BIODIVERSITAS
Volume 26, Number 2, February 2025
Pages: 831-836

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d260231

Analysis of miR-21-5p and miR-144-5p expression as biomarkers in active lung tuberculosis and home contacts

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Manuscript received: 13 May 2024. Revision accepted: 17 February 2025.

Abstract. *Savitri AP, Massi MN, Hatta M, Santoso A, Fachri M, Djaharuddin I, Wahyuni S, Ilyas M, Iskandar H, Pattelongi I, Handayani I, Iskandar IW, Hidayah N, Angria N, Halik H. 2025. Analysis of miR-21-5p and miR-144-5p expression as biomarkers in active lung tuberculosis and home contacts. Biodiversitas 26: 831-836.* Considering the central role of microRNAs (miRNAs) in development and disease, researchers have proposed that specific circulating miRNAs affect the outcome of tuberculosis (TB) infection and that blood miRNA levels might reflect the course of the disease. This study analyzed the expression of miR-21-5p and miR-144-5p as potential biomarkers in patients with active TB and their household contacts (individuals with latent TB and healthy contacts). This study used a cross-sectional design and enrolled 20 people with active TB, 22 household contacts with positive interferon-gamma release assay results, and 22 healthy controls. miR-21-5p and miR-144-5p expression was examined using quantitative real-time PCR. miR-21-5p expression was more than 37-fold higher in patients with active TB than in healthy contacts. Meanwhile, miR-21-5p expression was approximately 15-fold higher in patients with active TB than in those with latent TB. miR-21-5p expression was 2.5-fold higher in patients with latent TB than in healthy contacts, whereas miR-144-5p expression was 35-fold higher in patients with active TB than in healthy contacts, and approximately 52-fold higher in patients with active TB than in contacts with latent TB. miR-144-5p expression in latent TB was approximately 1.5-fold higher in healthy contacts than in contacts with latent TB. Receiver operating characteristic analysis illustrated that miR-21-5p and miR-144-5p could distinguish latent TB from active TB with areas under the curve of 0.811 (95% Confidence Interval (CI) = 0.67-0.953) and 0.818 (95% CI = 0.689-0.947), respectively. miR-21-5p and miR-144-5p expression was elevated in active TB, highlighting their potential as diagnostic biomarkers.

Keywords: Active tuberculosis, household contacts, latent tuberculosis, microRNA, miR-144-5p, miR-21-5p

INTRODUCTION

Tuberculosis (TB), which is caused by *Mycobacterium tuberculosis* (Mtb), is a significant cause of mortality globally. Mtb infection is a primary global public health concern, especially when it affects the lungs (pulmonary TB). Approximately 50% of people with TB die of the disease without treatment (Fachri et al. 2018; WHO 2021). Furthermore, TB is transmitted by the inhalation of Mtb from the air, followed by entry into the respiratory tract. People exposed to Mtb might become infected and develop TB, becoming potential sources of new infections. Interestingly, not all people exposed to Mtb become infected or develop active TB. This highlights the complexities of host-pathogen interactions. Various elements, including nutritional conditions, coinfections, interactions with environmental microorganisms, and prior immunizations, impact the outcome of an infection. Genetic factors within the host also hold significance in regulating an individual's vulnerability to intracellular pathogens and controlling disease susceptibility (Hatta et al. 2010). Approximately 5%-10% of individuals infected with Mtb develop active TB at specific points. The remaining 90%-95% of infected individuals exhibit no symptoms, and they are considered to have latent TB infection. This state is defined by an immune response to mycobacterial proteins without

noticeable clinical indications or active disease symptoms (Flynn and Chan 2001; Penn-Nicholson et al. 2020).

Identifying and treating latent TB are major obstacles to eliminating TB because of the insufficient clinical and microbiological data (Muñoz et al. 2015). Misdiagnosis associated with TB remains a global problem that can result in increased rates of mortality and morbidity. Solid cultures, direct smear microscopy, radiographic tests, and the Tuberculin Skin Test (TST) are used in national TB programs in endemic countries (Ndzi et al. 2019; Susilawati and Larasati 2019). Accurate diagnosis, suitable and standardized treatment, monitoring and treatment evaluation, and public health responsibility are the cornerstones of TB diagnosis and management (Wikanningtyas et al. 2018).

