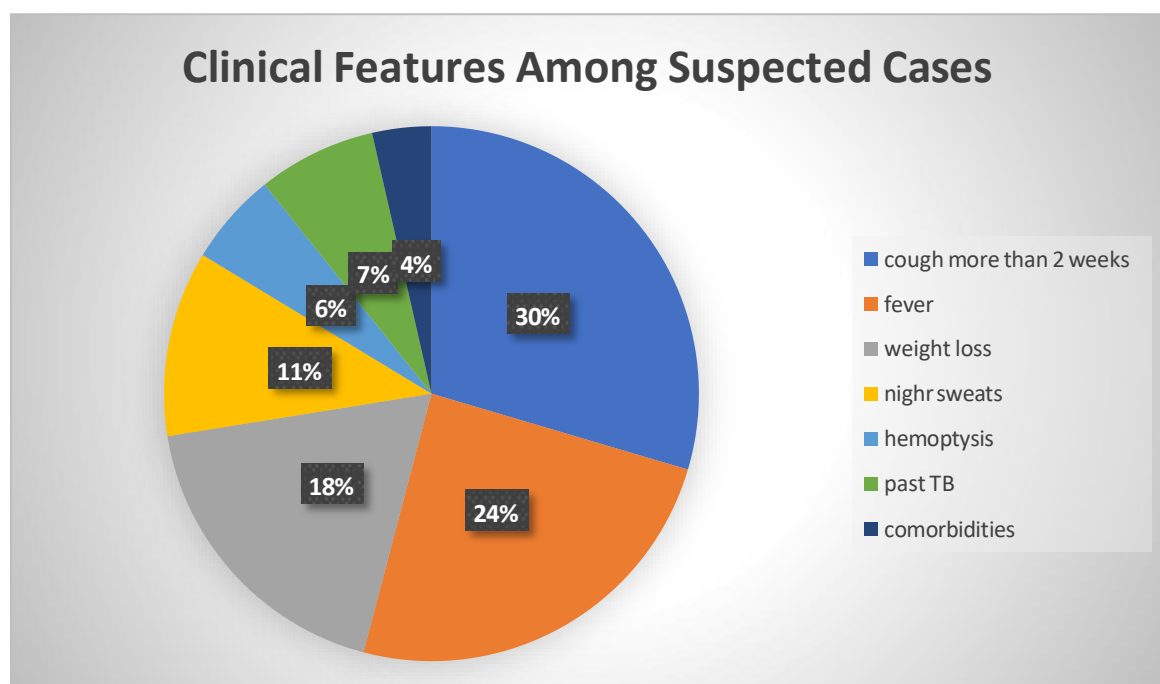


Fig -4 Clinical Features Among Suspected Cases (N = 350)

Discussion

In this study, 350 clinically and radio logically suspected smear-negative pulmonary TB patients from Index Medical College, Hospital & Research Centre, and Indore (M.P.) were evaluated using RT-PCR and Middlebrooks 7H9 broth culture. RT-PCR detected TB in 28.6% of cases, outperforming culture, which identified 20.9%. Among sample types, BAL specimens showed the highest RT-PCR positivity (31.4%), followed by gastric aspirates (28.6%) and sputum (27.8%). The majority of patients were male (58.6%), with the most affected age group being 20–40 years (40%). Clinically, the most common features were cough >2 weeks (82.8%), fever (68.6%), weight loss (51.4%), night sweats (31.4%), and haemoptysis (15.7%). Radiological findings among RT-PCR positive cases included cavitory lesions (40%), infiltrative lesions (32%), patchy changes (12%), fibrotic or old lesions (10%), and normal imaging (6%). Overall, RT-PCR identified 100 positive cases, accounting for 70.8% of clinically confirmed TB cases.

These findings are comparable to Nakiyingi L et al. (2012) ⁸ in Uganda, where among 205 smear-negative suspects, 72 (35%) were LJ culture-positive and 128 (62.4%) PCR-positive. Their in-house PCR showed 75% sensitivity and 35.9% specificity, with positive and negative predictive values of 39% and 72.4%, respectively. Similarly, Maria M Y et al. (2014) ⁹ reported q-PCR detection in 42/140 sputum samples (30%) among HIV/AIDS patients, with sensitivity, specificity, and accuracy of 87.2%, 98.9%, and 95%, respectively, closely aligning with the present study's higher detection rate by RT-PCR compared to culture (28.6% vs. 20.9%). Comparisons with radiological and clinical patterns in other studies show notable differences. Tayfun C et al. (2014) ¹⁰ reported a younger cohort (mean age 22.5 years) with HRCT findings dominated by micronodules (87%), large nodules (63%), and centrilobular nodules (62%), mainly in the upper lobes. In their study, systemic symptoms such as fever and weight loss were less common, and cavitory or infiltrative lesions were uncommon, unlike the present study where such lesions were observed in a significant proportion of cases. Rufai SB et al. (2014) ¹¹ at AIIMS, New Delhi, evaluated drug-resistant TB using LPA and Xpert MTB/RIF, highlighting limitations in rapid molecular assays for detecting resistance. In comparison, the present study employed RT-PCR for detection of MTB in smear-negative patients, demonstrating its reliability in identifying cases with low bacillary load. Gnoma LL Stella et al. (2015) ¹² reported that among 772 participants in the Republic of Congo, 36% of pulmonary TB cases were smear-negative, often presenting with milder symptoms such as anorexia and asthenia. In HIV-positive patients, 57% were SNPT, with shorter cough duration and systemic symptoms serving as important predictors. The present study also found prolonged cough (>2 weeks) as the most common symptom (82.8%), along with weight loss, fever, and night sweats. Radiologically, cavitory and infiltrative lesions were prominent among RT-PCR positive cases, reinforcing the utility of combining clinical assessment with molecular diagnostics for smear-negative TB. Kim M J et al. (2015) ¹³ compared Xpert MTB/RIF with real-time PCR and reported overall sensitivities of 80% for RT-PCR and 75.5% for Xpert, with lower detection in smear-negative specimens. Discrepant results, including rifampicin resistance detection, were noted, emphasizing that while rapid molecular assays are valuable, real-time PCR provides reliable detection, particularly in smear-negative patients.

Conclusion

This study confirms that RT-PCR demonstrates higher sensitivity than culture for detecting smear-negative pulmonary TB, particularly in patients with low bacillary load or subtle radiological findings. Integration of molecular diagnostics with clinical and radiological assessment significantly improves diagnostic accuracy and facilitates early TB detection.

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