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Original Research Article

microRNA hsa-miR-425-5p and hsa-miR-4523 expressions as biomarkers of active pulmonary tuberculosis, latent tuberculosis infection, and lymph node tuberculosis

Muhammad Nasrum Massi [a,b,c,*], Najdah Hidayah [d], Irda Handayani [e], Israini Wiyulanda Iskandar [c], Fathul Djannah [f], Nirmawati Angria [g], Handayani Halik [b,g]

[a] Department of Clinical Microbiology, Faculty of Medicine, Universitas Hasanuddin, Makassar, 90245, South Sulawesi, Indonesia
[b] Hasanuddin University Medical Research Center Laboratory, Faculty of Medicine, Universitas Hasanuddin, Makassar, 90245, South Sulawesi, Indonesia
[c] Institute Research and Community Services Universitas Hasanuddin, Makassar, 90245, South Sulawesi, Indonesia
[d] Research Center for Vaccine and Drugs, National Research and Innovation Agency (BRIN), Tangerang Selatan, 15314, Banten, Indonesia
[e] Department of Clinical Pathology, Faculty of Medicine, Universitas Hasanuddin, Makassar, 90245, South Sulawesi, Indonesia
[f] Department of Anatomy Pathology, Faculty of Medicine, Universitas Mataram, Mataram, 83126, Indonesia
[g] Department of Biomedical Sciences, Faculty of Health Technology, Megarezky University, Makassar, 90234, South Sulawesi, Indonesia

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ABSTRACT

Background: Studies on miRNA highlight its significance as an immunomarker for several diseases, including tuberculosis. This study aimed to determine the difference between miR-425-5p and miR-4523 expressions in patients with active pulmonary TB (PTB), latent TB infection (LTBI), and lymph node TB (LNTB), whose diagnosis remains challenging.

Methods: This case–control study was performed on blood samples obtained from 23 patients with PTB, 21 with LTBI, 21 with LNTB, and 25 healthy controls (HC). miRNA hsa-miR-425-5p and hsa-miR-4523 expression levels were measured by RT-qPCR. Statistical analyses were performed using SPSS version 25.0.

Results: RT-qPCR showed that hsa-mir-425-5p and hsa-mir-4523 expression levels were significantly different among the four groups (PTB, LTBI, LNTB, and HCs). The hsa-mir-425-5p miRNA expression level in LNTB was higher than that in LTBI ($p = 0.003$). Meanwhile, the hsa-mir-4523 miRNA expression was downregulated in PTB and LNTB than in LTBI ($p < 0.0001$ and $p = 0.015$, respectively). The ROC analysis of a single sample showed that only mir-4523 could discriminate LTBI and HCs, with an AUC of 0.829 ($p < 0.001$). The ROC curve of each miRNA was further analyzed after logistic regression by adjusting for sex and age. The combination of both miRNAs was also analyzed. The model that analyzed the combination of both miRNAs after adjusting for age had the best performance in differentiating LNTB from LTBI, with an AUC of 0.97 ($p < 0.001$).

Conclusion: miRNA hsa-mir-425-5p was upregulated and miRNA hsa-mir-4523 was downregulated in PTB and LNTB than in LTBI.

1. Introduction

As the biggest cause of infection-related mortality, tuberculosis (TB) continues to have an impact on world health. It is difficult to understand how *Mycobacterium tuberculosis* (MTB), the life-threatening pathogen that causes TB, subverts the human immune system. Owing to the intracellular nature of MTB and slowed cell division, it has more opportunities to avoid the host's mycobactericidal action and survive. The course of TB infection is determined by the host's natural defense mechanisms and pathogen's tactics for avoiding the host's protection.

The illness trajectory becomes increasingly complicated as the phases of infection change, from latency to extrapulmonary. A quarter of the global population is estimated to have latent TB infection (LTBI), where MTB is long inactive and susceptible to active disease when the immune system is weakened. Due to this latency, the disease must be diagnosed and effectively treated to stop future disease development. However, current diagnostic tests for LTBI (e.g., the tuberculin skin test and interferon gamma [(IFN)-γ] release assays) could not distinguish

* Corresponding author. Department of Clinical Microbiology, Faculty of Medicine, Universitas Hasanuddin, Makassar, 90245, South Sulawesi, Indonesia.
E-mail address: nasrumm@unhas.ac.id (M.N. Massi).