The potential of microRNA (miRNA) as a biological marker for diagnosing TB is being investigated. miRNA detection has broad roles in the diagnosis of various illnesses, such as infectious diseases, diabetes, psoriasis, cancer, and heart disease. During infection, multiple microorganisms, such as Mtb, alter DNA through epigenetic modifications, which are inheritable changes to DNA that do not alter a gene's sequence. Examples of these modifications include histone modifications and DNA methylation (Behrouzi et al. 2019). miRNAs are helpful biomarkers for prognosis, treatment outcomes, and diagnosis. Apart from establishing the association between miRNAs and various illnesses such as cancer, heart disease, immune dysfunction, and infections, small RNA molecules have a substantial impact on the occurrence and progression of these ailments (Zhou et al. 2016).

Because of its increased expression in human and murine macrophages upon Mtb infection, miR-21 has been identified as an essential factor in Mtb infection. miR-21 upregulation suppresses the expression of TLR-4 and Bcl-2, thereby blocking the release of inflammatory cytokines. Furthermore, miR-21 improves the survival of mycobacterial cells by suppressing apoptosis. Another study found that patients with untreated TB had significantly higher miR-21-5p levels than healthy controls (Ruiz-Tagle et al. 2020). Conversely, miR-144-5p has been linked to the inhibition of autophagosome formation and reduction of the capacity of macrophages to efficiently eliminate Mtb (Liu et al. 2011; Sinigaglia et al. 2020). Based on these findings, the current study investigated the expression patterns of miR-21-5p and miR-144-5p in individuals with active TB and their household contacts. In addition, this study evaluated biomarkers for differentiating latent and active TB. In addition to offering potential candidate diagnostic biomarkers and therapeutic targets, this study aimed to provide essential insights into the molecular mechanisms underlying TB infection and its pathogenesis by examining the roles of miR-21-5p and miR-144-5p.

MATERIALS AND METHODS

Sample collection

This cross-sectional study was conducted at the Public Health Lung Center (BBKPM) and HUM-RC Laboratory (Hasanuddin University Medical Research Center) of the Faculty of Medicine, Universitas Hasanuddin (Makassar, Indonesia). Blood and sputum samples were collected from patients with active TB, while blood samples were collected from their household contacts. Active TB was defined as a new diagnosis of TB, the presence of active TB symptoms, positive acid-fast bacillus smear and culture, and findings suggestive of TB on thoracic X-ray. The exclusion criteria for the active TB group were as follows: previous receipt of anti-TB treatment and comorbidities such as diabetes mellitus, human immunodeficiency virus infection, or cancer. Contacts with latent TB included household contacts living with patients with active TB for at least 6 months who did not exhibit active TB symptoms despite having a positive Interferon-Gamma (IFN-γ) Release Assay (IGRA) result. The healthy contacts group consisted of individuals who lacked active TB symptoms and had a negative IGRA result despite contact with patients with active TB; household contacts with indeterminate IGRA results were excluded. Additionally participants younger than 15 years were excluded from all groups.

IGRA assay

The QuantiFERON-TB Gold Plus (Qiagen, Hilden, Germany) was used for this investigation. Four milliliters of blood were drawn from each household contact, placed in four different tubes, and incubated for 16-24 h at 37°C. After incubation, the four tubes were centrifuged for 15 min at 2000-3000 RCF (×g) to separate the plasma. Carefully, 200-300 µL of plasma were placed into different microtubes and stored at -20°C until used in Enzyme-Linked Immunosorbent Assay (ELISA) to assess IFN-γ levels in plasma stimulated with Mtb antigens. Using an ELISA reader equipped with a 450-nm filter and a reference filter ranging from 620 to 650 nm, every well's Optical Density (OD) was determined within 5 min after terminating the reaction. Based on the obtained OD, IFN-γ levels were computed using QuantiFERON-TB Gold Plus Analysis Software version 2.71 (Qiagen). The results were interpreted as positive, negative, or indeterminate.

