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Research Article

A pilot study of circulating *miR-361-5p* and *miR-3125* in active tuberculosis patients in Babylon Province, Iraq

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Abstract
Tuberculosis (TB) remains a major global health challenge and is closely linked to host immune responses to *Mycobacterium tuberculosis*.MicroRNAs (miRNAs) have emerged as key post-transcriptional regulators of immune and inflammatory pathways and may serve as potential diagnostic biomarkers.This pilot study aimed to explore the diagnostic potential of serum miR-361-5p and miR-3125 in active pulmonary tuberculosis included 150 clinically suspected TB cases, of which 35 were laboratory-confirmed, along with 25 healthy controls. Diagnosis was performed using Acid-Fast Bacilli (AFB) smear microscopy (20.6% detection), Löwenstein–Jensen culture, and the GeneXpert MTB/RIF assay (23.3% detection). Serum levels of *miR-361-5p* and *miR-3125* were measured by RT-qPCR and normalized using the 2^−ΔΔCt method. Both miRNAs were significant level upregulated in TB patients compared to controls. *miR-361-5p* levels were 6.73 ± 13.21 vs. 1.57 ± 1.7 ($p = 0.0487$), while *miR-3125* levels were 3.48 ± 1.87 vs. 1.24 ± 0.62 ($p < 0.0001$). Median (IQR) values further supported these findings, particularly for miR-3125, which showed more consistent upregulation. ROC analysis demonstrated high diagnostic accuracy for miR-3125 (AUC = 0.92, sensitivity = 89%, specificity=79%), whereas *miR-361-5p* showed limited performance (AUC = 0.547). Additionally, *miR-3125* levels were significantly higher in rifampicin-resistant patients than in sensitive cases (4.92 ± 2.1 vs. 3.12 ± 1.7; $p = 0.007$), where *miR-361-5p* showed no significant association. This pilot study shows that serum *miR-361-5p* and *miR-3125* are elevated in active TB, with *miR-3125* potentially serving as a biomarker for rifampicin resistance, indicating it could be useful as a non-invasive tool for early TB detection.

Keywords: Biomarkers, *Mycobacterium tuberculosis*, *microRNA-361-5p*, *microRNA-3125*, Tuberculosis

INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease caused by the bacterium *Mycobacterium tuberculosis* (Mtb) and remains a major public health concern worldwide. Approximately a quarter of the global population is affected by latent Mtb infection, resulting in millions of deaths annually (Sinigaglia *et al*., 2020). Mtb is a slow-growing, acid-fast bacillus with a lipid-rich cell wall that enhances its resistance to host immune defenses and chemical agents (Mohammad nabi *et al*., 2024). The primary route of transmission is through inhalation of airborne droplets from individuals with active pulmonary TB, particularly in overcrowded or poorly ventilated environments (Sarkar and Sarkar, 2025). Clinically, active TB is characterized by chronic cough, hemoptysis, fever, night sweats, weight loss, and fatigue, whereas latent infection is asymptomatic and noncommunicable (Davidson *et al*., 2024). Pulmonary TB (PTB) is the most common form, primarily affecting the lungs (WHO, 2024).

MicroRNAs (miRNAs) are small non-coding RNAs (~20–24 nucleotides) that regulate gene expression post-transcriptionally by binding to the 3′-untranslated regions of target mRNAs, leading to translational inhibition or mRNA degradation (Ergin and Çetinkaya, 2022). They play crucial roles in cellular differentiation, proliferation, apoptosis, metabolism, and immune modulation (Mehta, 2021).In TB ,dysregulated host microRNAs have been shown to influence immune responses and contribute to pathogen survival, highlighting their potential as non-invasive diagnostic biomarkers (Agrawal *et*

al., 2024).Circulating miRNAs have been shown to differ between TB patients and healthy individuals, highlighting their potential as non-invasive diagnostic markers (Qi *et al*., 2012).

Among these, *miR-361-5p* has been identified as a regulator of apoptosis, cell proliferation, and immune-related pathways, particularly the NF-κB signalling pathway, which controls cytokine production and inflammation (Zhou *et al*., 2018). Similarly, *miR-3125* contributes to post-transcriptional regulation of immune and inflammatory signaling by modulating macrophage activity, phagocytosis, and cytokine expression via NF-κB and Toll-like receptor pathways (Han *et al*., 2022).

The present study aimed to conduct a pilot investigation to evaluate the serum expression of *miR-361-5p* and *miR-3125* in active TB patients compared to healthy controls. To the best of our knowledge, it represents the first experimental study in Iraq examining these circulating miRNAs, providing preliminary data on their potential utility as non-invasive diagnostic biomarkers in this population.

