

Gene Xpert MTB/RIF Assay and Conventional Microscopy in Tuberculosis among Silicosis Patients of Western Rajasthan

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Abstract:

Tuberculosis remains a major public health challenge in silicosis patients due to altered lung pathology and low bacillary load, leading to diagnostic difficulties. To study detection of MTB by Microscopy (ZN & FM) and molecular detection by CBNAAT in silico tuberculosis patients. A total of 410 patients were included, predominantly male (96.34%), with a higher representation from rural areas (71.46%). ZN microscopy revealed acid-fast bacilli in only 14.88% of cases, with 85.12% remaining smear-negative because of its limited sensitivity, and FM demonstrated improved detection, with 37.32% positivity across varying smear grades. CBNAAT detected MTB in 55.85% of cases. Among CBNAAT-positive samples, rifampicin sensitivity was observed in 85.15%, while 14.84% showed resistance. Of the resistant cases, medium-level resistance was most common (50%), followed by high-level resistance (38.23%). Our findings highlight the superior sensitivity of CBNAAT in silico tuberculosis and reinforce its routine use for early diagnosis and drug-resistance detection.

Keywords: ZN, FM, CBNAAT, MTB

Introduction

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis*, also known as Koch's bacillus. Silicosis, the most common form of pneumoconiosis, results from the inhalation of respirable silica-containing dust generated from materials such as coal, asbestos, glass, and stone. When tuberculosis and silicosis coexist in an individual, the resulting clinical condition is referred to as silico-tuberculosis (silico-TB). The term silicosis was first introduced by Visconti in 1870, while *M. tuberculosis* was first identified by Robert Koch in 1882. (1)

The Government of India, through the Ministry of Health and Family Welfare, has set a goal oriented target to eradicate TB by the year 2025 under the National Strategic Plan (2017–2025) (2,3). Simultaneously, the International Labour Organization and the World Health Organization aim to eliminate silicosis from developing countries by 2030 (4). Complete elimination of TB without addressing silicosis, or vice versa, remains an unachievable objective. TB is one of the most frequent diseases associated with silicosis in low and middle income countries, second only to HIV/AIDS. Silicosis is recognized as a predisposing factor for TB, significantly enhancing the probability of both pulmonary and extrapulmonary tuberculosis compared with individuals without silicosis. (5)

Silica, particularly crystalline silica in the form of silicon dioxide or quartz, is a naturally occurring compound found plentifully in the earth's crust. Various industrial activities including mining, sandblasting, quarrying, and the manufacture of synthetic stone generate airborne silica dust, leading to occupational exposure. Silica exists in both crystalline and amorphous forms, each possessing special physicochemical properties. Among these, crystalline silica exhibits the highest pathogenic potential due to its polymerized tetrahedral framework, rendering it more threatening than amorphous silica. The crystalline structure enhances its biological reactivity and contributes to severe health hazards upon respiration.(6)

Industries such as ceramics, construction, and foundries are well recognized for their association with silica related occupational risks. Within these sectors, certain job roles carry an increased risk of silica exposure, including the use of silica-based fillers and abrasives, stone cutting (including monumental masonry and tombstone preparation), polishing and cutting of gemstones, masonry work in furnaces, and abrasive blasting. Sandblasting is widely employed in engineering, shipbuilding, and metal and automotive repair industries but poses significant occupational health risks due to intense silica exposure.(7)

Accurate and early diagnosis of tuberculosis (TB) remains a basis of TB control, specifically in high-burden countries like India. The present study evaluated the diagnostic yield of Ziehl–Neelsen (ZN) microscopy, fluorescent microscopy (FM), and cartridge-based nucleic acid amplification test (CBNAAT), along with the detection of rifampicin resistance among presumptive silico tuberculosis patients.

Method and material

Aim and objective

To study detection of MTB by Microscopy [Ziehl–Neelsen (ZN) staining, fluorescent microscopy (FM)] and molecular detection by Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) in silico tuberculosis patients.

Study Design: Hospital-based cross-sectional study

Study Duration: The study was conducted over a period from June 2024 to May 2025 after taking permission from the institutional ethical committee.

Study Population: The Study Population consists of patients with a confirmed diagnosis of silicosis who were subsequently evaluated for suspected active pulmonary tuberculosis based on the emergence of new or progressively worsening clinical symptoms and radiological findings.