miRNA extraction and profiling

The miRNeasy Serum/Plasma Kit (Cat. No. 217184, Qiagen) was used to extract RNA from serum samples, including positive controls. The miRCURY LNA RT Kit (catalog number 339340, Qiagen) was used to amplify cDNA from the extracted total RNA. Real-time quantitative PCR (qPCR) was used to quantify miR-21 and miR-144-5p levels using the CFX96 Touch Real-time PCR Detection System (Bio-Rad, Hercules, CA, USA). The targeted miRNA was amplified using the miRCURY LNA miRNA PCR Assay Kit (Qiagen). The master mix consisted of 5 µL of 3 × miRCURY SYBR Green Master Mix, 1 µL of PCR Primer mix, 3 µL of the cDNA template, and 1 µL of RNase-free water. The primer sequences (5′ to 3′) for miR-21-5p and miR-144-5p were UAGCUUAUCAGACUGAUGUUGA (miRBase accession: MIMAT0000076) and GGAUAUCAUCAUAUACUGUAAG (miRBase accession: MIMAT0004600), respectively. mir-103a-3p (5′-AGCAGCAUUGUACAGGGCUAUGA-3′, miRBase accession: MIMAT0000101) was used as the reference RNA target

based on earlier studies indicating its excellent statistical performance in qRT-PCR investigations of miRNA using human samples (Peltier and Latham 2008; Veryaskina et al. 2022; Massi et al. 2023; Shepelkova et al. 2023). The samples were collected in triplicate. Relative miRNA expression was calculated as the fold change compared to a reference sample using the $2^{-\Delta\Delta CT}$ method, which allows the comparison of miRNA expression among different samples by normalizing to reference RNA (Qureshi and Sacan 2013).

Data analysis

SPSS Statistics version 26.0 (IBM Co., Armonk, NY, USA) was used to analyze the data. For nominal variables, comparisons of characteristics among the groups were assessed using the chi-squared test and Fisher's exact test. One-way ANOVA was used to compare numerical variables among the groups. The Mann-Whitney U test and Kruskal-Wallis test were used to compare the miRNA expression between two and three groups, respectively. In all analyses, a p-value less than 0.05 was considered significant (Ndzi et al. 2019). Using Receiver Operating Characteristic (ROC) curve analysis, the diagnostic performance of the significantly expressed miRNAs was estimated. An ROC curve is a graphical depiction that evaluates a parameter's capacity to discriminate between two groups (Ndzi et al. 2019). The Area Under the ROC Curve (AUC) was created by graphing sensitivity against 1 - specificity for all potential cutoffs. The optimal cutoff points were determined using Youden's index, ensuring appropriate statistical rigor.

RESULTS AND DISCUSSION

A total of 64 subjects were included in the study (Table 1), comprising 20 patients with positive smear results, 22 latent TB contacts, and 22 healthy contacts. As presented in Table 1, there were no significant differences in age and the age distribution among the groups ($p>0.05$); however, the active TB group had a higher percentage of males than the other groups ($p<0.05$).

The qPCR results revealed that miR-21-5p expression was 37.5-fold higher in patients with active TB than in healthy contacts ($p<0.001$). Meanwhile, miR-21-5p expression was approximately 15-fold greater in patients with active TB than in individuals with latent TB ($p = 0.001$). Contacts with latent TB exhibited 2.5-fold higher miR-21 expression than healthy contacts; however, this difference was not significant ($p = 0.324$). miR-144-5p expression in active TB was 52-fold higher and nearly 36-fold higher than in latent TB ($p<0.001$) and healthy contacts ($p<0.001$), respectively. A 0.7-fold decrease in miR-144-5p expression was recorded in contacts with latent TB compared with that in healthy contacts, although this difference was not statistically significant ($p = 0.76$). Figure 1 presents a graphic comparison of miRNA expression among the three groups.

The sensitivity and specificity of miR-21-5p and miR-144-5-p expression were assessed using ROC curve analysis. As presented in Figure 2, miR-21-5p could differentiate latent and active TB with an AUC of 0.811 (95% CI = 0.67-0.953, $p = 0.001$). With a cutoff of 1.011, the sensitivity was 90%, and the specificity was 72.7%. ROC curve analysis revealed that miR-144-5p could differentiate latent and active TB with an AUC of 0.818 (95% CI = 0.689-0.947, $p<0.001$). With a cutoff of 0.691, the sensitivity and specificity were 80% and 72.7%, respectively.