MATERIALS AND METHODS

Sample collection

In this cross-sectional study,150 clinical specimens were collected from patients across different age groups and both genders .Only 35 cases were laboratory confirmed by different diagnostic methods as shown in (Table-1).All specimens were obtained from the Dr. Saleh Al-Mukhtar Consultant Centre for Chest and Respiratory Diseases in Al-Hillah/Babylon Province, between December 2024 and May 2025.Five millilitres of venous blood were drawn aseptically from each participant.After disinfecting the skin over the vein with 70% ethanol, the blood was put into gel tubes to separate the serum. Specimens were centrifuged for 5 minutes at 3000 rpm after standing at room temperature for 30 minutes. The obtained sera were visually inspected for hemolysis, and only non-hemolyzed samples were used. The sera were then divided into two sterile Eppendorf tubes and stored at -20°C until further molecular analysis.

Inclusion and exclusion criteria for pulmonary tuberculosis patients

Inclusion criteria: Patients aged 18-65 years with a new diagnosis of active PTB, confirmed by at least one of the following: positive sputum smear microscopy for acid-fast bacilli (AFB), positive culture for Mycobacterium tuberculosis (Mtb), or a positive GeneXpert MTB/RIF assay. Patients also presented with clinical symptoms consistent with PTB (e.g., persistent cough >2 weeks, fever, night sweats, weight loss).

Exclusion criteria: Patients with a history of anti-TB treatment, co-infection with Human Immunodeficiency Virus (HIV), evidence of extrapulmonary TB, other concurrent respiratory diseases, diabetes mellitus, autoimmune diseases, malignancy, or current use of immunosuppressive drugs.

Inclusion and exclusion criteria for healthy controls

Inclusion criteria: Apparently healthy individuals aged 18-65 years, with no clinical signs or symptoms of TB or other infectious diseases, and no history of TB. Controls were recruited from the same geographical area as the patients.

Exclusion criteria: Individuals with a history of TB, known contact with an active TB case, any chronic or acute illness, or those who were pregnant.

Ribonucleic acid (RNA) extraction and quality assessment

Total RNA was extracted from serum samples following the manufacturer's protocol using the RNA Extraction Kit (GENEzol^{TM} Reagent [Geneaid, Taiwan]).The quantity and quality of RNA were measured using a NanoDrop spectrophotometer, employing diode-array scanning over the wavelength range of 200–320 nm.The absorbance profile was analyzed to calculate A260/280 and A260/230 ratios. Samples with ratios less than 1.8 were re-extracted to ensure high-quality RNA for downstream analysis.

Primer preparation

All primers (*miR-361-5p*, *miR-3125*, PU universal primer, and housekeeping gene *U6*) were provided in lyophilized form and reconstituted with nuclease-free water to a final concentration of 10 picomoles/µL.

Primer design

Specific primers for *miR-361-5p*, *miR-3125*, and *U6* were designed using the sRNA Primer Database to ensure compatibility with the stem-loop RT-qPCR method, enabling specific detection of mature miRNAs. Primer characteristics, including melting temperature (Tm), GC content, and potential secondary structures, were evaluated using OligoCalc. Primer pairs demonstrating optimal thermodynamic properties were selected for experimental analysis (Yan *et al*., 2013).

CDNA synthesis was performed using the stem-loop reverse transcription method with a PU universal primer to allow specific and reproducible reverse transcription of mature miRNAs. The design and configuration of the stem-loop structure and PU universal primer used for cDNA synthesis and subsequent PCR amplification are illustrated in Fig. 1.

Reverse transcription and quantitative PCR (RT-qPCR)

Target microRNAs were amplified and quantified using the TransScript® Green One-Step RT-qPCR Kit

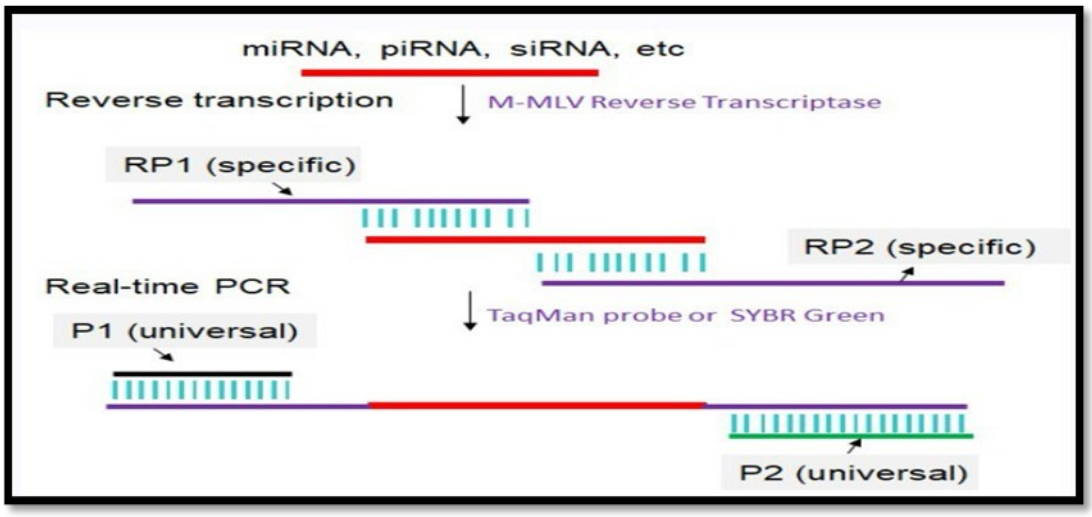

Fig.1. *Schematic of miRNA primer design*

(TransGen, China) according to the manufacturer's instructions. Specific primer sequences used in PCR (*miR-361-5p, miR-3125*, and *U6*) are listed in Table 2.

