Expression Patterns of microRNA-26a and IFN-γ in Tuberculosis Patients

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المستخلص

الخلفية:
يحدث السل (Tuberculosis - TB) بسبب بكتيريا المتفطرة السلية (Mycobacterium tuberculosis - Mtb)، وما يزال يمثل مشكلة صحية عالمية. يرتبط الميكرو RNA-26a (miR-26a) بتفاعلات العائل مع الممرض، إلا أن دوره في مرض السل وعلاقته بتعبير الإنترفيرون-غاما (IFN-γ) في الدم المحيطي ما تزال غير مفهومة بشكل كامل.

الهدف:
هدفت هذه الدراسة إلى التحقيق في العلاقة بين تعبير جيني miR-26a و IFN-γ لدى مرضى السل.

الطرائق:
شملت هذه الدراسة 100 مريضًا مشخصين بالسل النشط (تتراوح أعمارهم بين 20–58 سنة) و50 شخصًا سليمًا كمجموعة سيطرة متطابقين في العمر والجنس، وتم تجنيدهم خلال الفترة من ديسمبر 2024 إلى مايو 2025. تم استخلاص RNA الكلي من مصل الدم وتحليله باستخدام تقنية تفاعل البوليميراز المتسلسل الكمي في الزمن الحقيقي (qRT-PCR) لتقييم مستوى تعبير miR-26a و IFN-γ.

النتائج:
أظهرت النتائج أن مستويات miR-26a في الدم كانت مرتفعة بشكل معنوي لدى مرضى السل (3.49 ± 0.57 ضعف) مقارنة بمجموعة السيطرة. كما أظهر تعبير IFN-γ زيادة لدى المرضى (1.82 ± 0.22 ضعف) مقارنة بالأصحاء. وأظهر تحليل المجموعات الفرعية أن مستويات miR-26a كانت أعلى في حالات السل الرئوي (3.15 ± 0.26) مقارنةً بـ السل خارج الرئة (2.42 ± 0.18؛ P = 0.0498)، في حين بقيت مستويات IFN-γ متقاربة بين أنواع السل المختلفة.

الاستنتاجات:
يرتبط السل النشط بزيادة ملحوظة في مستويات miR-26a في الدم، خاصة في حالات السل الرئوي، مما يشير إلى احتمال وجود دور تنظيمي له في المناعة المتوسطة بـ IFN-γ. وقد يعمل miR-26a كمؤشر حيوي جديد يمكن استخدامه في تشخيص السل. كما قد يكمّل miR-26a أدوات التشخيص الحالية، من خلال توفير مؤشر جزيئي إضافي يساهم في تحسين دقة الكشف عن المرض وتصنيفه. ومع ذلك، هناك حاجة إلى مزيد من الدراسات لتوضيح تأثيراته الجزيئية وإمكانية استخدامه علاجيًا.

الكلمات المفتاحية:
miR-26a، IFN-γ، السل، المتفطرة السلية، الميكرو RNA.

Abstract

Background: The Tuberculosis (TB) is induced by *Mycobacterium tuberculosis* (Mtb), persists as a worldwide health issue. MicroRNA-26a (miR-26a) is associated with host-pathogen interactions, nevertheless, its function in TB and its correlation with interferon-gamma (IFN-γ) expression in peripheral blood are not well understood.

Aim: To investigate the relationship between miR-26a and IFN-γ genes expression in TB patients.

Methods: In this study a with 100 persons diagnosed with active TB (ages 20–58 years) and 50 healthy controls matched for age and sex, recruited from December 2024 to May 2025. Total RNA from serum was extracted and analyzed using quantitative real-time PCR to assess miR-26a and IFN-γ expression.

Results: Circulating miR-26a was significantly elevated in TB patients (3.49 ± 0.57-fold) relative to controls, as well IFN-γ expression exhibited a increase in patients (1.82 ± 0.22-fold) comparing with control. Subgroup analysis indicated elevated miR-26a levels in pulmonary TB (3.15 ± 0.26) compared to extrapulmonary TB (2.42 ± 0.18; $P = 0.0498$), although IFN-γ levels were consistent across TB types.

Conclusions: Active TB is correlated with a substantial increase in circulating miR-26a, especially in pulmonary cases, indicating a possible regulatory function in IFN-γ–mediated immunity. miR-26a may function as a new biomarker that can be used for the TB diagnose. Importantly, miR-26a may complement existing diagnostic tools, providing an additional molecular biomarker that enhances the accuracy of TB detection and disease classification. Additional research is necessary to clarify its molecular effects and therapeutic applicability.

Keywords: miR 26a; IFN-γ; Tuberculosis; *Mycobacterium tuberculosis*; MicroRNAs.