Table 1. Study participants' characteristics

Characteristics	Group			*p*
	Active TB (n = 20)	Latent TB (n = 22)	Healthy contacts (n = 22)	
Age; mean ± SD (years)	41.65 ± 12.20	38.95 ± 12.56	36.5 ± 14.94	0.618
Age category; n (%)				0.459
<26	4 (20.0)	6 (27.3)	7 (31.8)	
26-35	4 (20.0)	3 (13.6)	4 (18.2)	
36-45	10 (50.0)	5 (22.7)	4 (18.2)	
>45	2 (10.0)	8 (36.3)	7 (31.8)	
Sex; n (%)				<0.05
Male	15 (75.0)	5 (22.7)	2 (9.1)	
Female	5 (25.0)	17 (77.3)	20 (90.9)	

Table 2. miR-21 and miR-144-5p expression in the active TB, latent TB, and healthy contacts groups

Group	Active TB (n = 20)	Latent TB (n = 22)	Healthy contacts (n = 22)	*p*			
				Active TB vs. Latent TB	Active TB vs. Healthy contacts	Latent TB vs. Healthy contacts	All groups
miR-21-5p expression (95% CI) [fold change]	5.281 (1.805-24.85)	0.359 (0.088-0.998)	0.141 (0.037-0.35)	0.001	<0.001	0.324	<0.001
miR-144-5p expression (95% CI) [fold change]	4.617 (1.034-24.504)	0.089 (0.031-0.642)	0.13 (0.105-0.396)	<0.001	<0.001	0.76	<0.001

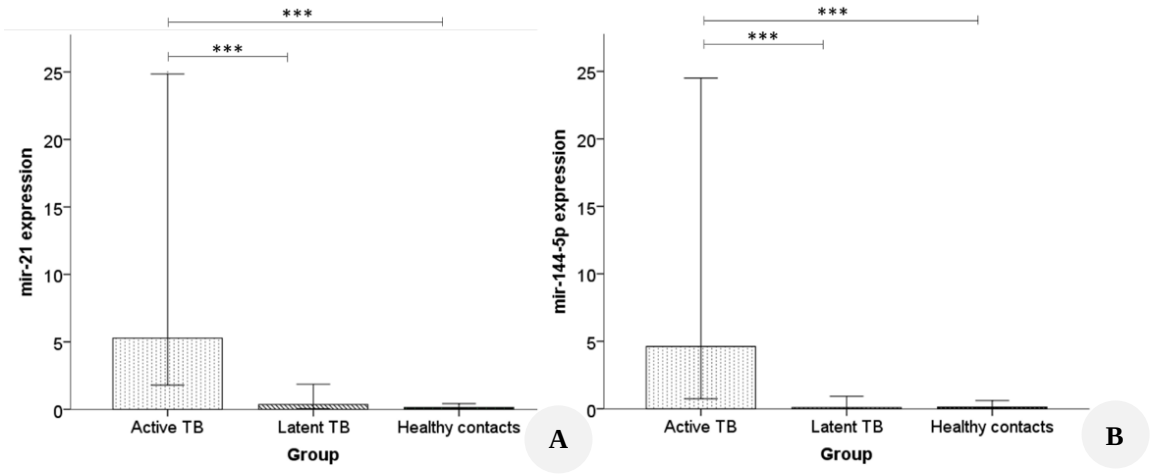

Figure 1. A. miR-21-5p; and B. miR-144-5p expression in sera from subjects in the active TB, latent TB, and healthy contacts groups. *** $p \leq 0.01$

