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Research Article

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) remains a major global health concern, with India bearing the highest disease burden. Early diagnosis of pulmonary TB (PTB) is critical, but conventional smear microscopy often lacks sensitivity in patients with low bacillary loads. Culture methods, while sensitive, are time-consuming. Real-Time PCR (RT-PCR) provides a rapid and sensitive alternative for detecting *Mycobacterium tuberculosis* (MTB) in smear-negative cases.

Aims and Objectives: To evaluate the diagnostic performance of RT-PCR in clinically and radiologically suspected smear-negative PTB patients and compare its efficacy with Ziehl–Neelsen smear microscopy and Middlebrook 7H9 broth culture.

Materials and Methods: A cross-sectional observational study was conducted on 350 clinically suspected, ZN smear-negative samples (sputum, bronchoalveolar lavage [BAL], and gastric aspirate) collected at Index Medical College, Indore, from July 2019 to July 2021. Samples were processed for smear, culture, and RT-PCR using the Qiagen Care TB PCR Kit. Sensitivity, specificity, predictive values, overall accuracy, and concordance with clinical and radiological findings were analyzed.

Results: RT-PCR detected 28.6% of smear-negative cases, outperforming culture (20.9%). BAL specimens had the highest positivity (31.4%), followed by sputum (27.8%) and gastric aspirates (28.6%). RT-PCR positivity strongly correlated with radiological lesions, particularly cavitary and infiltrative patterns. Among 120 clinically confirmed TB cases, 85 (70.8%) were RT-PCR positive.

Conclusion: RT-PCR offers superior diagnostic performance compared to smear microscopy and culture in smear-negative PTB, with BAL specimens yielding the highest positivity. Integrating molecular diagnostics with clinical and radiological assessment can enhance early detection and optimize patient management in tertiary care settings.

Keywords: Pulmonary tuberculosis, Smear-negative TB, Real-Time PCR, Middlebrook 7H9 culture, Bronchoalveolar lavage

INTRODUCTION

Tuberculosis (TB) remains a severe global health crisis, with India bearing the highest disease burden worldwide. [1] Timely and accurate diagnosis of pulmonary TB (PTB) is crucial for effective disease control and management. [2] Conventional sputum smear microscopy, the mainstay of diagnosis, has low sensitivity, particularly in patients with reduced bacillary loads. [3] This deficiency results in a significant number of **smear-negative PTB suspects**—patients with suggestive clinical and radiological findings but negative smears—posing a persistent diagnostic challenge. [4] Culture methods, though more sensitive, are time-consuming, taking weeks to yield definitive results, thereby delaying the initiation of essential treatment.[5] Delayed treatment in smear-negative patient's risks disease progression, poor outcomes, and continued community transmission. [6] Nucleic Acid Amplification Tests (NAATs), specifically **Real-Time Polymerase Chain Reaction (RT-PCR)**, offer a rapid and sensitive alternative. [7,8] RT-PCR directly detects *Mycobacterium tuberculosis* (MTB) DNA, effectively bypassing the limitations associated with slow microbial growth. [9] This study aims to evaluate the diagnostic performance and clinical utility of RT-PCR for the rapid confirmation of TB in smear-negative PTB patients within a tertiary care setting in Central India. [10]

AIMS AND OBJECTIVES – To assess the efficacy of real-time PCR in clinically and radiologically suspected smear-negative pulmonary tuberculosis patients. To compare RT-PCR with smear microscopy and Middlebrook 7H9 broth culture for detecting *Mycobacterium tuberculosis* in sputum, BAL, and gastric aspirate samples.

MATERIAL AND METHODS –

A total of 350 clinically suspected, ZN smear-negative samples (sputum, BAL, and gastric aspirate) were collected from outpatients and inpatients at Index Medical College, Indore, following ethical approval. The observational study was conducted from July 2019 to July 2021.

Study design: Cross-sectional observational study.

Sample size - Calculated based on expected sensitivity, specificity, desired precision, and prevalence among smear-negative patients.

- Sensitivity = 85%, Specificity = 90%
- Precision = ±7%

- Prevalence of confirmed TB among smear-negative suspects = 30%

$$N = \frac{Z^2 \times Sn \times (1-Sn)}{d^2 \times p}$$

$$N = \frac{1.96^2 \times 0.85 \times (1-0.85)}{0.07^2}$$

$$N = \frac{99.96}{0.30}$$

So you need ≈**333 suspected smear-negative PTB patients**.