Introduction

Mycobacterium tuberculosis (Mtb) remains a major global health concern, responsible for millions of new infections annually. Despite extensive research, the molecular interactions between Mtb and host immune regulators are not fully elucidated. In particular, how host microRNAs, such as miR-26a, modulate interferon-gamma (IFN-γ)–mediated immunity during active tuberculosis remains unclear, representing a key gap in understanding disease pathogenesis (1). World Health Organization's (WHO) Global TB Report indicated that there were 10.8 million new TB cases worldwide, with an incidence rate of 134 cases per 100,000 population (2). Surveillance data indicates that around 25% of the global population possesses immunological indication of the precede infection with Mtb. Mortality figures indicate that 1.4 million individuals succumbed to tuberculosis in 2020,

rendering it the second most prevalent infectious cause causing death globally, following COVID-19 (3).

The host immune response to Mtb is predominantly governed by cellular immunity, wherein T helper 1 (Th1) cells and their corresponding cytokines are essential. IFN-γ is acknowledged as the primary cytokine coordinating the immunological response to the mycobacteria primarily functions to activate macrophages, enabling them to perform their microbicidal roles against intracellular Mtb. IFN-γ signaling not only augments the direct cytotoxic ability of macrophages but also facilitates antigen presentation and the development of CD4+ T cells towards the protective phenotype. The pivotal significance of IFN-γ is highlighted by various clinical and experimental studies that illustrate its crucial function in regulating Mtb infection (4).

Many microRNAs such as miR-155, miR-29a, and miR-21 are involved in tuberculosis-related immune responses. However, miR-26a was chosen in this study because it has a specific link to the IFN-γ signaling pathway, which is essential for host defense against *Mycobacterium tuberculosis*. A research showed that *M. tuberculosis* increases miR-26a levels to suppress the coactivator p300, reducing macrophage response to IFN-γ and helping the bacteria survive (5), Unlike other TB-related miRNAs, miR-26a shows consistent upregulation in pulmonary TB and can be detected easily in serum samples (6).

The MicroRNAs (miRNAs) are categories of small, endogenous, non-coding RNA molecules, generally 18-23 nucleotides long, that serve as essential for the post-transcriptional regulators of the gene expression (6). They generally exert regulatory effects by attaching to complementary regions in target messenger RNAs (mRNAs), leading to mRNA degradation, decapping, deadenylation, or translation suppression. Through these pathways, miRNAs regulate a various cellular activities, comprising proliferation, differentiation, development, metabolism, apoptosis, and immunological responses (7). The complex role of miRNAs in influencing immune cell formation and function has become increasingly evident. As a result, pathogenic

bacteria, such as Mtb, have demonstrated the ability to manipulate host miRNA expression profiles to their benefit, thereby undermining host protection and enhancing their survival. A single miRNA can regulate numerous target genes (8), thus, the expression manipulation of even a few miRNAs by a pathogen can significantly impact host cellular pathways and the overall immunological environment. Therefore, this study aimed to investigate whether Mtb infection modulates miR-26a expression and whether this has an impact on IFN-γ expression.

Material and methods

Patients

The current study was conducted at the Tuberculosis Health Centre within Medical City, Baghdad, Iraq, and the Microbiology Laboratory of College of Medicine in the University of Anbar, Anbar, Iraq. The Ethics Committee of University of Anbar approved current investigation (approval no. 176, 2024). All the participants provided written informed consent after the being apprised of study's purpose. Male or female, suffering from chronic TB infection, and diagnosed with TB for a minimum of 6 months were included in current study, while patients with complications or infections in the heart, liver, kidney, diabetes, or tumors were not eligible for participation in the study.

Samples collection:

A total of 3–5 mL of peripheral venous blood was collected from 100 TB patients, aged 20 to 58 years, who received treatment at Tuberculosis Health Centre within Medical City from early December 2024 to May 2025. The study also includes 50 participants who were deemed to be in good health, serving as a control.

RNA Extraction:

A 0.25 ml of whole blood was transferred from anticoagulant tube immediately after collection and mixed with 0.75 ml. TRIzol for RNA purification. Total RNA was isolated using a commercial phenol–guanidine-based kit (TransGen Biotech Co.) In accordance with the manufacturer's guidelines, the RNA was eluted in 50 µl of

nuclease-free water. Yield and purity were assessed via Nanodrop, the RNA was stored at –70 ° C (9).

miR-26a and IFN-γ expression:

Detection of miR-26a and IFN-γ expression was performed via a twostep qPCR approach. The first step included the conversion of RNA to complementary DNA (cDNA) using an AddScript Reverse Transcriptase kit (addbio, Korea) according to protocol of: Priming 25ºC for ten min., Reverse transcription 50 ºC for sixteen min., RT inactivation 80 ºC for 5 min and Hold 12 ºC. Subsequently, the second step was carried out by employing the specific primers obtained from Macrogen (Korea) as detailed in Table 1. Each 20 µL reaction solution contained 5 µL cDNA template, 2.5 µL forward and 2.5 µL reverse primers, and 5 µL SYBR Green master mix and 5 µL nucleus free water. Mic qPCR Cycle (Bio Molecular System/ Australia) was programmed with a thermo cycling protocol as follows: 1 cycle of initial the denaturation at 95ºC, 45 cycles of denaturation at 95ºC, and annealing at 60ºC, while the temperature of melting curve was 60-95ºC. The expression levels of miR-26a and IFN-γ genes were normalized to U6 and GAPDH respectively as the internal control (10).

Table 1: primers of gene expression

Genes	Sequences (5`- 3`)	References
miR-26a	**F: CTGTCAACGATACGCTAC R: GTAATCCAGGATAGGCTG**	**(11)**
U6	**F: CTTCGGCAGCACATATAC R: GAACGCTTCACGAATTT GC**	**(11)**
IFN-γ	**F: CTCTGCATCATTTTGGGTTCT R: ATCCGCTACATCTGAATGACCT**	**(12)**
GAPDH	**F: CAAGATCATCAGCAATGCCTCC R: GCCATCACGCCACAGTTTCC**	**(12)**

Statistical Analysis

The Statistical Packages for the Social Sciences (SPSS) program (2019) was employed to identify the impact of various groups/factors on study parameters. We

performed a T-test to compare the means in a meaningful way. The chi-square test (χ^2) was utilized to conduct a significant comparison of percentages at the 0.05 and 0.01 probability levels.

Results

The demographical parameters of current study (Table 2) showed no significant differences between male and female TB patient. While there is significant differences in TB infection among age groups, where the highest incidence was in 20-29 (36%) and the lowest incidence in ≥50 (18%). The present finding showed significant differences (P= 0.0051) concerning the types of infection where the highest incidence was in pulmonary TB (64%).

Table 2: The demographic distribution of TB patients

Sex No. (%)						
Male	**Female**			**Total**	**χ2**	**P-value**
52 (52)	**48 (48)**			**100 (100)**	**0.160**	**0.689 NS**
Age group (years) No. (%)						
20-29	**30-39**	**40-49**	**≥50**			
36 (36)	**24 (24)**	**22 (22)**	**18 (18)**	**100 (100)**	**7.210**	**0.0499 ***
Location of infection No. (%)						
Pulmonary		**Extra pulmonary**				
64 (64%)		**36 (36%)**		**100 (100)**	**7.840**	**0.0051 ****
NS Non-Significant, * (P≤0.05), ** (P≤0.01).						

The miR-26a was upregulated in TB patients (3.49 ±0.57) comparing to healthy control (Table 3). As well, the INF-γ expression was augmented in TB patient comparing to control (Table 4). The levels of miR-26a were considerably ($p = 0.0498$) elevated in pulmonary TB (3.15 ± 0.26) compared to extrapulmonary TB (2.42 ± 0.18), conversely, IFN-γ expression exhibited no significant ($p = 0.769$) disparity between pulmonary and extrapulmonary TB patients (Table 5).

Table 3: Fold change of miRNA-26a gene expression in TB patient and control groups

Table 4: Fold change of INF-γ gene expression in TB patient and control groups.

Group	Ct of U6	Ct of miRNA-26a	ΔCT	ΔΔCT	Fold change
Patients	17.76	22.21	4.45	-1.32	3.49 ±0.57
Control	17.86	23.63	5.77	0.00	1.07 ±0.20

Table 5: Distribution of miR-26a and INF-γ gene expression according to types of TB

Group	Ct of GAPDH	Ct: INF-γ	ΔCT	ΔΔCT	Fold change
Patients	17.60	22.21	4.56	-0.19	1.82 ±0.22
Control	19.39	23.63	4.24	0.00	1.08±0.04

Genes	Extra Pulmonary TB	Pulmonary TB	T-test	P-value
miR-26a	2.42 ±0.18	3.15 ±0.26	0.607	0.0498*
INF-γ	0.88 ±0.13	0.91 ±0.16	0.081	0.7691 NS
* (P≤0.05), NS Non-Significant.				