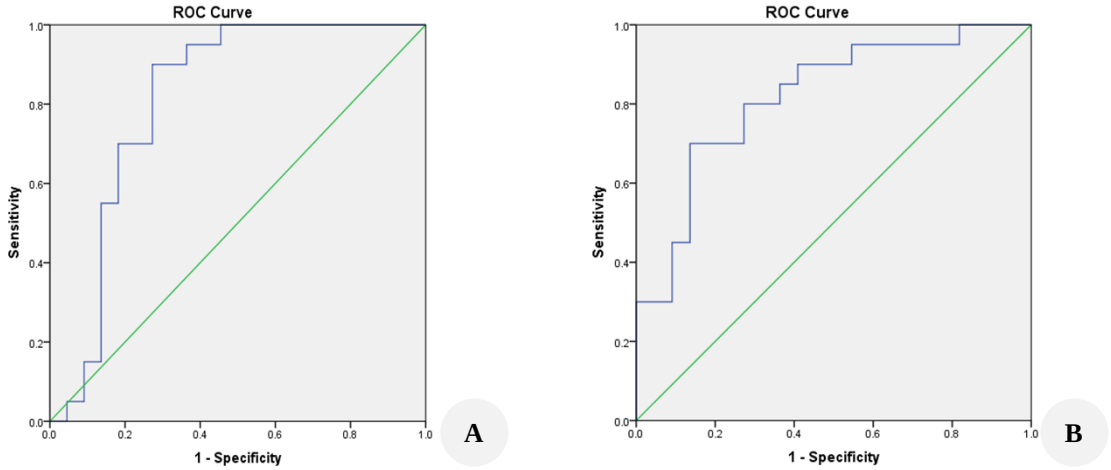

Figure 2. ROC curves of A. miR-21-5p; and B. miRNA 144 5-p for differentiating latent and active TB

Discussion

Diagnosing TB remains a significant problem globally, with incorrect diagnoses contributing to higher rates of illness and mortality. National TB programs rely heavily on techniques such as direct smear microscopy, solid cultures, radiographic examinations, and the TST to facilitate diagnosis in countries in which TB is endemic (Ndzi et al. 2019). Mtb culture is time-consuming and impractical as the definitive diagnostic standard for TB cases outside the lungs. The identification of smear-negative and extrapulmonary TB remains a significant clinical hurdle, particularly in pediatric cases because of the challenges in obtaining sputum samples. While the IGRA is useful for diagnosing latent TB, it cannot differentiate between latent and active TB. It is also not suitable for monitoring the treatment response in patients with active TB. Consequently, a more efficient diagnostic strategy that utilizes substitute specimens such as blood, feces, and urine, which can be collected from people of all ages, is required.

miRNA is a noteworthy biological marker currently being evaluated for its potential use in TB diagnosis. Interestingly, various microorganisms, including Mtb, induce epigenetic alterations during infection. While there are no definitive biomarkers that can reliably distinguish between latent and active TB, emerging research, including miRNA studies, shows promise in filling this diagnostic gap. Several research has been conducted to compare miRNA expression between individuals with infectious diseases and healthy controls. This expression difference, termed “differential expression,” is assessed using qPCR based on normalization or reference genes. miRNAs are endogenously expressed, highly conserved, noncoding sequences of 18-24 nucleotides that control the expression of posttranscriptional genes in a range of organisms under both normal physiological and pathological circumstances (Ratti et al. 2020).

miRNAs are essential for controlling the basic functions of dendritic cells, macrophages, and Natural Killer (NK) cells. Several studies revealed differences in the expression of specific miRNAs in NK cells and macrophages between

patients with latent TB and healthy individuals. miRNAs control T-cell function and differentiation by efficiently modifying gene expression (Behrouzi et al. 2019). Several studies revealed differences in gene expression patterns in NK cells and macrophages between people with latent or active TB and healthy controls. In patients with TB, miRNAs appear to orchestrate changes in cellular composition and related gene expression. In addition, macrophages, dendritic cells, and NK cells, which participate in the innate immune response, are crucial for regulating miRNAs. The robust stability of serum miRNAs makes miRNA profiling a proper diagnostic method in clinical laboratories. These molecules are robust against acidic and alkaline conditions, resistant to enzymatic degradation, and unaffected by changes in temperature and time. Serum miRNAs have garnered attention as possible biomarkers for TB and other pathological conditions because of their significant diagnostic potential (Pierce et al. 2020).