RT-qPCR amplification was carried out in a total reaction volume of 20 µL containing SYBR Green dye, specific primers, RNA template, enzyme mix, and RNase-free water. Amplification was performed in sequential cycles of reverse transcription, denaturation, annealing, and extension, with real-time detection by SYBR Green fluorescence. The primer details and thermal cycling conditions are provided in Tables 3 and 4, respectively.

Relative expression levels of miRNAs were calculated using the 2^−ΔΔCt method, with U6 serving as the endogenous control. The stability of U6 across TB patients and healthy controls was confirmed in this study and supported by previous research (de Santana Silva *et al*., 2025).

All reactions were conducted in triplicate, and no-template controls were included to monitor potential contamination. Melt curve analysis was performed to verify amplification specificity. Although minor secondary peaks were observed, which can occur in SYBR Green-based assays, gel electrophoresis confirmed a single band of the expected size for each target amplicon. The dominant peaks corresponded to the expected targets, indicating minimal non-specific amplification and no significant effect on Ct values or downstream fold-change calculations.

Ethical approval consideration

Sputum and blood samples were collected from all participants after obtaining informed consent. The study was approved by the Publication Ethics Committee of the Babylon Health Directorate, Ministry of Health, Iraq (approval number M241201, 8 December 2024). Demographic and clinical information, including age, sex, date of infection, and presence of chronic illnesses, was recorded for each participant in accordance with ethical guidelines.

Statistical analysis

The descriptive statistics were performed using IBM SPSS Statistics 26.0 (Armonk, NY: IBM Corp.) and Graph Pad Prism version 9.5.0 (San Diego, California, USA). Cycle threshold (Ct) values obtained from RT-qPCR were used to calculate ΔCt and ΔΔCt, and relative expression levels were determined using the 2^-ΔΔCt method. The Mann–Whitney U test was used to compare miRNA expression between two independent groups, and a p-values of ≤ 0.05 were considered statistically significant.

RESULT AND DISCUSSION

Diagnostic performance of acid-fast bacilli (AFB), Culture, and GeneXpert in tuberculosis

The present study revealed clear differences in the diagnostic performance of Acid-Fast Bacilli (AFB) smear microscopy, culture, and GeneXpert MTB/RIF in detecting Mtb.In this study, AFB smear microscopy identified MTB in only 31 out of 150 samples (20.6%), while 119 samples (79.3%) were smear-negative. Although AFB smear is rapid and cost-effective, its sensitivity is inherently limited by the waxy, lipid-rich mycobacterial cell wall, which restricts the penetration of the carbol-fuchsin dye and reduces microscopic visibility even in the presence of bacilli. Moreover, detection is limited by a high bacillary load of about 10,000 bacilli/mL in the Ziehl–Neelsen method so this technique is not reliable, especially for paucibacillary cases (Feng *et al*., 2025). In the present study, positivity on culture (LJ) was 23.3% (35/150) and remained the gold standard, as it can detect viable bacilli even at low bacterial counts. Nevertheless, its clinical applicability is limited because 4 to 8 weeks are required for bacterial growth; very strict lab conditions are required, and

Table 1. Identification of *Mycobacterium Tuberculosis* by different tests

Test	Positive	Negative
AFB smear	31 (20.6%)	119 (79.3%)
Culture	35 (23.3%)	115 (76.6%)
GeneXpert	35 (23.3%)	115 (76.6%)

Table 2. Primers sequences

Gene	Primer sequence (5' -3')	Reference
miR-361-5p	F:GGACGGTAGCAAGCAAAGAGTGTGGTACCCCTGGA R:GGGATTCTGGAAGATGATGATGACTTATCAGAATC	Designed by this study
miR-3125	F:GGACGGTAGCAAGCAAAGAGTGTGTCTCTCCACAG R:GGGATTCTGGAAGATGATGATGACTAGAGGAAGCT	Designed by this study
Housekeeping *U6*	F:CTCGCTTCGGCAGCACA R:AACGCTTCAGAATTTGCGT	Designed by this study
Pu (Universal)	F:GGACGGTAGCAAGCAAAGAGTGTG R:GGGATTCTGGAAGATGATGATGAC	Designed by this study