Inclusion Criteria

- Patients presenting with symptoms suggestive of pulmonary silico-tuberculosis at tertiary care hospitals in western Rajasthan.
- Patients who have been suspected with silico-tuberculosis based on clinical evaluation , imaging studies with history

Exclusion Criteria

Patients with a confirmed diagnosis of a respiratory disease other than pulmonary and extrapulmonary TB.

Sample Collection & Transportation

Suspected silico-TB patients from clinical diagnosis and patient result history review were given a sputum, BAL, and pleural fluid sample collection container, with instructions on how to collect and deliver sputum and other pulmonary samples to the facility. The pulmonary sample, together with the SRF from each diagnostic site, was packed in a standard triple packaging container and an ice bag was inserted to keep the sample at 2-8 °C. All the samples were transported to the molecular diagnostic testing laboratory.

Sputum and other pulmonary samples, together with the lab request form, were received and checked for completeness of the laboratory request form, correct sample tube labelling and leakage. Those accepted were given laboratory numbers for processing to perform microscopy and CBNAAT (GeneXpert) (Cepheid, Sunnyvale, CA) MTB/RIF based on NTEP guideline (8)

Microscopy

The smear were prepared from samples and directly stained by Ziehl-Neelsen or Auramine O Fluorescent staining and was microscopically examined.

CBNAAT - GeneXpert MTB/RIF

The GeneXpert MTB/RIF (Cepheid, Sunnyvale, CA) test was performed as per the manufacturer's instruction. Aliquots of decontaminated samples were taken out of 4 °C storage, together with the sample reagent buffer containing NaOH and isopropanol was mixed at the ratio of 1:2 followed by incubation at room temperature for 15 min. Two millilitres of the sample were then transferred into the GeneXpert MTB/RIF cartridge (Cepheid, Sunnyvale, CA) and loaded into the GeneXpert MTB/RIF (Cepheid, Sunnyvale, CA). The results were generated after 2 hours, reported as both M. tuberculosis negative or positive, and whether those positive were RIF (Rifampicin) susceptible or resistant.

Results:

In our study, gender-wise distribution Male (96.34%) was predominance as compared to females (3.60%) and 71.46% of patients were from rural areas and 28.53% were from urban settings. (Fig1&2) The age-wise distribution of the majority of TB cases occurred in the 31–50 year age group, with peak prevalence in the 41–50 years (25.12%) followed closely by the 31–40 years group (24.14%). (Table:-1) Sputum was the most commonly received and preferred specimen (91.70%), followed by bronchoalveolar lavage (6.82%) and pleural fluid (1.46%). (Table :-2) ZN staining showed a high proportion of smear-negative cases (85.12%), with only 14.88% demonstrating varying grades of acid-fast bacilli positivity and only 62.68% of cases were FM-negative, while 37.32% showed bacillary positivity with various such as 1,2,3,+ grades, including higher proportions of scanty (12.43%) followed by 1+ (9.02%), 2+ (10.48%) and 5.36% 3 grade positives. (Table:-3 & 4)

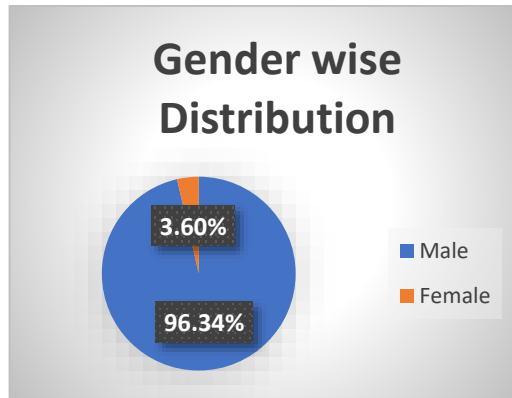


Figure :1 Gender wise distribution

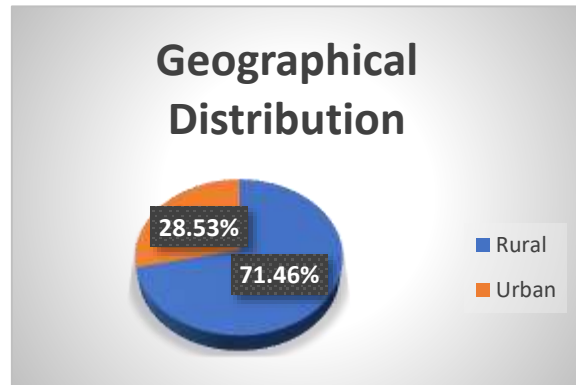


Figure:2 Geographical Distribution

Table:1 Age group wise Distribution

Age group	Male	Female	Total	Percentage
<10	0	0	0	0%
11-20	4	0	4	0.97%
21-30	29	1	30	7.31%
31-40	95	4	99	24.14%
41-50	100	3	103	25.12%
51-60	71	3	74	18.04%
61-70	69	4	73	17.80%
71-80	25	0	25	6.09%
>90	2	0	2	0.48%
Total	395	15	410	100%