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between LTBI and active TB. In addition, the emergence of extrapulmonary TB (EPTB) poses another threat. EPTB has become a significant issue in the last 10 years as tubercular infection trends have changed. Intestinal TB, bone TB, and cervical lymphadenitis TB are examples of extrapulmonary TB (EPTB), which can also present beyond the lungs. Cervical lymphadenitis TB is the most prevalent kind of EPTB observed in Indonesia, and the majority of cases were well-organized granulomas [1,2]. Approximately 15%–20% of all TB cases in individuals with immunocompromised status and >50% of cases in HIV-positive individuals are caused by EPTB [3]. Given its various forms and vague initial symptoms, EPTB diagnosis is challenging for clinicians because the patient is not seen at an early stage of the disease. Delayed diagnosis is further attributed to the absence of typical clinical symptoms and frequently negative results of traditional diagnostic procedures (smear microscopy and culture) because of the paucibacillary nature of samples.

The "end TB strategy" emphasizes the importance of easily available biomarkers for the early detection of TB. microRNA (miRNA) exploration offers a technique to find potential treatment targets. miRNAs are short noncoding RNAs that are biologically conserved, ranged in length from 18 to 25 nucleotides, and characterized by gene regulation, transcription, and immunomodulation [4]. By effectively attaching to the 3′ UTR of mRNA, miRNAs control nearly 60% of mammalian genes, which causes translational repression and mRNA degradation [5]. Studies on miRNA have highlighted its significance as an immunomarker for the course of active diseases and an immunomodulator for innate systems including apoptosis and autophagy.

Numerous research have been done during the past ten years to emphasize the significance of looking at TB infection at the RNA level. miRNAs are emerging as crucial regulators in key biological processes and are engaged in the post-transcriptional regulation of several protein-coding genes in tissue-specific ways. Previous studies have shown that miRNAs play a critical role in the outcome of many viral diseases by modulating the host immune system. According to recent data, specific human miRNAs may play a role in TB by either increasing the immune response or aiding pathogen immune evasion [6]. Selected miRNAs have demonstrated their relevance in adaptive and innate immune responses against MTB through functional analysis, revealing their potential as diagnostic and prognostic markers in active TB. The ability to identify differentially expressed miRNAs in bodily fluids such serum, saliva, and urine makes them a prime candidate marker to track TB and discriminate between current and latent infections [7].

So far, there is still few studies have focused on miRNA expressions in TB cases in the Indonesian population. In this study, we investigated two miRNAs, namely, miR-425-5p and miR-4523, which were previously observed to be upregulated and downregulated, respectively, in EPTB [7]. This study aimed to determine the difference between miR-425-5p and miR-4523 in active pulmonary TB (PTB), latent TB (LTBI), and extrpulmonary TB (EPTB), whose diagnosis remains challenging. We also examined lymph node TB (LNTB), which is the most frequent EPTB found in Indonesia [1,2]. The results of this study are expected to assist in the development of biomarkers for the early diagnosis of active PTB, EPTB, and LTBI.

2. Material and methods

2.1. Participant selection and sample collection

This case–control study was performed on blood samples obtained from 23 patients with active PTB, 21 with LTBI, 21 with LNTB, and 25 HCs). Patients with PTB were recruited from the Community Lung Health Center Makassar and had not received anti-TB drugs at the time of recruitment. LTBI was recruited from the households of patients with PTB. LNTB was recruited from several primary healthcare centers in Mataram, West Nusa Tenggara. The diagnosis of TB was implemented according to a positive microscopic smear and liquid culture (MGIT, BD Bactec). Individuals with LTBI and HCs were household contacts of patients with TB, who were distinguished by interferon gamma (IFN-γ) release assay. In this study, household contacts with positive IGRA were categorized as LTBI, and those negatives were considered HCs. LNTB was diagnosed histopathologically, using tissue from a neck lump surgery, or cytologically, using the fine-needle aspiration biopsy, using aspirate from the lump. The presence of Datia Langhans cells, epithelioid cells, mature lymphoid cells, and caseous necrosis supports the diagnosis.