Discussion

Tuberculosis remains a considerable public health concern, and is a primary cause of mortality, it is a social health concern that can lead to stigma and economic detriment, since individuals may be unable to work owing to the fatigue induced by the illness. Current investigation performed on 100 active TB patients, the finding demonstrated non-significant relation between sex and TB infection in spite of that male was more susceptible to TB infection with percentage of 52%, while the age group 20-29 years was most susceptible to infection. These finding were consistent with previous local study (13). The activity of immune system is highest in female than male, further, behavioral habits physiological differences between the two-sex effect the susceptibility to TB (14, 15). The highest incidence of TB infections worldwide is observed in the young age, indicating significant transmission throughout communities and workplaces, on the other hand the smoking prevalence has risen markedly among young adults in recent years, especially the using of electronic cigarette and hookahs in addition to declining of BCG immunity with age, which may increase the risk of TB (16, 17).

Regarding the infection types, present study found a significant differences (p= 0.0051) between pulmonary and extra pulmonary TB. Gopalaswamy, *et al.* found that extrapulmonary TB constitutes approximately 20 – 30% of all active TB cases, primarily impacting children and immunocompromised people(18). Extrapulmonary TB impacting diverse organs and tissues beyond the pulmonary system. The predominant manifestation is lymph node tuberculosis, which generally appears as

painless, swollen lymph nodes, particularly in the cervical region. Pleural tuberculosis impacts the pleura of the lungs, leading to thoracic discomfort, cough, and pleural effusion. Skeletal tuberculosis, encompassing Pott's disease, affects bones and joints, predominantly the spine, resulting in back discomfort, deformity, or neurological symptoms due to spinal cord compression. Genitourinary tuberculosis affects the kidneys, urinary tract, and reproductive organs, resulting in symptoms including hematuria, pelvic discomfort, or infertility (19). The predominant of pulmonary TB is likely due to mode of transmission via inhalation of droplet nuclei (1–5 μm), which lodge deep within the alveoli, establishing the lungs as the initial site of contact. Mtb is highly aerobic and flourishes in areas with elevated O_2 tension, specifically the lung parenchyma (20).

Mycobacterium tuberculosis utilizes strategies to evade immune surveillance often linked to the resolution of inflammation. Currently, there is limited understanding of the regulatory processes utilized by Mtb to initiate infection during its intracellular existence. miRNAs significantly influence the regulation of immune responses (8), thus, we postulated that miR-26a may serve as a potential mechanism for the divergent expression levels of INF-γ following Mtb infection. Current findings indicate that IFN-γ expression is significantly elevated in the tuberculosis (TB) patient cohort relative to controls (table 4). IFN-γ is a pivotal effector cytokine synthesized by Th1 CD4+ T cells, CD8+ T cells, and NK cells, essential for macrophage activation and the intracellular eradication of Mtb, thus, elevated IFN-γ level in active disease is biologically rational and aligns with its established function in host defense (21). Numerous clinical investigations and biomarker assessments indicate increased levels of IFN-γ in active pulmonary TB relative to uninfected controls or latent infections, hence endorsing the application of IFN-γ–based assays for immunodiagnosis and immunological surveillance (22-24). The intensity of IFN-γ responses may fluctuate based on illness stage, bacterial load, host genetics, and concomitant immune modulators. Consequently, although our observed upregulation

corresponds with several findings, the variability among research is well established (24).

Current results indicated upregulated miR-26a in TB patients and its noticeably upregulated in pulmonary comparing to extra pulmonary TB. Many previous studies indicating the upregulation of miR-26a in TB patients (5, 25). A study by Ni *et al*. demonstrated that Mtb causes the high expression of miR-26a and miRNA-132, which reduces the p300 transcriptional coactivator level, consequently impairing the transcription of the IFN-γ genes and the immune cells response to this cytokine (5). They demonstrated that the knockdown of miR-132 and miR-26a in human macrophages enhances the transcriptional replies to the IFN-γ through enhanced expression of p300, indicating that the activation of the two miRNAs by Mtb may serve as an effective survival mechanism for the pathogen (5). Regarding current results, miR-26a may work as positive regulator of circulating INF-γ that involve in immune response suggesting their potential application in diagnostic or therapeutic approaches of TB.

Conclusion

The *M. tuberculosis* is an ancient microorganism that had evolved over time to humans and has specifically adapted for survival within its internal environment. Among present cohort, there was no significant relation between sex and TB infection while the age rang 20-29 years was most susceptible to TB. Pulmonary TB was significantly dominant than extra pulmonary type. Circulating miR-26a expression significantly elevated leading to IFN-γ upregulation in TB patients, underscores its potential as both a biomarker for TB and a differentiator of disease location where it was upregulated in pulmonary comparing to extra pulmonary TB. These results encourage further exploration of the molecular function of miR-26a in TB pathogenesis and its potential application in diagnostic or therapeutic approaches.

Declaration:

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Competing interest:

The researchers state that they do not have any conflicting interests.

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