Stastical analysis -

Data were analyzed using SPSS, with continuous variables expressed as mean ± SD or median (IQR) and categorical variables as frequencies (%). RT-PCR diagnostic performance was assessed by sensitivity, specificity, PPV, NPV, overall accuracy, and Kappa agreement, with $p < 0.05$ considered significant.

Inclusion criteria: The study included patients of all ages and genders with signs and symptoms associated with PTB, such as cough lasting more than 2 weeks, weight loss, fever, chest pain, abnormal chest X-ray findings, and two negative sputum smear results.

Exclusion criteria: Patients already diagnosed with PTB and receiving anti-TB treatment.

Sample collection and processing-

All specimens sputum, BAL, or gastric aspirates were transported promptly in sealed, leak-proof containers on ice, following biosafety protocols, and processed within a few hours to preserve viability for smear microscopy, culture, and real-time PCR. Direct smears were prepared from sputum, BAL, or gastric aspirates, heat-fixed, and stained using the Ziehl–Neelsen method with carbol fuchsin, acid-alcohol decolorization, and methylene blue counterstain. Slides were examined under oil immersion (100x) for acid-fast bacilli (AFB) and graded as scanty, 1+, 2+, or 3+ per RNTCP guidelines, with patients showing two negative smears classified as smear-negative for further molecular testing. For culture, specimens were decontaminated with 4% NaOH (2–3 times the sample volume) at 37°C for 30 minutes, centrifuged at 13,000 rpm for 10 minutes, resuspended in sterile saline, and washed twice to remove residual NaOH. Processed samples were labeled and inoculated into Middlebrook 7H9 broth supplemented with OADC (Oleic acid, Albumin, Dextrose, and Catalase) enrichment and PANTA

(Polymyxin B, Amphotericin B, Nalidixic acid, Trimethoprim, and Azlocillin,)antibiotics, incubated at 37 ± 1°C, and monitored twice weekly for six weeks, with positive growth confirmed by Z-N staining for AFB.

Real-Time PCR (RT-PCR) for TB Detection

Molecular detection of *Mycobacterium tuberculosis* in smear-negative pulmonary TB suspects was performed using the Qiagen Care TB PCR Kit (sensitivity: 10 bacteria/mL) on the Stratagene MX300P real-time PCR system. DNA was extracted from decontaminated specimens by adding 30 µL of TB DNA Extraction Solution, followed by incubation at 37°C for 30 minutes, heat treatment at 100°C for 10 minutes, and centrifugation at 13,000 rpm for 10 minutes. PCR amplification was carried out with TB PCR Solution, Taq polymerase, and Uracil-N-glycosylase (UNG) in a dedicated workstation. Each run included negative, high positive, and critical positive controls, and cycling conditions were followed as per the manufacturer's instructions.

RESULT-

Throughout this cross sectional study, various clinical specimens were collected from patients and analyzed in the Department of Microbiology at Index Medical College, Indore (Madhya Pradesh). Out of the 350 smear-negative samples tested using RT-PCR, 100 (28.6%) were positive, while 250 (71.4%) were negative, indicating that nearly one-third of the clinically suspected patients were detected by molecular methods. When analyzed by sample type, RT-PCR positivity was observed across all specimens, with 27.8% (68/245) of sputum samples, 31.4% (22/70) of bronchoalveolar lavage (BAL) samples, and 28.6% (10/35) of gastric aspirates testing positive, highlighting the relatively higher diagnostic yield of BAL specimens. Radiological correlation showed that RT-PCR positivity was highest in patients with cavitary lesions (40/70), followed by infiltrative lesions (32/77), patchy/minimal changes (12/92), fibrotic or old lesions (10/35), and normal X-ray/HRCT findings (6/76). Comparison with clinical diagnosis revealed that out of 120 clinically confirmed TB cases, 85 (70.8%) were concordant with RT-PCR results, while 35 clinically suspected patients tested negative by RT-PCR, underscoring the role of RT-PCR in enhancing detection among smear-negative pulmonary TB patients.

Table 1- RT-PCR Results in Smear-Negative Patients (N = 350)