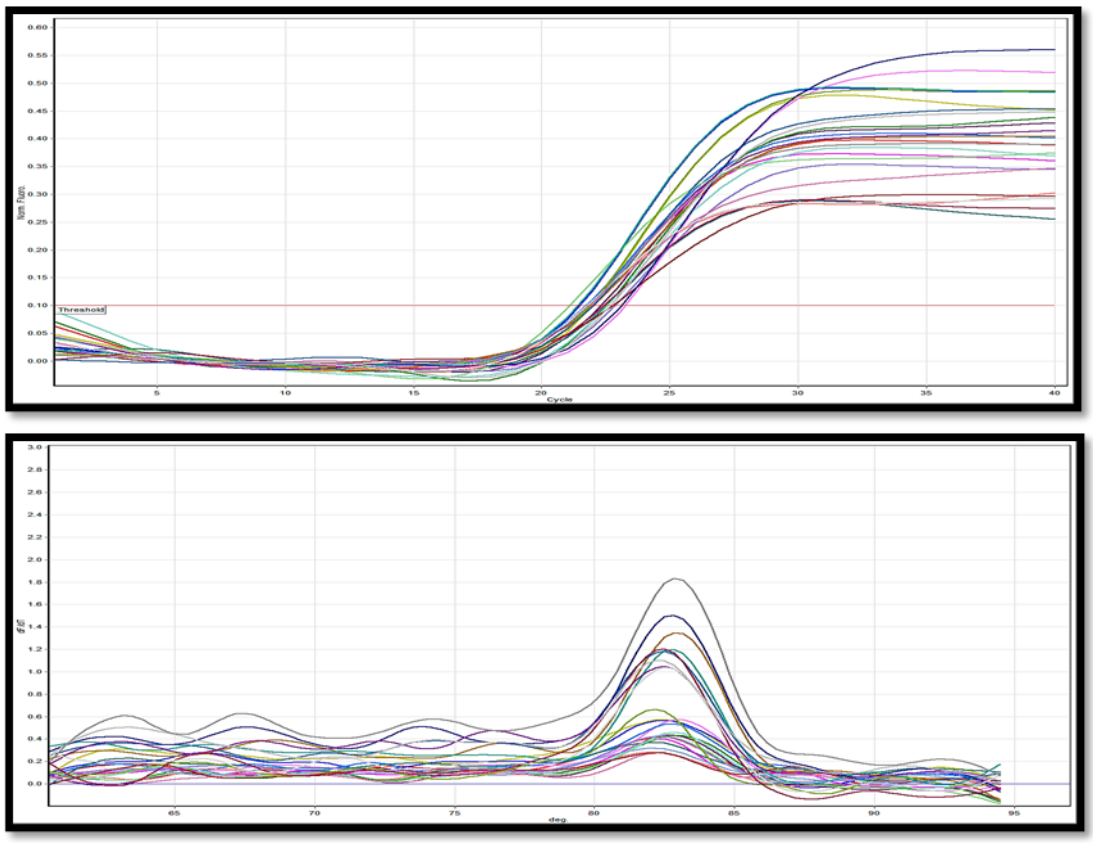

Fig. 2. *Housekeeping gene U6 amplification(A) and melt curve analysis (B) by RT-qPCR for normalization of miRNA expression.*

specimens must be processed optimally to avoid contamination that can delay treatment (Zaporojan *et al*., 2024). In contrast, the GeneXpert MTB/RIF assay identified MTB in 35 (23.3%) specimens with a diagnostic yield equivalent to culture and significantly higher than smear microscopy in this study. GeneXpert provides a molecular result in hours and has the potential to detect MTB at much lower bacterial loads than smear, with real-time detection of rifampicin resistance, which is crucial for prompt management of multidrug-resistant TB (Chen *et al*., 2023). These results further support the role of GeneXpert as a frontline diagnostic tool due to its rapid and accurate detection capabilities.

Clinical and demographic features of tuberculosis patients

Demographic and clinical profiles of the TB patients considered here were characterized by several epidemiological patterns as indicated in (Table-5). While there was no significant difference between the sexes, men (51.4%) were more likely to be affected. This pattern has been widely described and could be due to greater exposure to infectious environments, higher

Table 3 Components of quantitative RT- qPCR assay

Component	Volume	Final concentration
Green one -step- qPCR super mix- (SYBR)	10 µl	1X
Forward primer	2 µl	10 pmol/ML
Revers primer	2 µl	10 pmol/ML
TransScript® Green One-Step RT/RI Enzyme Mix	0.4 µl	-
Template RNA	1 µl	as required
Water, RNase-Free	4.6 µl	-
Total volume	20 µl	