Table:2 Samples wise Distribution

Types Of Sample	No. Of Patient's	Percentage
Sputum	376	91.70%
BAL	28	6.82%
Plural Fluid	6	1.46%
Total	410	100%

Table:3 ZN Staining Results

ZN Staining Result	No. of Patient's	Percentage
Negative	349	85.12%
Scanty	31	7.5%
1	26	6.34%
2	4	0.97%
3	0	0.00%
Total	410	100%

Table:4 FM Staining Results

FM Staining Result	No. Of Patient's	Percentage
Negative	257	62.68%
Scanty	51	12.43%
1	37	9.02%
2	43	10.48%
3	22	5.36%
Total	410	100%

Table:5 CBNAAT Results

CBNAAT Result	No. Of Patient's	Percentage
Detected	229	55.85%
Non Detected	181	44.14%
Total	410	100%

Table:6 Detected CBNAAT Results

Detected CBNAAT Result	No. Of Patient's	Percentage
Sensitive	195	85.15%
Resistance	34	14.84%
Total	229	100%

Table:7 RIF Resistant CBNAAT Results

Resistance CBNAAT	No. OF Patient's	Percentage
High Level Resistance	13	38.23%
Medium Level Resistance	17	50.00%
Low Level Resistance	4	11.76%
Total	34	100%

Table 8: Comparative analysis Between ZN Microscopy , Fluorescence Microscopy and CBNAAT (Cartridge Based Nucleic Acid Amplification Test) (n=410)

Results	ZN Microscopy		Fluorescence Microscopy		CBNAAT	
	Positive	Negative	Positive	Negative	Positive	Negative
Total Samples (n=410)	61(14.87%)	349(85.12%)	153(37.31%)	257(62.68%)	229(55.85%)	181(44.14%)

Table 9: Positive Predictive Value (PPV)

Diagnostic Method	True Positive (TP)	False Positive (FP)	PPV (%)
ZN Microscopy	61	0	100%
Fluorescence Microscopy	153	0	100%

Formula used: $PPV = TP / (TP + FP) \times 100$

Table 10: Negative Predictive Value (NPV)

Diagnostic Method	True Negative (TN)	False Negative (FN)	NPV (%)
ZN Microscopy	181	168	51.86%
Fluorescence Microscopy	181	76	70.43%

Formula used: $NPV = TN / (TN + FN) \times 100$

Table 11: Statistical Significance (Chi-Square Test)

Comparison	χ^2 Value	df	p-value	Statistical Significance
ZN Microscopy vs CBNAAT	56.67	1	<0.0001	Highly Significant
Fluorescence Microscopy vs CBNAAT	192.90	1	<0.0001	Highly Significant

Discussion:

The present study was conducted on 410 patients with suspected silico tuberculosis attending tertiary care hospital in western Rajasthan. In our study A male (96.34%) predominance this was similar to Rao VG et al (9) and Horton et al(10) reported significantly higher TB burden among males because of occupational exposure, higher prevalence of smoking and alcohol consumption among males, increased healthcare-seeking behavior and sociocultural barriers limiting access to diagnostic services for females. In our study greater population of rural areas (71.46%), which was similar to Sathiyamoorthy et al. (11) and Chadha et al. (12) study due to burden of TB in rural populations especially in resource limited region increase because of overcrowding, poor ventilation, malnutrition, and limited healthcare infrastructure remain. The age group wise findings indicate that TB predominantly affects individuals in their economically productive years, leading to substantial socioeconomic consequences. (13) Sputum sample is easy to collect for pulmonary TB samples due to its accessibility and cost effectiveness.(14) ZN Staining finding elevates that the limited sensitivity of conventional ZN microscopy specially in patients with paucibacillary load, early disease, extrapulmonary involvement, or co-infected with HIV. Lina et al. (15) reported FM more sensitive than ZN microscope with higher positivity. Fluorescent microscopy indicated that a significantly higher detection rate compared to ZN staining. The improved sensitivity of FM can be attributed to its ability to screen a larger smear area at lower magnification and detect lower bacillary loads. (16,17) These findings Strengthen WHO and NTEP recommendation for the replacement of conventional ZN microscopy with LED-based fluorescent microscopy wherever practicable.(18,8) CBNAAT detected Mycobacterium tuberculosis in 55.85% which was consistent with findings from Boehme et al (19) and Theron et al (20) who reported detection rates of approximately 60% in similar to symptomatic cohorts. This establish the superior sensitivity of CBNAAT, especially in smear-negative and paucibacillary cases. The ability of CBNAAT to provide rapid results within two hours, along with simultaneous detection of rifampicin resistance.

