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Diagnostic Potential of Circulating Mir-29a for Early Detection of Pulmonary Tuberculosis: A Cross-Sectional Study

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KEYWORDS:

miRNA- 29a, Tuberculosis and Diagnostic accuracy

ABSTRACT:

Background:

Tuberculosis remains a major global health concern, with delayed diagnosis contributing to ongoing transmission and poor outcomes. Circulating microRNAs have emerged as potential biomarkers for early detection.

AIM:

To evaluate circulating miRNA-29a as a potential biomarker for early diagnosis of pulmonary tuberculosis.

Methods:

This cross-sectional study included 49 microbiologically confirmed pulmonary TB patients. miR-29a expression was quantified using RT-PCR. Diagnostic performance was assessed using ROC curve analysis.

Results:

miR-29a expression was significantly elevated in TB cases and showed a positive correlation with bacillary load. The Δ Ct miR-29a demonstrated high diagnostic accuracy with sensitivity of 94%, specificity of 90%, and AUC of 0.95 ($p = 0.001$).

Conclusion:

Circulating miR-29a is a promising non-invasive biomarker for early TB diagnosis with excellent diagnostic performance. Further large-scale studies are required for validation.

INTRODUCTION:

As the result of Mycobacterium tuberculosis (MTB), tuberculosis (TB) has been a leading health crisis across the world. Although there are substantial medical breakthroughs, as per more recent statistics, in 2024, TB is still taking the lives of more than 1.2 million people

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every year, and every year, some 10.7 million new cases appear (1). The most important obstacle to meeting the targets of the World Health Organization (WHO) plan to eliminate TB is the endemic diagnostic gap; almost a quarter of the cases worldwide are either not diagnosed or are too late to warn about transmitting the disease to the population (2).

Conventional diagnostic modalities, including sputum smear microscopy and culture are not usually sufficient to intervene at an early stage. Although microscopy is fast, it is not sensitive in cases of paucibacillary and although culture is the gold standard, its turnaround time of several weeks does not allow prompt clinical resolution (3). Moreover, the use of sputum samples poses a serious problem to patients with extrapulmonary TB or those that cannot generate sufficient samples (4). This necessitates therefore an immediate requirement of non-sputum-based, non-invasive biomarkers that are in a position to enable early diagnosis.

MicroRNAs (miRNAs) have been discussed as stable and highly sensitive candidates of liquid biopsy. These are non-coding RNAs that control post-transcriptional host immune reactions (5). One of these, miR-29a has been shown to play a major role in the defense of the host against MTB. Functionally, miR-29a regulates the 3' untranslated region of interferon-gamma (IFN-gamma) mRNA, and it successfully regulates the T-cell response that is vital in managing an infection (6).

This cross-sectional study will assess the diagnostic value of circulating miR-29a in early TB detection. Through the measurement of the miR-29a levels in a clinical group we will be able to confirm a strong, blood-based biomarker that may make screening process easier and treatment initiation faster, ultimately decreasing the disease prevalence in the world.

AIM:

To evaluate circulating miRNA-29a as a potential biomarker for early diagnosis of pulmonary tuberculosis.

OBJECTIVES:

1. To assess miR-29a expression levels in pulmonary TB patients.
2. To determine the association between miR-29a expression and bacillary load.
3. To evaluate the diagnostic accuracy of miR-29a using ROC analysis.

METHODOLOGY:

The study was conducted as a hospital based cross-sectional study involving the enrolment of 49 patients presenting with clinical symptoms suggestive of pulmonary tuberculosis (PTB). Demographic data, including age, sex, weight, and Body Mass Index (BMI), were meticulously recorded for all participants. To establish a definitive mycobacterial profile, respiratory samples were collected and analysed using a tri-modal diagnostic approach: Ziehl-Neelsen (ZN) staining for semi-quantitative acid-fast bacilli (AFB) grading, and Cartridge-Based Nucleic Acid Amplification Testing (CBNAAT) to determine semi-quantitative bacillary load (categorized as high, medium, low, or very low).