Table 4. RT-qPCR of MiRNA(*361-5p,3125*) and(*U6*) amplification program

Step	Temperature	Time	Cycles no.
Hold temperature1	45ºC	5 min	1
Hold temperature 2	94 ºC	30 sec	
Denature	94ºC	15 sec	40
Annealing/extension	60 ºC	45 sec	
Melting temperature	60-95 ºC	2-5 sec/step	

prevalence of smoking and alcohol use, as well as men's delay in seeking healthcare. Sociocultural and biological characteristics may also account for the observed variations (Peer *et al*., 2023).A higher percentage of TB cases in this study were from rural areas (60%) than from urban/residential area (40%).This pattern is consistent with findings from Iraqi epidemiological research indicating that TB prevalence tends to be higher in rural communities, where access to healthcare services is more limited and socioeconomic challenges are more pronounced. For example, a study conducted in Basra, Iraq reported similar trends and highlighted that delayed diagnosis and treatment in rural populations contribute to ongoing transmission and greater disease burden (Mohammed *et al*., 2022) The reinforcement of medical resources in rural areas continues to be a priority to lower disease transmission rates and improve treatments. While no significant difference in BMI between groups was observed, TB patients trended towards lower BMI,a known consequence of chronic infection. Weight loss in TB is often attributed to increased metabolic requirement, reduced appetite, and systemic inflammation (Musuenge *et al*.,2020). Rifampicin - resistant TB was detected in 17.1% of patients, a clinically significant finding, as rifampicin resistance is the primary marker of multidrug-resistant TB (MDR-TB). This prevalence is consistent with patterns reported in the WHO Global Tuberculosis Report 2024, which indicates that RR/MDR-TB proportions can reach high levels in high-burden countries, particularly among previously treated patients and in specific regions (WHO,2024). Rifampicin resistance mainly results from mutations in the *rpoB* gene, some of which preserve or enhance bacterial fitness, facilitating ongoing transmission (Islam *et al*., 2024).

This finding further underscores that multidrug-resistant TB is a persistent issue, leading to challenges for the treatment and public health burden.The prevalence we found is similar to national estimates, underscoring the importance of continued resistance surveillance and the availability of rapid molecular diagnostics including GeneXpert for timely treatment decisions (Abdullah, 2022).

Stability of U6 as an endogenous control

In this study, U6 small nuclear RNA (snRNA) was used as an endogenous control for normalization of miRNA expression. As shown in Table 6 and Fig. 2, no significant difference in U6 expression levels was observed between tuberculosis patients and the low standard deviations indicate minimal variability between groups, confirming the stability of U6 expression. This stability supports its use as a reliable reference gene for normalization and for calculating the relative expression levels of *miR-3125* and *miR-361-5p* using the 2^−ΔΔCt method.

The consistent expression of *U6* in the present study cohort supports its use as a normalizer in serum-based miRNA quantification, consistent with previous studies reporting its suitability for circulating miRNA analysis (de Santana Silva *et al*., 2025). However, for future studies, the inclusion of additional endogenous controls or synthetic spike-ins could further enhance normalization accuracy and reproducibility, particularly in larger or multi-center cohorts.

Serum expression of *miR-361-5p* and *miR-3125* in tuberculosis

In this study, the serum levels of *miR-361-5p* and *miR-3125* were measured in patients with active pulmonary

Tuberculosis (PTB) and compared with those of healthy controls. As shown in Table 7, Fig. 3 and 4, *miR-361-5p* exhibited a mean level of 6.73 ± 13.21 in TB patients vs 1.57 ± 1.7 in controls (P = 0.0487), while *miR-3125* showed 3.48 ± 1.87 in patients compared to 1.24 ± 0.62 in controls (P < 0.0001). Median (IQR) values (*miR-361-5p*: 1.35 ± 4.01 vs. 0.98±1.41; *miR-3125*: 2.92±2.3 vs. 1.189 ±1.69) indicate that *miR-361-5p* displays a skewed distribution with substantial inter-individual variability, whereas *miR-3125* shows a more homogeneous upregulation.

The upregulation of *miR 361-5p* aligned with previous reports (Draz *et al*., 2014), which documented higher serum levels in patients with active TB compared to healthy controls. Functionally, *miR 361-5p* has been implicated in regulating apoptosis and cell survival pathways in macrophages, processes critical for controlling intracellular *Mycobacterium tuberculosis* replication while preventing excessive tissue damage (Wu *et al*., 2023).

Similarly, the increased expression of *miR-3125* in TB patients supports its role in modulating macrophage polarization and cytokine production, potentially contributing to a regulated inflammatory response during infection (Kimura *et al*., 2023). Nevertheless, variability in *miR-3125* expression has been reported in some studies, including downregulation in certain cohorts (Fu *et al*., 2011), reflecting differences in methodology, sample type, population genetics, or disease severity (Wang *et al*., 2022).

Overall, the combined presentation of mean ± SD and median (IQR) in Table 7 provides a clear depiction of both the average expression and variability of these miRNAs, highlighting *miR-3125* as a consistently elevated biomarker and *miR-361-5p* as a potentially variable yet significant marker in TB.

Receiver operating characteristic (ROC) curve analysis for serum and gene expression for miRNA (*361-5p,3125*)

ROC curve analysis was performed to evaluate the potential of serum *miR-361-5p* and *miR-3125* as non-invasive diagnostic biomarkers for active pulmonary tuberculosis (PTB). The differential expression of these miRNAs in TB reflects host pathogen interactions and the modulation of immune pathways, making ROC analysis a widely used tool in TB biomarker research as show in (Table 8), Fig. (5,6).