Rifampicin resistance pattern Among CBNAAT-detected cases, 14.84% were found to be rifampicin resistant. This prevalence is clinically significant and indicates an ongoing burden of drug-resistant TB in the study population. In India TB report and NTEP Annual reported RR/MDR TB prevalence among new TB patients 5-10% and more in retreatment patients (18,8). In occupational and higher risk setting RR can be 10-15% increases. Singh et al(21) was reported RR/MDRTB 10-18% whereas WHO Global TB Report reported new TB cases often lower RR (3-6%) prevalence rate which was dissimilar to our finding (18) Rifampicin resistance is considered a surrogate marker for multidrug-resistant TB, and its early detection is necessary for initiating appropriate second-line treatment regimens.(19,22)

Distribution of rifampicin resistance levels Further analysis of rifampicin-resistant cases revealed that medium-level resistance was the most common (50%), followed by high-level resistance (38.23%) and low-level resistance (11.76%). This distribution suggests heterogeneous resistance patterns, possibly reflecting different mutation profiles within the *rpoB* gene.(22) The prevalence of medium- and high-level resistance is of particular concern, as these mutations are often associated with poor treatment outcomes if not managed appropriately.(21,22) CBNAAT's potentiality to categorize resistance levels provides opinions for clinicians and supports the need for confirmatory drug susceptibility testing and individualized patient management.(19,8)

The findings of this study indicate a significant male and rural prevalence of tuberculosis, with the disease primarily affecting individuals in their productive age groups. The data emphasize the need for improved access to rapid molecular diagnostics in rural settings and highlight the continued relevance of sputum-based testing. Integration of CBNAAT with conventional microscopy significantly improves diagnostic yield and facilitates early detection of drug resistance, which is critical for TB control under the National Tuberculosis Elimination Programme (NTEP).

The comparative analysis of diagnostic procedures in our study clearly establish that reliance on microscopy alone would have resulted in substantial failure to diagnose of TB. While FM improves detection over ZN staining, CBNAAT offers the highest diagnostic yield along with the critical advantage of rapid drug resistance detection. These findings strongly support the integration of CBNAAT as a primary diagnostic test for presumptive TB cases as a current NTEP and WHO guidelines. The GeneXpert MTB/RIF assay is a simple, rapid, and accurate method for detecting *M. tuberculosis*, is less dependent on the operator's skills, and staff with minimal training can use the equipment easily. Although the GeneXpert MTB/RIF assay has these advantages, similar to other tests for *M. tuberculosis*, a negative result cannot exclude the diagnosis of TB, and patients with positive results can also be estimated thoroughly with results of the ZN smear test, culture, clinical symptoms, and radiographic evidence. (Table 8)

In Conclusion, the present study demonstrates the limitations of conventional microscopy and emphasize the diagnostic superiority of CBNAAT in detecting both tuberculosis and rifampicin resistance. The conclusion focus on the need for strengthening molecular diagnostic infrastructure to achieve timely diagnosis and effective TB control. The CBNNAT MTB/RIF Assay method has a short turnaround time and simultaneously detects *M. tuberculosis* and RIF resistance in less than 2 hours. It could be a useful tool for rapid identification of *M. tuberculosis*, mostly in smear-negative clinical samples.

Reference:

1. Pandit, Shally; Chandan, Sudatta; Barik, Braja S.; Hussain, Tahziba; Pati, Sanghamitra. Tuberculosis and Silicosis: an insightful perspective. The Egyptian Journal of Chest Diseases and Tuberculosis 74(2): p 134-146, April-June 2025. | DOI: 10.4103/ecdt.ecd
2. Sachdeva KS, Parmar M, Rao R, Chauhan S, Shah V, Pirabu R, et al. Paradigm shift in efforts to end TB by 2025. Indian Journal of Tuberculosis 2020; 67: S48–S60.
3. Khanna A, Saha R, Ahmad N. National TB elimination programme – what has changed. Indian Journal of Medical Microbiology 2023; 42:103–107.
4. Bhargava A, Bhargava M, Juneja A. Social determinants of tuberculosis: context, framework, and the way forward to ending TB in India. Expert Review of Respiratory Medicine 2021; 15:867–883.