Table 1
miRNA sequenced used in this study.

Target miRNA	Accession Number (miRbase)	Sequence (5′–3′)
hsa-miR-103a-3p [9]	MIMAT0000101	AGCAGCAUUGUACAGGGCUAUGA
hsa-miR-425-5p [7]	MIMAT0003393	AAUGACACGAUCACUCCCGUUGA
hsa-miR-4523 [7]	MI0016890	GACCGAGAGGGCCUCGGCUGU

2.2. Interferon-gamma release assay

IGRA was performed using QuantiFERON Gold Plus TB Test Kit (QFT-Plus); 4 mL of venous blood was extracted from household contact participants, and 0.8–1.2 μL was transferred to QFT-Plus tubes (nil, TB1, TB2, and mitogen). These four tubes were simultaneously shaken until covered in blood. These four tubes were incubated at 37 °C for 16–24 h, followed by 15 min of centrifugation to separate the plasma. Plasma samples were stored at −20 °C until employed in the IFN-γ enzyme-linked immunosorbent assay (ELISA) procedure, according to the manufacturer's protocols. The optical density (OD) was measured using an ELISA reader with a 450-nm filter and 620–650-nm reference filter. QuantiFERON TB Gold Plus Analysis Software ver. 2.71 (Qiagen) was used to calculate the IFN-γ from the OD readings. All the tested samples had Nil values ≤ 8 IU/mL and mitogen–Nil difference of ≥0.5 IU/mL, indicating that all samples were valid. The difference value of Tb1–Nil and/or Tb2–Nil of ≥0.35 IU/mL was considered positive [8].

2.3. RNA isolation and complementary DNA synthesis

The total RNA was extracted using the RNA Extraction Kit (Qiagen) following the protocols of the manufacturer. The total RNA was reverse-transcribed into cDNA using the miRCURY LNA RT Kit (Cat. no. 339340, Qiagen). cDNA was quantified by Biodrop LITE. The blood samples from the patients were extracted using 300 μL. To reverse transcribe into cDNA, adding 2 μL of RNA isolation (based on a concentration of 5 ng/μL) and 8 μL of the enzyme kit, resulting in a total volume of 10 μL. In the RT-PCR assay, 3 μL of cDNA was mixed in. The reverse transcription step was performed for 60 min at 42 °C, followed by a 5-min inactivation of the reaction at 95 °C. The resulting cDNA was stored at 4 °C indefinitely.

2.4. miRNA profiling

The expression levels of miRNA hsa-miR-425-5p and hsa-miR-4523 were measured using reverse-transcription quantitative polymerase chain reaction (RT-qPCR). The miRCURY LNA SYBR® Green PCR Kit (Qiagen) was employed, along with specific primers listed in Table 1 (obtained from miRBase: the microRNA database, available at https://www.mirbase.org/). The reference gene chosen for this analysis was mir-103a-3p. mir-103a-3p was selected based on previous research, which demonstrated its superior statistical performance compared to the commonly used reference RNAs in miRNA qRT-PCR experiments involving human samples. The data analysis revealed a standard deviation of Cq: 4.06, with a mean of 30.2 and a median of 29.4 [9,10]. The CFX96 Touch Real-time PCR Detection System (Bio-Rad, CA, USA) was

Table 2
Demographic information and clinical characteristics of the study participants.