Among the evaluated miRNAs, *miR-3125* demonstrated high diagnostic accuracy, with an area under the curve (AUC) of 0.92 (95% CI: 0.85–0.98), a sensitivity of 89%, and a specificity of 79% (p < 0.0001). These metrics indicate excellent discriminative ability between PTB patients and healthy controls, suggesting that miR

Table 5. Demographic and clinical characteristics of participants

Variable	Category	Tuberculosis patient (n=35)	Healthy controls (n=25)	p-value
Age (years)	Mean ± SD	34.0 ± 9.8	33.6 ± 9.2	0.3664
Gender	Male	18 (51.4%)	13 (52.0%)	0.96
	Female	17 (48.6%)	12 (48.0%)	
BMI (kg/m²)	Mean ±SD	21.7 ± 5.8	23.5 ± 2.2	0.49
Geographic Area	Rural	21 (60.0%)	-	-
	Urban	14 (40.0%)	-	-
Rifampicin Resistance	Sensitive (S)	29 (82.7%)	-	-
	Resistant (R)	6 (17.1%)	-	-

Data presented as Mean ± Standard Deviation or Count. p-value from an independent sample t-test or a Chi-square test

Table 6. Comparison of U6 levels between cases and controls

Biomarker	Tuberculosis patient (n=35)	Healthy controls (n=25)	p-value
U6	22.43 ± 0.47	22.38 ± 0.43	0.68

Table 7. Comparison of biomarker levels between cases and controls

Biomarker		Tuberculosis patient (n=35)	Healthy controls (n=25)	p-value
miR-361-5P	Mean ±SD	6.73 ± 13.21	1.57 ± 1.7	
	Median (IQR)	1.35 ± 4.01	0.98 ± 1.41	0.0487
miR-3125	Mean ±SD	3.48 ± 1.87	1.24 ± 0.62	
	Median (IQR)	2.92 ± 2.3	1.189 ± 1.69	<0.001

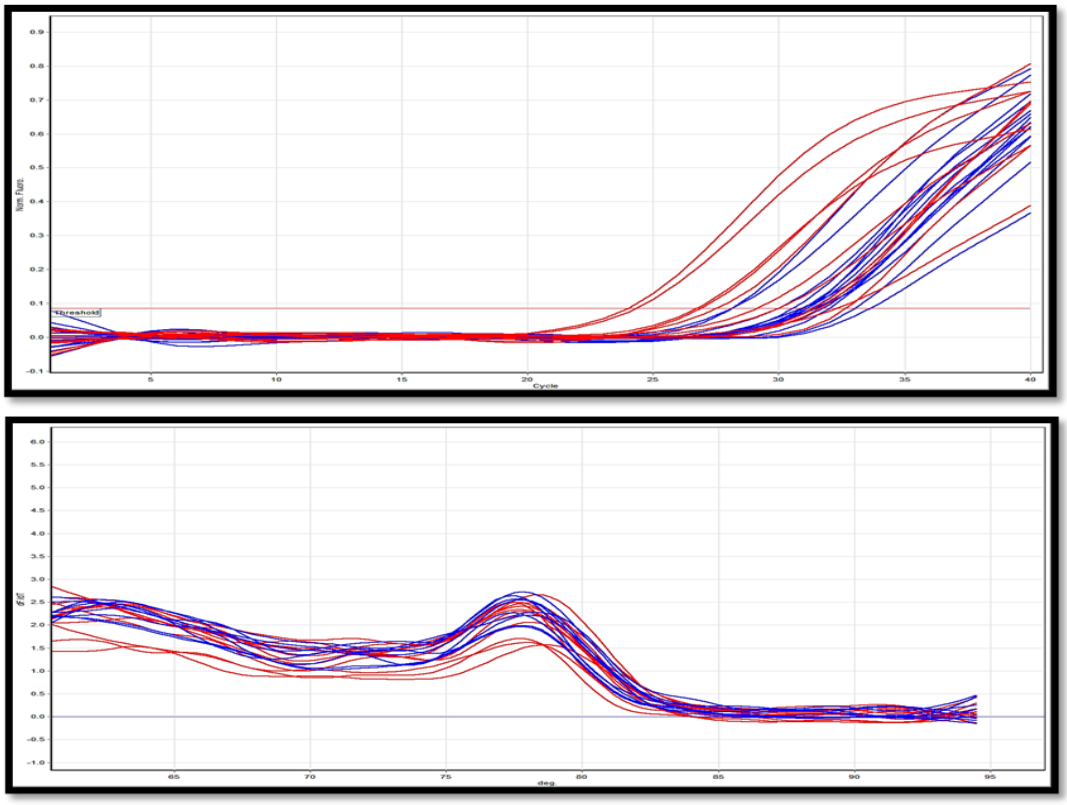

Fig. 3. *miRNA361-5p gene amplification(A) and melt curve analysis (B) by RT-qPCR , red and blue curves for case and control samples respectively*