RT-PCR Result	Number (n)	Percentage (%)
Positive	100	28.6%
Negative	250	71.4%

Total	**350**	**100**

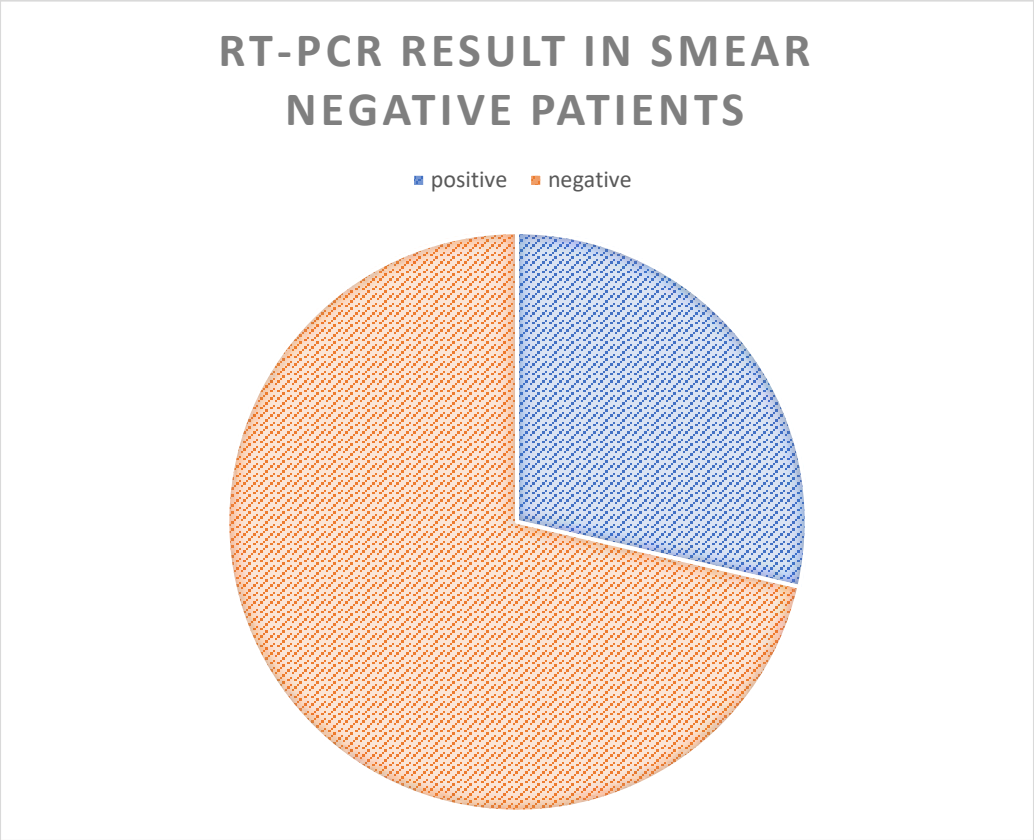

Fig 1- RT-PCR Results in Smear-Negative Patients (N = 350)

Table 2- RT-PCR Positivity by Sample Type

Sample Type	Total	RT-PCR Positive	RT-PCR Negative
Sputum	245	68	177
Bronchoalveolar Lavage	70	22	48
Gastric Aspirate	35	10	25
Total	350	100	250