Characteristics	LTBI (n = 21)	LNTB (n = 21)	PTB (n = 23)	HC (n = 25)	p-value
Age (median (IQR)), years	42 (19.5)	21 (12.5)	43 (14)	40 (29)	0.001
Sex					
Male (n, %)	5 (23.8%)	6 (28.6%)	16 (69.6%)	6 (24%)	0.002
Female (n, %)	16 (76.2%)	15 (71.4%)	7 (30.4%)	19 (76%)	
Granuloma (LNTB)					
WOG		17 (68%)			
POG		8 (32%)			

Abbreviations: HC, healthy controls; IQR, interquartile range; LTBI, latent TB infection; LNTB, lymph node TB; PTB, pulmonary TB; POG, poorly organized granuloma; WOG, well-organized granuloma. The p-value based on chi-square test.

used to conduct RT-qPCR. Following an initial denaturation at 95 °C for 2 min, the qPCR cycles were as follows: 45 cycles of denaturation at 95 °C for 10 s, annealing at 56 °C for 1 min, and plate reading. The melt curve was produced by raising the temperature from 65.0 to 95.0 °C with an increase of 0.5 °C every 5 s, then reading the plate. The relative expression level of each miRNA in individual samples was calculated based on Equation $2^{-\Delta\Delta Ct}$, where $\Delta Ct = Ct_{miRNA} - Ct_{endogenous\ control}$. All the data was provided in the Supplementary file 1.

2.5. Statistical analysis

The statistical analysis was performed using IBM SPSS Statistics for Windows version 26.0 (IBM Corp., Armonk, NY, USA). Categorical data were compared using the chi-square test or Fisher exact test. Student's t-test or one-way analysis of variance was performed to determine the difference in miRNA expressions among groups, followed by Tukey's multiple comparison tests. The receiver operating characteristic (ROC) curve was used to analyze the sensitivity and specificity of the single miRNA. The ROC curve was also used after logistic regression models adjusted for a significant independent variable to evaluate the ability of a single miRNA or combination of both miRNAs in discriminating among groups, PTB, LTBI, and LNTB. For all statistical analyses, a p-value <0.05 was considered significant.

3. Results

3.1. Characteristics of the participants

The total study sample included 23 patients with active PTB, 21 with LTBI, 21 with EPTB, and 25 HCs. The characteristics of the participants are shown in Table 2.

3.2. miRNA expression

RT-qPCR results showed that hsa-mir-425-5p expression levels were significantly different ($p = 0.006$) among the four groups (active PTB, LTBI, LNTB, and HCs). However, this difference was due to the significant difference in hsa-mir-425-5p miRNA expression levels between LTBI and LNTB. mir-425-5p miRNA was upregulated by 19.8 times in LNTB compared with that in LTBI ($p = 0.003$). Details are shown in Table 3 and Fig. 1.

Fig. 2 shows the expression levels of hsa-mir-425-5p and hsa-mir-4523 in patients with active PTB, LTBI, and LNTB with LTBI as control. The expression level of hsa-mir-425-5p miRNA in LNTB was higher (upregulated) than that in LTBI. Meanwhile, the expression level of hsa-mir-4523 miRNA was lower (downregulated) in active PTB and LNTB than in LTBI.

The sensitivity and specificity analyses of mir-425-5p and mir-4523 expressions were assessed using the ROC curve (Table 4 and Fig. 3). The ROC analysis of a single miRNA (model I) showed that only mir-4523

Table 3
Differences in expressed miRNAs in the serum samples of LTBI, LNTB, and PTB.

Target	P-value ANOVA	Contrast group	Ratio	Lower bound (95%)	Upper bound (95%)	P-value Tukey
mir-425-5p	0.006*	PTB–LNTB	0.18501	0.022	1.525	0.163
		PTB–TBI	3.66035	0.394	34.030	0.428
		PTB–HC	0.75657	0.092	6.238	0.986
		LNTB–LTBI	19.78472	2.270	172.423	0.003*
		LNTB–HC	4.08935	0.531	31.488	0.277
		LTBI–HC	0.20669	0.024	1.801	0.233
mir-4523	0.0001*	PTB–LNTB	0.35184	0.033	3.783	0.658
		PTB–LTBI	0.02049	0.002	0.227	<0.0001*
		PTB–HC	0.93319	0.096	9.085	1.000
		LNTB–LTBI	0.05825	0.005	0.663	0.015*
		LNTB–HC	2.65232	0.265	26.572	0.685
		LTBI–HC	45.53270	4.406	470.576	<0.0001*

Abbreviations: HC, healthy controls; LTBI, latent TB infection; LNTB, lymph node TB; PTB, pulmonary TB.