Several studies identified miRNAs as promising biomarkers in TB (Sabir et al. 2018). A study using miRNA microarray revealed the changes in miRNA expression profiles during the transition from latent to active TB (Wang et al. 2011; Yi et al. 2012). A previous study reported that miR-29a-3p expression was increased in patients with active and latent pulmonary TB, highlighting its potential as a biomarker for latent and active pulmonary TB (Angria et al. 2022). Another study demonstrated the high expression of miR-378 in TB and that this miRNA assisted in TB diagnosis in active and latent groups along with predicted adverse symptoms (Sun et al. 2022). Moreover, the profile of some miRNAs such as miR-29, miR-31, miR-125b, miR146a, and miR-155 have been reported. Their overall sensitivity, specificity, and Diagnostic Odds Ratio (DOR) meet the minimal target product profile for TB diagnostics (Daniel et al. 2022).

The importance of miR-21 and miR-144-5p expression arises from their roles in identifying the persistence of *Mycobacterium* in people exposed to these pathogens. One of the first oncomiRs discovered to be upregulated in a variety of cancers was miR-21. Consequently, miR-21 has emerged as a potential target for therapeutic interventions in various forms of cancer, and it has been proposed as a promising biomarker for both diagnosis and prognosis. Despite its established role in cancer diagnosis, more research is required to determine the relationship between miR-21 and infectious pathogens, mainly Mtb. Similarly, miR-144-5p is crucial in the diagnosis of several cancers. Several studies discussed the function of miR-144-5p in Mtb infection. However, this miRNA only differentiates between active and latent TB. Meanwhile, several reports have addressed the distinction between latent and active TB (Bautista-Sánchez et al. 2020; Ruiz-Tagle et al. 2020).

There was no significant difference in age and the age distribution among the active TB, latent TB, and healthy contacts groups in this study, although the sex distribution differed among these groups. Specifically, the proportion of males was higher in the active TB group than in the latent TB and healthy contacts groups. This finding aligns with WHO data indicating that more men are diagnosed with TB via smear positivity than women (WHO 2021). Moreover, this study found a significant increase in the expression of miR-21-5p and miR-144-5p in patients with active TB compared with that in contacts with latent TB. This result aligns with existing theories and earlier studies indicating that miR-21-5p expression is increased in macrophages infected with Mtb. By downregulating Bcl-2 and TLR-4, this upregulation simultaneously suppresses inflammatory cytokines and promotes apoptosis and mycobacterial survival. Similarly, in human monocytes infected with Mtb, miR-144-5p targets DRAM2 mRNA, inhibiting autophagy and promoting *Mycobacterium* survival (Sabir et al. 2018; Sinigaglia et al. 2020).

This study described the potential of serum miRNAs as helpful biomarkers in TB. However, miR-21-5p is more sensitive than miR-144-5p for diagnosis, which could be the basis for further, more specific research with a larger number of samples. Such research should examine the levels of inflammatory cytokines involved in the inflammatory pathway. Because of the sensitive nature of the two miRNAs, it is hoped that these findings will be useful for differentiating patients with active TB from those with latent TB and healthy controls and provide data for biomarkers for the early diagnosis of active and latent TB, in line with the Sustainable Development Goals. To the best of our knowledge, no prior study examined miR-21-5p and miR-144-5-p expression in patients with TB in the Indonesian population. Therefore, unlike previous studies that focused on global or regional TB biomarkers, our findings highlight unique expression patterns that may enhance early detection in Indonesia. These results have significant implications for developing diagnostic tools in the region. The latent TB infection status in household contacts was based on medical history, physical examination, and IGRA findings without radiological evaluation; hence, subclinical TB might not have been excluded in this investigation. Furthermore, we did not examine disease severity in patients with active TB, which might have contributed to their miRNA expression (Qureshi and Sacan 2013). Further cohort studies with larger sample sizes are required to verify the role of miR-21-5p and miR-144-5p in TB severity and treatment outcomes.

In conclusion, the expression of miR-21-5p was approximately 15-fold higher in patients with active TB than in contacts with latent TB. Additionally, miR-144-5p expression was approximately 52-fold higher in patients with active TB than in contacts with latent TB. Therefore, miR-21-5p and miR-144-5p show promise as biomarkers for distinguishing active TB from latent TB, but further research with larger sample sizes and additional validation is necessary.

ACKNOWLEDGEMENTS

We want to thank the laboratory staff of the Hasanuddin University Medical Research Center, Makassar, Indonesia. We would also like to express our gratitude to all the study participants.