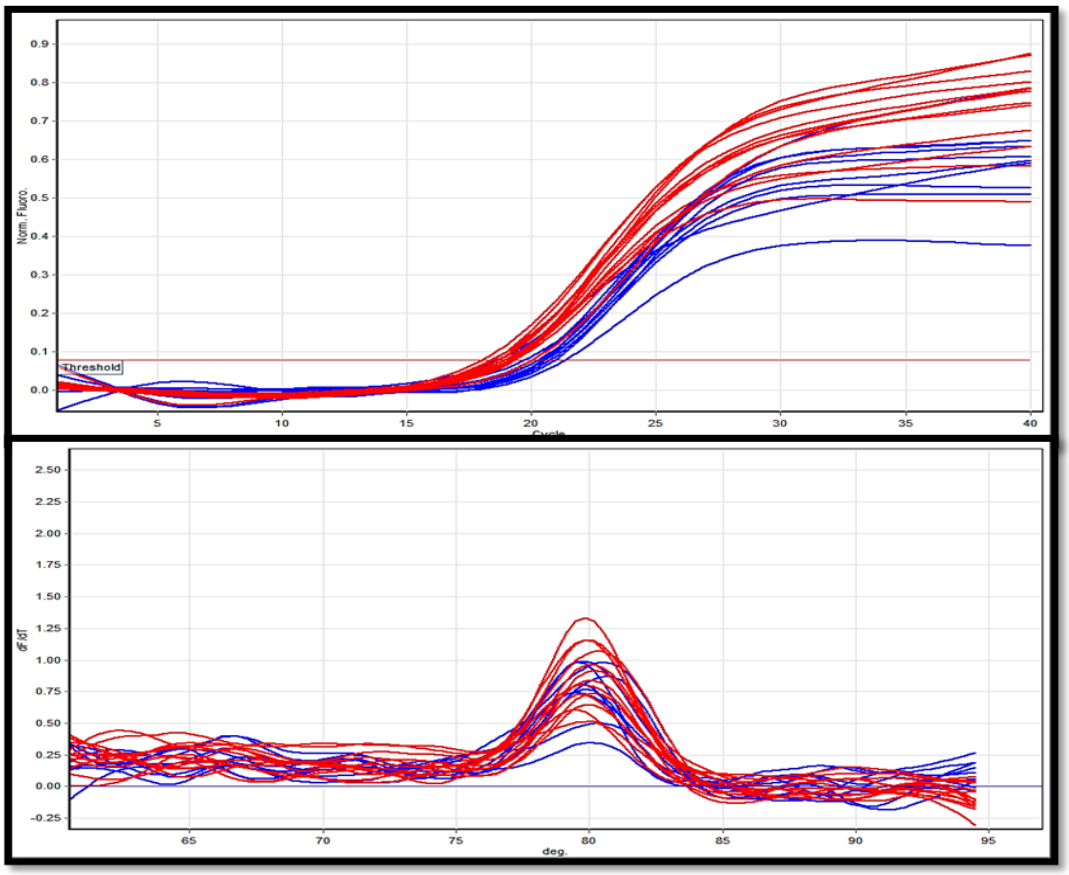

Fig 4. *miRNA3125 gene amplification(A) and melt curve analysis (B) by RT-qPCR, red and blue curves for case and control samples respectively*

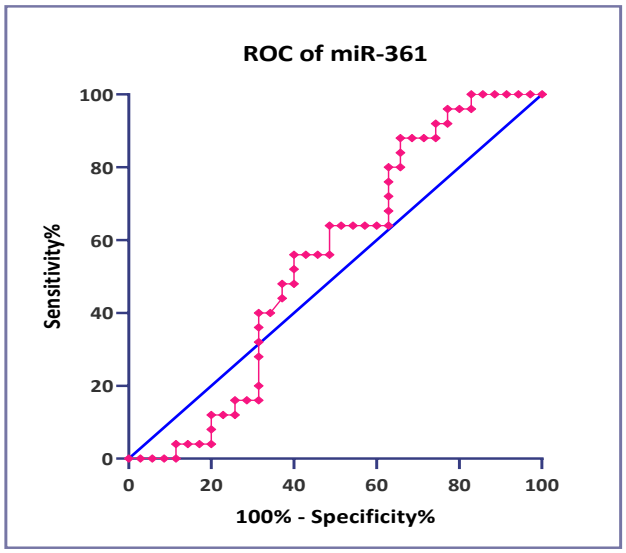

Fig. 5. *Sensitivity and specificity of serum miR 361-5P in tuberculosis (TB) patients*