5. Lanzafame M, Vento S. Mini-review: Silico-tuberculosis. *Journal of Clinical Tuberculosis and Mycobacterial Disease* 2021; 23:100218.
6. Adamcakova J, Mokra D. New insights into Patho-mechanisms and treatment possibilities for lung silicosis. *International Journal of Molecular Sciences* 2021; 22:4162.
7. Rees D, Murray J. Silica, silicosis and tuberculosis. *Occupational Health Southern Africa* 2020; 26:266–276.
8. National TB Elimination Program (India). NTEP overview. <http://en.wikipedia.org/wiki/>
9. Rao VG, Bhat J, Yadav R, Gopalan GP, Nagamiah S, et al. Prevalence of Pulmonary Tuberculosis - A Baseline Survey In Central India. *PLoS ONE* (2012)7(8): e43225. doi:10.1371/journal.pone.0043225
10. Horton KC, MacPherson P, Houben RMGJ, White RG, Corbett EL, Sex Differences in Tuberculosis Burden and Notifications in Low- and Middle-Income Countries: A Systematic Review and Meta-analysis. *PLoS Med* (2016)13(9): e1002119. doi:10.1371/journal.pmed.1002119
11. Sathiyamoorthy Ramadass, Kalaivani, Mani, Aggarwal Praveen, Gupta Sanjeev Kumar, Prevalence of pulmonary tuberculosis in India A systematic review and meta-analysis *Lung India* 37(1):p 45-52, Jan-Feb 2020.
12. Chadha VK, Kumar P, Anjinappa SM, Singh S, and Narasimhaiah S et al., Epidemiological situation of tuberculosis in India *Indian Journal of Medical Research* 2012;135:678-688.
13. Dye Christopher and William BG. The Population dynamics and control of Tuberculosis *Science* 2010;328:856-861.
14. Steingart KR, Ng V, Henry M, Hopewell P C, Ramsey A, Jane Cunningham J et al. Sputum Processing methods to improve the sensitivity of smear microscopy for tuberculosis :- A systemic review *The Lancet infectious Diseases*.2006;6(10); 664-74
15. Lina TT, Rahman MA, Arifeen SE, et al. Comparison of Ziehl- Neelsen Microscopy and Fluorescence Microscopy for the diagnosis of pulmonary tuberculosis. *BMC infection Disease*.2009;9:191.
16. Steingart KR, Megan Henry, Vivienne Ng, Prof Philip C Hopewell, Andrew Ramsey, Jane Cunningham et al. Fluorescence versus conventional sputum microscopy for tuberculosis a systemic review. *The Lancet infectious Diseases*.2006;6(9);570-581
17. Cattamanchi A, Dowdy DW, Davis JL, Worodria W, Yoo SD, Joloba M , et al. Performance of LED Fluorescence microscopy for diagnosis of pulmonary tuberculosis in HIV infected patients *American Journal of Respiratory Critical Care Medicine* 2009;179(6)527-534
18. World Health Organization (WHO) Global Tuberculosis Report 2024. Geneva; WHO; 2024 Supports limitations of smear microscopy ,role of CBNAAT and drug resistant TB burden.
19. Catharina C. Boehme, Pamela Nabeta, Doris Hilleman, Mark P. Nicol, Shubhada Shenai, Fiorella Krapp, et al . Rapid molecular detection of tuberculosis and Rifampicin Resistance. *New England journal of Medicine*.2010;363:1005-1015.
20. Theron G, Zijenah L, Chanda D, Clowes P, Rachow A, Lesosky M, et al. The impact of Xpert MTB/RIF for tuberculosis diagnosis :a systemic review. *European Respiratory Journal*.2014;44 (3)670-684.
21. Singh UB, Mehta G, Singla N, Jain A, Gupta P, Behera D, et al. Drug resistant tuberculosis in India: current status and challenges. *Indian Journal of Medical Research*.2018;147(6)527-539
22. Armand Van Deun, Kya J. M. Aung, Valentin Bola, Rossin Lebeke, Mohamed Anwar Hossain, Willem Bram de Rijk et al., Rifampicin Drug resistance tests for tuberculosis challenging the gold standard *Journal of clinical Microbiology* 2013;51(8)2633-2640.