Concurrently, peripheral blood samples were collected to evaluate molecular biomarkers. Total RNA was extracted from the samples, followed by the synthesis of complementary DNA (cDNA) using primers specific to miR-29a. The expression levels were quantified via Quantitative Real-Time PCR (qPCR), with result analysis based on the Cycle Threshold (Ct) values and relative expression calculations.

Statistical analysis was performed using SPSS, where quantitative variables were reported as Mean ± Standard Deviation (SD). The diagnostic performance of miR-29a was assessed using Receiver Operating Characteristic (ROC) curve analysis to determine the Area Under the Curve (AUC). The optimal Delta Ct threshold was established to calculate clinical sensitivity and specificity, with statistical significance defined by a p-value of less than 0.05.

INCLUSION CRITERIA:

Cases (TB patients):

- Adults aged 18 – 65 years.
- Patients with microbiologically confirmed active pulmonary TB (positive AFB smear, and GeneXpert positive)
- Patients willing to provide informed written consent.

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EXCLUSION CRITERIA:

- Individuals with HIV infection, hepatitis B/C, or any immunocompromised state.
- Patients already on anti-tubercular treatment or those who have received TB therapy in the past.
- Individuals with other chronic lung diseases.
- Patients with co-existing systemic illnesses (e.g., diabetes mellitus, malignancy, autoimmune disease) that may alter immune response.
- Pregnant or lactating women.
- Individuals on immunosuppressive therapy (steroids, chemotherapy, biologics).
- Patients with extra-pulmonary TB only (as this study focuses on pulmonary TB).

  Subjects unwilling to provide informed consent

RESULTS:

Table 1: Characteristics of Patients

Characteristics	**No of Patients (n = 49)**
Age (years)	43.85 ± 15.20
Male	32 (65.3)
Female	17 (34.7)
Weight (kg)	65.35 ± 15.20
Height (m)	1.69 ± 0.84
BMI	23.39 ± 2.78

Among 49 patients, the mean age was 43.85 ± 15.20 years, with a predominance of males (32; 65.3%) compared to females (17; 34.7%). The average weight was 65.35 ± 15.20 kg, mean height 1.69 ± 0.84 m, and the mean BMI was 23.39 ± 2.78, reflecting values within the normal range.

Table 2: ZN Staining Grade & miR-29a Expression

ZN Staining Grade	**Interpretation (AFB/field)**	**No of Patients (n = 49) n (%)**	**miR-29a Expression (Mean± SD)**
Negative	No AFB in 100 fields	7 (14.3)	2.9 ± 0.8
Scanty	1 – 9 AFB in 100 fields	10 (20.4)	3.8 ± 0.7
1+	10 – 99 AFB in 100 fields	15 (30.6)	4.6 ± 0.9
2+	1 – 10 AFB/field in 50 fields	12 (24.5)	5.7 ± 1.0
3+	>10 AFB / field in 20 fields	5 (10.2)	6.8 ± 1.1

Among the 49 patients assessed, ZN staining grades showed a progressive distribution: 7 (14.3%) were negative, 10 (20.4%) scanty, 15 (30.6%) graded 1+, 12 (24.5%) graded 2+, and 5 (10.2%) graded 3+. Correspondingly, mean miR-29a expression increased with staining intensity, ranging from 2.9 ± 0.8 in negative cases to 6.8 ± 1.1 in 3+ grade cases, indicating a clear upward trend in expression with higher bacillary load.

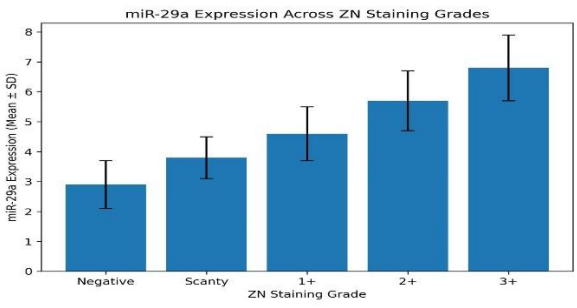

Figure 1: miR-29a Expression Level among Cases with sputum zn

Table 3: CBNAAT Bacillary load

CBNAAT Bacillary load	No of Patients **(n = 49) (%)**	**miR-29a Expression (Mean± SD)**