REFERENCES

Angria N, Massi MN, Bukhari A, Djaharuddin I, Jumadi O, Ahmad A, Miskad UA, Ladju RB, Santoso A, Halik H. 2022. Expression of miRNA-29a-3p and IFN-γ as biomarkers in active and latent pulmonary tuberculosis. Ann Med Surg 83: 104786. DOI: 10.1016/j.amsu.2022.104786.

Bautista-Sánchez D, Arriaga-Canon C, Pedroza-Torres A, De La Rosa-Velázquez IA, González-Barrios R, Contreras-Espinosa L, Montiel-Manríquez R, Castro-Hernández C, Fragoso-Ontiveros V, Álvarez-Gómez RM, Herrera LA. 2020. The promising role of miR-21 as a cancer biomarker and its importance in RNA-based therapeutics. Mol Ther Nucleic Acids 20: 409-420. DOI: 10.1016/j.omtn.2020.03.003.

Behrouzi A, Alimohammadi M, Nafari AH, Yousefi MH, Rad FR, Vaziri F, Siadat SD. 2019. The role of host mirnas on *Mycobacterium tuberculosis*. ExRNA 1: 40. DOI: 10.1186/s41544-019-0040-y.

Daniel EA, Sathiyamani B, Thiruvengadam K, Vivekanandan S, Vembuli H, Hanna LE. 2022. MicroRNAs as diagnostic biomarkers for tuberculosis: A systematic review and meta- analysis. Front Immunol 13: 954396. DOI: 10.3389/fimmu.2022.954396.

Fachri M, Hatta M, Abadi S, Santoso SS, Wikanningtyas TA, Syarifuddin A, Dwiyanti R, Noviyanthi RA. 2018. Comparison of Acid Fast Bacilli (AFB) smear for *Mycobacterium tuberculosis* on adult pulmonary Tuberculosis (TB) patients with type 2 Diabetes Mellitus (DM) and without type 2 DM. Respir Med Case Rep 23: 158-162. DOI: 10.1016/j.rmcr.2018.02.008.

Flynn JL, Chan J. 2001. Tuberculosis: Latency and reactivation. Infect Immun 69 (7): 4195-4201. DOI: 10.1128/IAI.69.7.4195-4201.2001.

Hatta M, Sultan AR, Tandirogang N, Masjudi, Yadi. 2010. Detection and identification of mycobacteria in sputum from suspected tuberculosis patients. BMC Res Notes 3: 72. DOI: 10.1186/1756-0500-3-72.

Liu Y, Wang X, Jiang J, Cao Z, Yang B, Cheng X. 2011. Modulation of T cell cytokine production by miR-144* with elevated expression in patients with pulmonary tuberculosis. Mol Immunol 48 (9-10): 1084-1090. DOI: 10.1016/j.molimm.2011.02.001.

Massi MN, Hidayah N, Handayani I, Iskandar IW, Djannah F, Angria N, Halik H. 2023. microRNA hsa-miR-425-5p and hsa-miR-4523 expressions as biomarkers of active pulmonary tuberculosis, latent tuberculosis infection, and lymph node tuberculosis. Noncoding RNA Res 8 (4): 527-533. DOI: 10.1016/j.ncrna.2023.07.001.

Muñoz L, Stagg RH, Abubakar I. 2015. Diagnosis and management of latent tuberculosis infection. Cold Spring Harb Perspect Med 5 (11): a017830. DOI: 10.1101/cshperspect.a017830.

Ndzi EN, Nkenfou CN, Pefura EWY, Mekue LCM, Guiedem E, Nguefeu CN, Ngoufack MN, Elong E, Yatchou LG, Ndjolo A, Kuiate J-R. 2019. Tuberculosis diagnosis: Algorithm that May discriminate latent from active tuberculosis. Heliyon 5 (10): e02559. DOI: 10.1016/j.heliyon.2019.e02559.

Peltier HJ, Latham GJ. 2008. Normalization of microRNA expression levels in quantitative RT-PCR assays: Identification of suitable reference RNA targets in normal and cancerous human solid tissues. RNA 14 (5): 844-852. DOI: 10.1261/rna.939908.