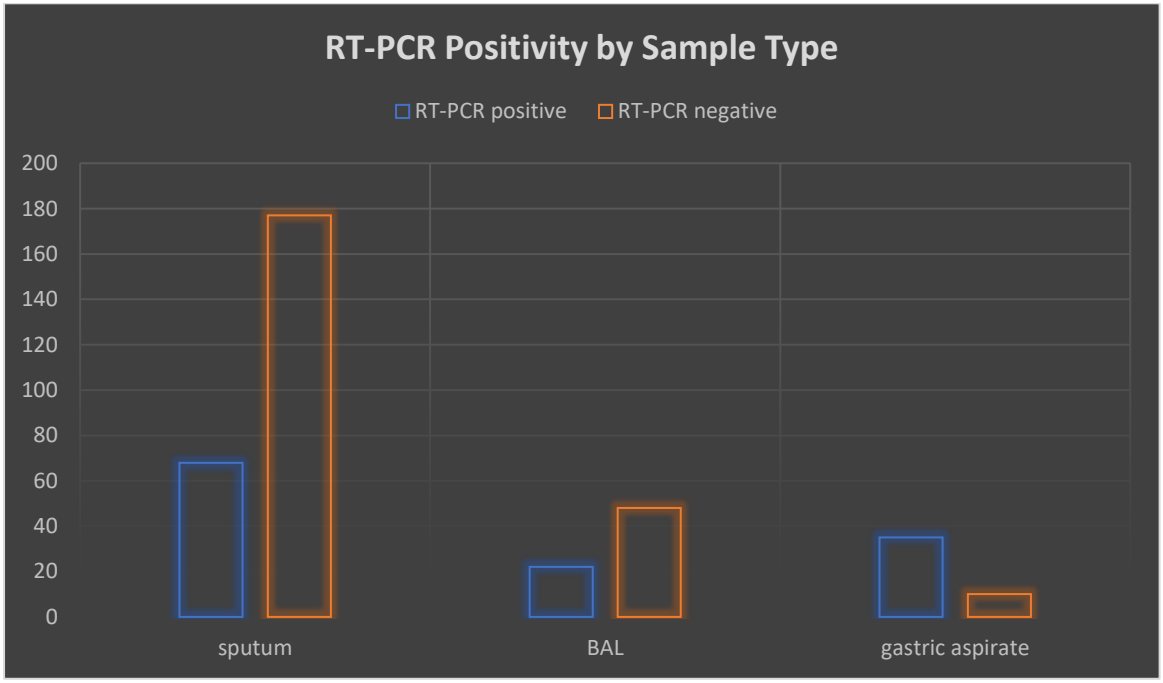

Fig 2- RT-PCR Positivity by Sample Type

Table 3- Radiological Correlation with RT-PCR Results

Radiologic Finding	RT-PCR Positive	RT-PCR Negative	Total
Cavitary lesions	40	30	70
Infiltrative lesions	32	45	77
Fibrotic/old lesions	10	25	35
Patchy/minimal changes	12	80	92
Normal X-ray/HRCT	6	70	76
Total	**100**	**250**	**350**

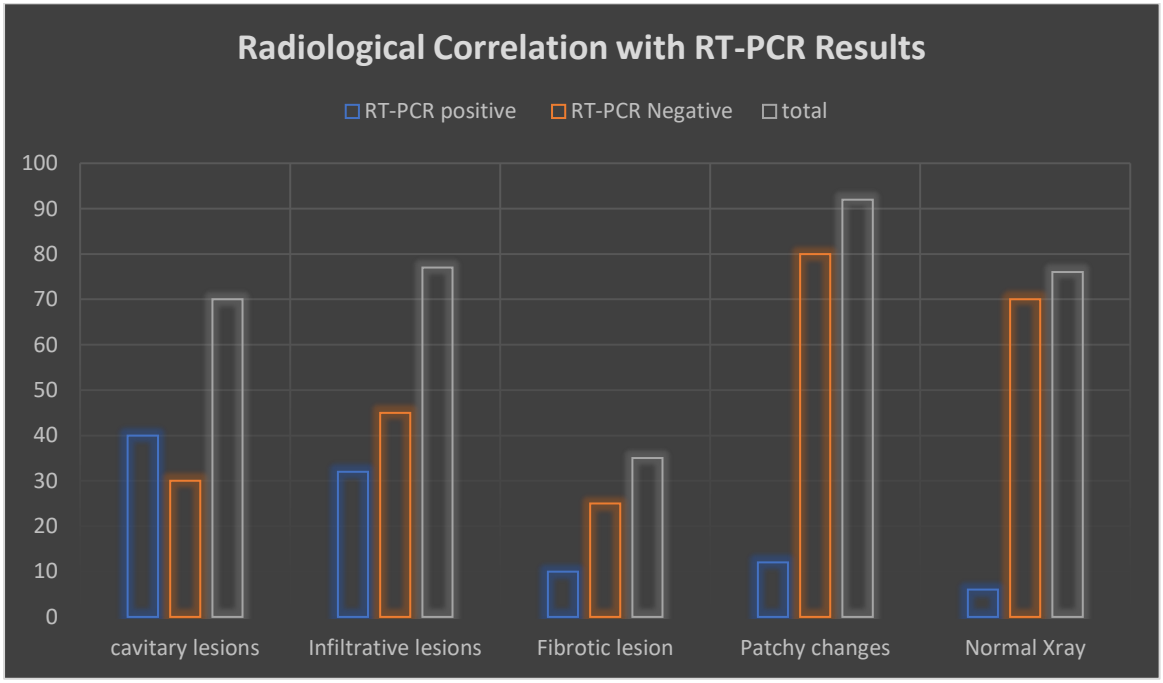

Fig 3- Radiological Correlation with RT-PCR Results

Table 4- Comparison of RT-PCR with Clinical Diagnosis

Category	Number (n)	Concordance with RT-PCR
Clinically suspected TB	350	-
Clinically confirmed TB	120	85 (70.8%)
RT-PCR confirmed TB	100	-
Clinically suspected but RT-PCR negative	35	-

DISCUSSION-

The present study evaluated the utility of real-time PCR (RT-PCR) in detecting Mycobacterium tuberculosis among clinically and radiologically suspected smear-negative pulmonary TB patients. RT-PCR detected 28.6% of cases, outperforming Middlebrook 7H9 broth culture, which identified 20.9% of cases, with the highest positivity observed in bronchoalveolar lavage (BAL) samples (31.4%). These findings are consistent with previous studies demonstrating the

superior sensitivity of molecular assays compared to conventional culture and smear methods.