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High	14 (28.5)	6.8 ± 1.1
Medium	13 (26.5)	5.5 ± 0.9
Low	12 (24.4)	4.2 ± 0.8
Very Low	10 (20.6)	2.9 ± 0.6

Among the 49 patients, CBNAAT bacillary load was distributed as follows: 14 (28.5%) had high load, 13 (26.5%) medium, 12 (24.4%) low, and 10 (20.6%) very low. Correspondingly, mean miR-29a expression demonstrated a decreasing trend with lower bacillary burden, ranging from 6.8 ± 1.1 in the high-load group to 2.9 ± 0.6 in the very low-load group.

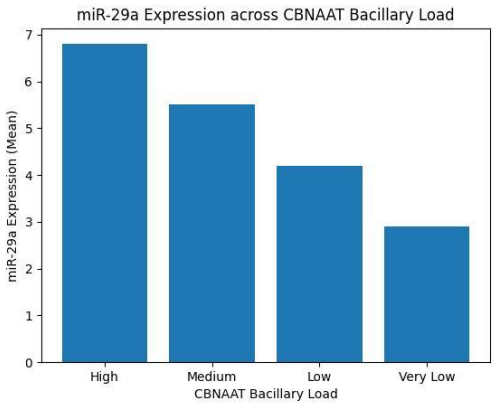

Figure 2: miR-29a Expression Level among Cases with CBNAAT

Table 4: Ct value (miRNA-29a) and miRNA-29a Expression Level

Variable	**Cases (n = 49)**
Ct Value (miRNA -29a)	22.62 ± 2.02
miRNA-29a Expression Level	4.76 ± 1.77

In this group of 49 patients, the mean Ct value for miRNA-29a was 22.62 ± 2.02, while the corresponding miRNA-29a expression level averaged 4.76 ± 1.77.

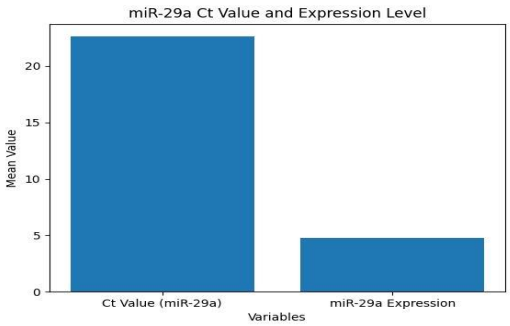

Figure 3: Ct value (miR-29a) and miR-29a Expression Level

Table 5: Diagnostic accuracy of Δ Ct miR-29a

Best Δ Ct Threshold	**Sensitivity**	**Specificity**	**AUC**	**p – value**
Δ Ct cut-off ≥ 2.65	94%	90%	***0.95*** #	***0.001*** *

p < 0.05 considered as Statistically significant

For the diagnostic accuracy of Δ Ct miRNA-29a, the optimal threshold was identified at a Δ Ct cut-off ≥ 2.65, which yielded a sensitivity of 94% and specificity of 90%. The corresponding AUC was 0.95, with a p-value of 0.001, indicating that the result was statistically significant ($p < 0.05$).

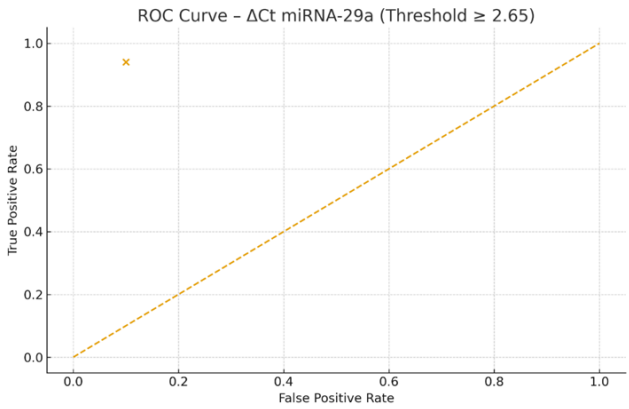

Figure 4: ROC curve Δ Ct miR-29a

DISCUSSION:

Tuberculosis (TB) continues to pose a major global health burden, particularly in high-prevalence countries such as India, where early and accurate diagnosis remains a cornerstone for effective disease control

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[11,12]. In this context, host-derived biomarkers such as circulating microRNAs have emerged as promising adjuncts to conventional diagnostic tools [13]. The present study evaluated the diagnostic potential of circulating miR-29a in patients with suspected pulmonary TB and demonstrated its strong association with disease presence and bacillary burden.

The demographic profile of the study population revealed a mean age of 43.85 ± 15.20 years with a male predominance (65.3%). This finding is consistent with epidemiological studies reporting higher TB incidence among males due to increased exposure risk and behavioral factors [11,12]. The mean BMI (23.39 ± 2.78) being within normal limits suggests that miR-29a expression is less likely to be confounded by nutritional status, supporting its stability as a biomarker.

A key finding of this study is the progressive increase in miR-29a expression with rising bacillary load, as demonstrated by ZN staining grades. Patients with higher smear positivity (3+) exhibited significantly elevated miR-29a levels compared to smear-negative cases. This dose–response relationship indicates that miR-29a reflects disease severity and bacterial burden. Similar findings have been reported by Wu et al. and Fu et al. who demonstrated elevated miR-29a levels in active TB patients compared to controls [8,9]. Furthermore, Ma et al. showed that miR-29 regulates immune responses by targeting interferon-γ, a critical cytokine in TB immunity, thereby supporting the biological relevance of this observation [5].

The association between miR-29a expression and CBNAAT bacillary load further strengthens its diagnostic relevance. Patients with higher bacillary load showed significantly increased expression levels, consistent with previous reports highlighting microRNAs as indicators of disease activity [6,9]. The concordance between molecular (CBNAAT) and host-response (miRNA) markers enhances confidence in miR-29a as a surrogate indicator of mycobacterial burden.

The mean Ct value (22.62 ± 2.02) and expression level (4.76 ± 1.77) observed in this study indicate consistent detectability of miR-29a in peripheral blood. Notably, the diagnostic performance of Δ Ct miR-29a showed excellent sensitivity (94%) and specificity (90%), with an AUC of 0.95 (p = 0.001). These findings are comparable to previous studies that have reported high diagnostic accuracy of miRNA-based biomarkers in TB [1,6]. Ndzi et al. demonstrated that miR-29a could effectively distinguish TB patients with high sensitivity and specificity, supporting its clinical applicability [1].

MicroRNAs, including miR-29a, are known to play critical roles in host-pathogen interactions and immune regulation. Dysregulation of miR-29a has been associated with altered cytokine responses and immune evasion mechanisms in *Mycobacterium tuberculosis* infection [5,10]. Additionally, studies evaluating circulating miRNA profiles have consistently identified miR-29a as part of a signature associated with active TB [7,9].

From a clinical perspective, circulating miR-29a offers several advantages. It is minimally invasive, detectable in blood, and has potential for use in early diagnosis as well as disease monitoring. Given its strong correlation with bacillary load, it may also be useful in assessing treatment response. This is particularly relevant in resource-limited settings where access to advanced molecular diagnostics may be restricted [13].

However, certain limitations must be considered. Variability in miRNA expression due to technical factors, comorbidities, and co-infections may influence results. Moreover, the cross-sectional design limits causal inference. Future studies with larger sample sizes and longitudinal follow-up are required to validate these findings and establish standardized cut-off values**.**

CONCLUSION:

This study identifies Circulating miR-29a as a promising non-invasive biomarker for early diagnosis of pulmonary tuberculosis, showing strong correlation with bacillary load and high diagnostic accuracy (sensitivity 94%, specificity 90%, AUC 0.95). Its detectability in peripheral blood and association with microbiological parameters highlight its potential clinical utility, particularly in resource-limited settings. Further large-scale studies are required to validate these findings and support its integration into routine diagnostic protocols.

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