Penn-Nicholson A, Mbandi SK, Thompson E et al. 2020. RISK6, a 6-gene transcriptomic signature of TB disease risk, diagnosis and treatment response. Sci Rep 10 (1): 8629. DOI: 10.1038/s41598-020-65043-8.

Pierce S, Geanes ES, Bradley T. 2020. Targeting natural killer cells for improved immunity and control of the adaptive immune response. Front Cell Infect Microbiol 10: 231. DOI: 10.3389/fcimb.2020.00231.

Qureshi R, Sacan A. 2013. A novel method for the normalization of microRNA RT-PCR data. BMC Med Genomics 6 (Suppl 1): S14. DOI: 10.1186/1755-8794-6-S1-S14.

Ratti M, Lampis A, Ghidini M, Salati M, Mirchev MB, Valeri N, Hahne JC. 2020. MicroRNAs (miRNAs) and long non-coding RNAs (lncRNAs) as new tools for cancer therapy: First steps from bench to bedside. Target Oncol 15 (3): 261-278. DOI: 10.1007/s11523-020-00717-x.

Ruiz-Tagle C, Naves R, Balcells ME. 2020. Unraveling the role of micrornas in *Mycobacterium tuberculosis* infection and disease: Advances and pitfalls. Infect Immun 88: e00649-19. DOI: 10.1128/IAI.00649-19.

Sabir N, Hussain T, Shah SZA, Peramo A, Zhao D, Zhou X. 2018. miRNAs in tuberculosis: New avenues for diagnosis and host-directed therapy. Front Microbiol 9: 602. DOI: 10.3389/fmicb.2018.00602.

Shepelkova GS, Evstifeev VV, Tarasov RV, Ergeshova AE, Bagirov MA, Yeremeev VV. 2023. MicroRNAs as biomarkers of active pulmonary TB course. Microorganisms 11 (3): 626. DOI: 10.3390/microorganisms11030626.

Sinigaglia A, Peta E, Riccetti S, Venkateswaran S, Manganelli R, Barzon L. 2020. Tuberculosis-associated MicroRNAs: From pathogenesis to disease biomarkers. Cells 9 (10): 2160. DOI: 10.3390/cells9102160.

Sun X, Liu K, Zhao Y, Zhang T. 2022. High miRNA-378 expression has high diagnostic values for pulmonary tuberculosis and predicts adverse outcomes. BMC Mol Cell Biol 23 (1): 14. DOI: 10.1186/s12860-022-00413-w.

Susilawati TN, Larasati R. 2019. A recent update of the diagnostic methods for tuberculosis and their applicability in Indonesia: A narrative review. Med J Indones 28: 284-291. DOI: 10.13181/mji.v28i3.2589.

Veryaskina YA, Titov SE, Zhimulev IF. 2022. Reference genes for qPCR-based miRNA expression profiling in 14 human tissues. Med Princ Pract 31 (4): 322-332. DOI: 10.1159/000524283.

Wang C, Yang S, Sun G, Tang X, Lu S, Neyrolles O, Gao Q. 2011. Comparative mirna expression profiles in individuals with latent and active tuberculosis. PLoS One 6 (10): e25832. DOI: 10.1371/journal.pone.0025832.

WHO [World Health Organization]. 2021. Global Tuberculosis Report 2021. World Health Organization, Geneva.

Wikanningtyas TA, Hatta M, Massi MN, Pratiwi I, Fachri M, Santoso SS, Syarifuddin A, Dwiyanti R, Noviyanthi RA. 2018. Diagnosis of a spectrum of pulmonary tuberculosis at Islam Hospital Sukapura, Jakarta, Indonesia: A retrospective study of 317 cases. J Med Sci 18 (3): 143-148. DOI: 10.3923/jms.2018.143.148.

Yi Z, Fu Y, Ji R, Li R, Guan Z. 2012. Altered microRNA signatures in sputum of patients with active pulmonary tuberculosis. PLoS One 7 (8): e43184. DOI: 10.1371/journal.pone.0043184.

Zhou M, Yu G, Yang X, Zhu C, Zhang Z, Zhan X. 2016. Circulating microRNAs as biomarkers for the early diagnosis of childhood tuberculosis infection. Mol Med Rep 13 (6): 4620-4626. DOI: 10.3892/mmr.2016.5097.