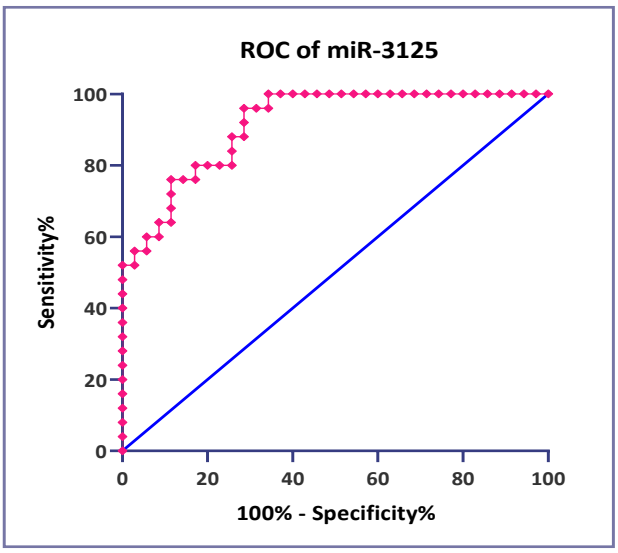

Fig. 6. *Sensitivity and specificity of serum miR 3125 in tuberculosis (TB) patients*

Table 8. Receiver operating characteristic (ROC) curve analysis for predicting tuberculosis

Biomarker	AUC (95% CI)	Optimal cutoff	Sensitivity (%)	Specificity (%)	p-value
miR-361-5p	0.547 (0.40 to 0.69)	>2.329	35.0	87.5	0.09
miR-3125	0.92 (0.85 to 0.98)	>1.68	89	79	<0.0001

Sens: Sensitivity; Spec: Specificity; Youden's Index [(Sensitivity + Specificity) - 1]; AUC: area under curve;

Table 9. Biomarker levels by rifampicin resistance status (cases only)

Biomarker	Sensitive (S) (n=29)	Resistant (R) (n=6)	p-value
miR-361-5p	7.769 ± 14.272	1.551 ± 2.473	0.1869
miR-3125	3.028 ± 1.448	5.645 ± 2.329	0.007

-3125 could serve as a promising non-invasive biomarker for TB detection. This finding aligneds with previous research, such as that of Sabir *etal*.,(2018) which reported that circulating miRNAs can achieve high diagnostic accuracy in distinguishing TB patients from healthy individuals.

In contrast, *miR-361-5p* showed limited diagnostic value, with an AUC of 0.547 (95% CI: 0.40–0.69; p = 0.09), low sensitivity (35%), and high specificity (87.5%). These results indicate poor overall discriminative performance when miR-361-5p is considered individually. Nevertheless, miRNAs with modest individual performance may still enhance diagnostic accuracy when included in combination panels, as demonstrated by Harapan *et al*. (2013), who found that where combined miRNA profiles improved overall diagnostic efficiency.

Association of serum *miR-3125* and *miR-361-5p* with rifampicin resistance in tuberculosis

In this exploratory study, as shown in Table 9, miR-3125 was significantly elevated in Rifampicin-resistant TB patients, with a mean expression of 5.645 ± 2.329 compared to 3.028 ± 1.448 in sensitive cases ($p = 0.007$), suggesting a potential association with drug resistance and indicating its value as a candidate biomarker. In contrast, miR-361-5p showed no significant difference between resistant and sensitive cases, with mean values of 1.551 ± 2.473 versus 7.769 ± 14.272 ($p = 0.187$), likely due to high variability and the small number of resistant patients.

Although studies directly linking these miRNAs to Rifampicin resistance are limited, circulating miRNAs have been reported to reflect host immune responses and may serve as non-invasive indicators of drug-resistant TB. For example, plasma exosomal miRNAs, including miR-122-5p, miR-23b-3p, and miR-15a-5p, have been associated with multidrug-resistant tuberculosis (Zhang *et al*., 2025).These preliminary findings support further investigation of miR-3125 in larger cohorts and integrated analyses with known genetic resistance markers, such as *rpoB* mutations, to validate its utility as a biomarker for Rifampicin-resistant TB.

Limitation of the study

This study has some limitations that should be considered. The sample size was relatively small (35 TB patients and 25 healthy controls), which may limit statistical power; however, the findings were consistent and statistically significant. The study did not include an

independent validation cohort, which may limit the generalizability of the proposed diagnostic performance and cut-off thresholds of the investigated miRNAs. Additionally, a clinically relevant control group such as suspected but non-TB cases was not included, limiting the assessment of biomarker specificity in distinguishing active TB from other respiratory illnesses. The case –control design may also introduce selection bias. Future larger, multi-center studies incorporating independent validation cohorts and symptomatic control groups, with more systematic evaluation, are warranted to confirm these findings and further assess the diagnostic specificity, reproducibility, and clinical applicability of circulating miRNAs.

Conclusion

This pilot study provides preliminary evidence that serum *miR-361-5p* and *miR-3125* are elevated in active pulmonary tuberculosis, highlighting their potential as non-invasive diagnostic biomarkers. *miR-3125* showed high diagnostic accuracy and was associated with rifampicin resistance, suggesting its utility in detecting drug-resistant TB. *miR-361-5p* exhibited greater variability in expression and limited individual diagnostic performance, but may still contribute in combination panels. These findings support the role of circulating miRNAs in TB diagnosis. Further large, multi-center studies with independent validation and a symptomatic control group are needed to confirm these results and assess their clinical applicability in the Iraqi population.

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Conflict of interest

The authors declare that they have no conflict of interest.

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