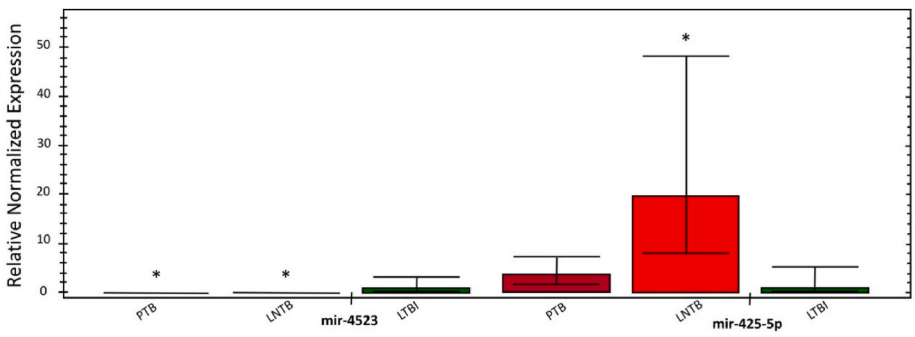

Fig. 1. Differences in the expression levels of hsa-mir-425-5p and hsa-mir-4523 microRNA in patients with PTB, LTBI, LNTB, and HC (star).

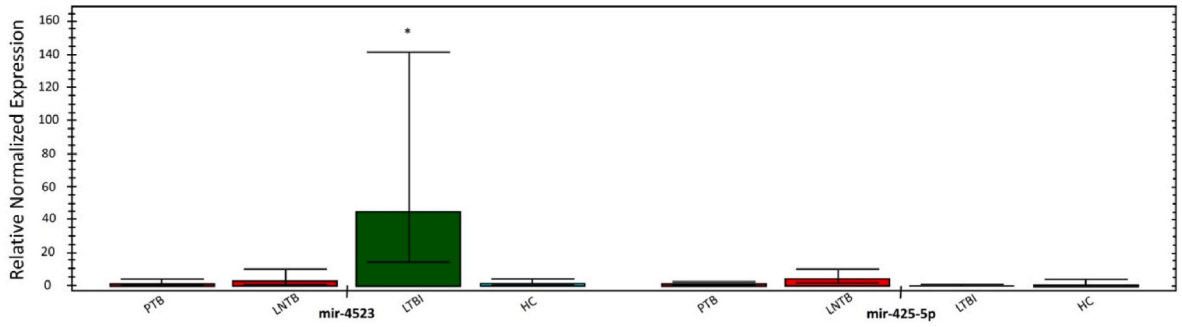

Fig. 2. Differences in the expression levels of hsa-mir-425-5p and hsa-mir-4523 in patients with active pulmonary tuberculosis (TB), LTBI, and LNTB using LTBI as control.

Table 4
Sensitivity and specificity analyses of the expression levels of hsa-mir-425-5p and hsa-mir-4523 miRNA.

miRNA	Contrast group	AUC	Cutoff	Sensitivity	Specificity	p-value
Model I [a]						
hsa-mir-425-5p	LNTB–LTBI	0.723	0.128	95.2%	47.6%	0.013
hsa-mir-4523	PTB–LTBI	0.199	–	–	–	0.001
	LNTB–LTBI	0.236	–	–	–	0.003
	LTBI–HC	0.829	0.041	85.7%	80%	<0.001
Model II[b]						
hsa-mir-4523	PTB–LTBI	0.859	0.478	87%	76.2%	<0.001
Model III[c]						
hsa-mir-4523	LNTB–LTBI	0.887	0.603	81%	81%	<0.001
Model IV[d]						
hsa-mir-4523 + hsa-mir-425-5p	LNTB–LTBI	0.846	0.435	95.2%	71.4%	<0.001
Model V[e]						
hsa-mir-4523 + hsa-mir-425-5p	LNTB–LTBI	0.907	0.598	90.5%	81%	<0.001