Several studies support the findings of the present research. Kiran Chawla et al. (2015) [11] reported PCR sensitivity of 74%, higher than culture (72%) and microscopy (56%), demonstrating the superior detection capacity of molecular assays. Similarly, Sah AK et al. (2017, Nepal) [12] observed that multiplex PCR had a higher sensitivity (95.2%) than GeneXpert (90.9%) in smear-negative pulmonary TB, with both methods achieving 100% specificity, consistent with the high accuracy of RT-PCR in this study. Chakraborty S et al. (2016, Kolkata) [13] highlighted the prevalence of rifampicin resistance in culture-positive pulmonary and extra-pulmonary samples and emphasized the advantage of Xpert MTB/RIF for rapid detection, comparable to how RT-PCR facilitated timely TB diagnosis in the current study.

Clinical presentation of smear-negative TB was also addressed in prior studies. Campos LC et al. (2016, Brazil) [14] and Jayamol R et al. (2017, India) [15] noted that these patients often exhibited milder symptoms, such as less frequent cough and hemoptysis, consistent with our observation that systemic features like weight loss and night sweats were more prominent among RT-PCR positive cases. High sensitivity of BAL-based molecular testing was observed both in the present study and in the work of Nikhil G C et al. (2018) [16], highlighting the utility of bronchoscopic sampling in sputum-scarce or challenging cases.

Further evidence supporting molecular methods comes from Chawala K et al. (2017, Central India) [17] and Lombardi et al. (2017, Italy) [18] who reported PCR sensitivities of 100% and 86.5%, respectively, outperforming conventional smear microscopy. Jayamol R et al. (2017) [15] and Gautam BP et al. (2018) [19] reported BAL GeneXpert sensitivity ranging from 31% to 92% and high rifampicin resistance prevalence (26.1%), emphasizing the importance of rapid molecular detection for both diagnosis and drug-resistance screening, in line with our finding that RT-PCR identified more cases than culture.

Comparative studies of molecular and culture-based methods in smear-negative TB further reinforce these observations. Agarwal A et al. (2018) [20] reported BAL culture sensitivity of 87.4%, PCR 73.8%, and AFB smear 61.2%, whereas the present study observed RT-PCR positivity of 28.6%, culture 20.9%, with BAL showing the highest positivity (31.4%). Similarly, Rasool G et al. (2019) [21] reported GeneXpert positivity of 28.57% and culture positivity of 34.52%, comparable to our findings. Priyatam K et al. (2019) [22] reported Xpert MTB/RIF positivity of 23.61%, slightly lower than the 28.6% RT-PCR positivity in this study. Jan F et al. (2020) [23] detected MTB in 20.29% of respiratory samples, while in our study, RT-PCR detected 28.6% and culture 20.9%. Predictive model

analyses by George M. Geert-Jan et al. (2020) [24] demonstrated the importance of clinical and radiological correlation, aligning with our observation that RT-PCR positivity strongly correlated with radiological lesions. Lee Han and Kim Jung et al. (2021) reported Xpert sensitivity of 41.1% and specificity of 100% for smear-negative PTB, comparable to our study findings.

Overall, RT-PCR in the present study detected 28.6% of smear-negative pulmonary TB cases, surpassing Middlebrook 7H9 culture (20.9%) and demonstrating the highest positivity in BAL specimens (31.4%). These findings are consistent with prior studies emphasizing the superior sensitivity of molecular assays over conventional methods. Evidence from Babafemi et al. [25] and Gholoobi et al. (2014) [26] further supports the increased diagnostic yield of bronchoscopic and PCR-based techniques, with sensitivities ranging from 58–86% and specificities of 77–97%. Meta-analyses confirm pooled pulmonary TB sensitivity at 82% (95% CI 0.81–0.83) and specificity 99% (95% CI 0.99–0.99), while extrapulmonary TB had sensitivity of 70% (95% CI 0.67–0.72) and specificity 99%, demonstrating the global reliability of molecular assays in detecting paucibacillary TB. Collectively, these data indicate that the RT-PCR results of the present study, though numerically lower, align with international evidence supporting molecular diagnostics as a highly sensitive tool, particularly when combined with radiological assessment and appropriate specimen selection.

Conclusion: RT-PCR provides superior diagnostic performance over conventional smear and culture methods in smear-negative pulmonary TB, with BAL specimens yielding the highest positivity. Integration of molecular diagnostics with clinical and radiological assessment can enhance early detection and improve patient management.

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