Abbreviations: AUC, area under curve; HC, healthy controls; LTBI, latent TB infection; LNTB, lymph node TB; PTB, pulmonary TB.
[a] Single miRNA.
[b] Adjusted for sex.
[c] Adjusted for age.
[d] Combination of hsa-mir-425-5p and hsa-mir-4523.
[e] Combination of hsa-mir-425-5p and hsa-mir-4523, adjusted to age.

could discriminate LTBI and HC, with an area under the curve (AUC) of 0.829 ($p < 0.001$). In addition, hsa-mir-425-5p had an AUC of >0.5, showing significance ($p = 0.013$). The specificity was only 47.6%, but the sensitivity reached 95.2%. We further analyzed the ROC curve of each miRNA after performing logistic regression by adjusting for sex and/or age. We also analyzed the combination of both miRNAs, and the results are shown in Table 4. Model V, which analyzed the combination of both miRNAs after adjusting for age, showed the best performance, with an AUC of 0.97 ($p < 0.001$). With a cutoff value of 0.598, the sensitivity and specificity were 90.5% and 81%, respectively, which were higher than those of a single miRNA. The regression equation built for each model is shown in the supplementary materials.

4. Discussion

More than half of all TB cases worldwide go undiagnosed, which shows it complex and complicated diagnosis [11]. A robust index of suspicion is necessary because a generalized debility may disguise symptoms. When other medical disorders were found to have produced aberrant chest X-ray findings and the culture findings returned negative for *M. tuberculosis*, the initial diagnosis of TB was deemed erroneous and amended. *M. tuberculosis* culture, the gold standard for TB diagnosis, takes time and is inappropriate for EPTB. The rates of identification with a positive result as high as 80% have been recorded for sputum culture for acid-fast bacilli (AFB), which is a relatively accurate and robust method of TB detection. When a patient's health improved or the patient died shortly after being diagnosed with pneumonia, but *M. tuberculosis* was not isolated, a diagnosis of pneumonia was confirmed [12]. Thus, an improved diagnostic method based on other specimens, such as blood, feces, and urine, is necessary. Nowadays, the most outstanding approach in diagnosing TB is to utilize miRNA gene expressions [13].

By regulating different gene expressions in the host cell, miRNAs play a role in some pathways that modify immune responses. In recent studies regarding the role of miRNAs in innate and adaptive immune responses during *M. tuberculosis* infection, the developing role of miRNAs in regulating adaptive and innate immunity has drawn increasing attention from numerous research groups [14,15]. According to several studies, the differential expression of miRNAs may be free to differentiate LTBI from active illness conditions and may indicate disease development. These results offer information on the possible use of miRNAs as TB biomarkers. Moreover, miRNAs and synthetic anti-miRNAs may have a bright future in human health despite being a relatively new technology that has yet to be extensively employed in clinical settings [15,16]. In addition, miRNAs are more acceptable candidates because of their excellent stability in bodily fluids and their association with various diseases [17]. Although this approach has certain limits, miRNAs can be employed as biomarkers for the categorization of infectious diseases and therapeutic reasons, such as TB.

A preliminary study on circulating miRNAs in patients with active PTB reported 59 upregulated and 33 downregulated signatures compared with non-TB controls [18]. In this study, we examined miRNA 425-5p and miRNA-4523, which were reported previously as the most upregulated and downregulated in EPTB [7], encouraging us to investigate their expressions in LTBI and active TB. Chakrabarty et al. (2019) identified that hsa-miR-425-5p was upregulated and hsa-miR-4523 was downregulated in EPTB when compared with those in healthy individuals [7]. In the present study, we found that hsa-miR-425-5p was upregulated and has-miR-4523 was downregulated in lymphadenitis TB compared with LTBI, but not in HCs.

MiRNA 425-5p and miRNA-4523 play a part in TB illness as well as the control of cancer. In liver hepatocellular carcinoma (LIHC), for instance, miRNA 425-5p is specifically targeted because it controls immune regulation in the microenvironment of LIHC [19]. Moreover, it has

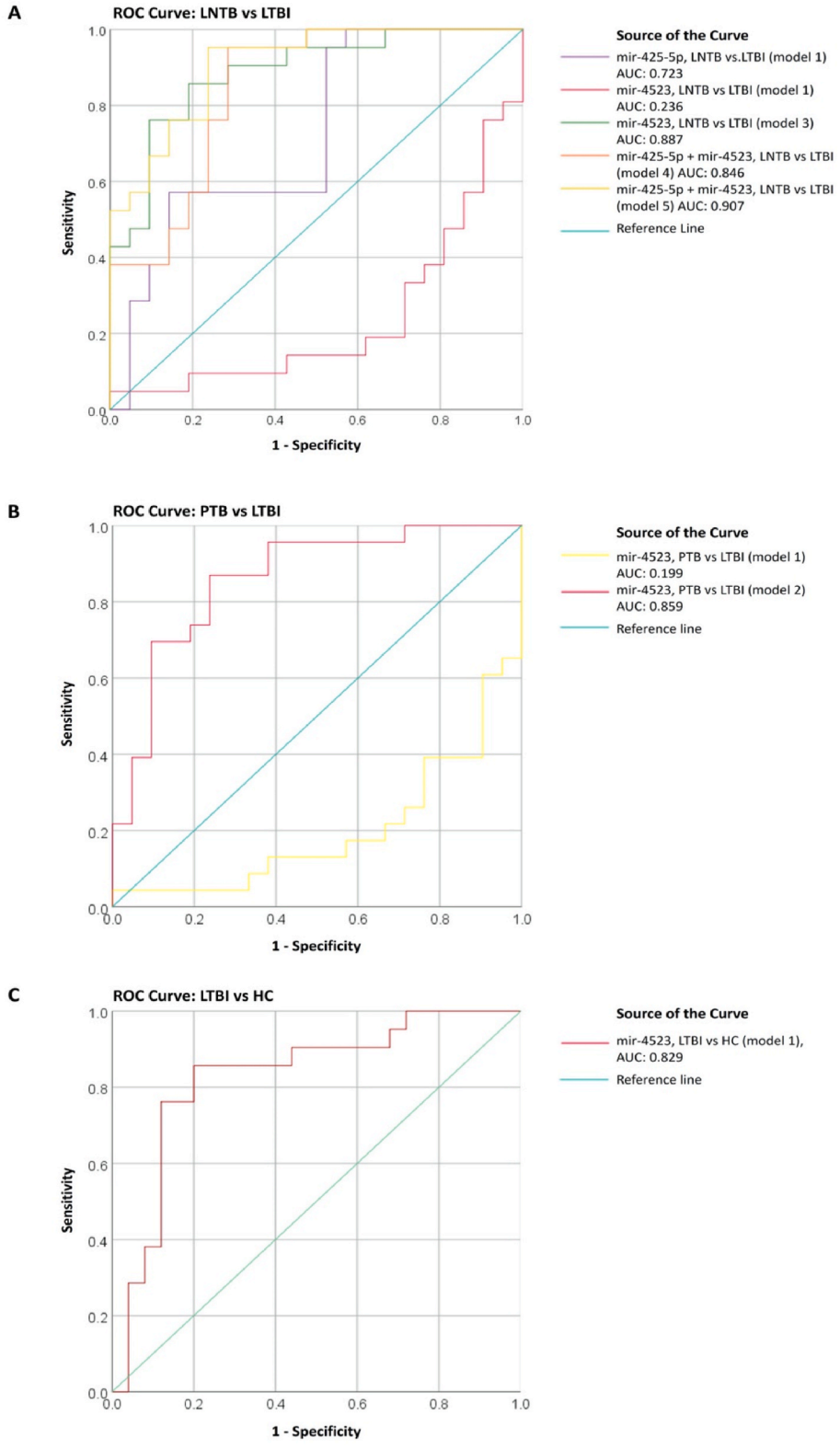

Fig. 3. Diagnostic power of hsa-miR-425 and hsa-4523 expression levels determined by the ROC curve analysis. The ROC curve was also used after the logistic regression models were adjusted for significant independent variables to evaluate the ability of a single miRNA or a combination of both miRNAs in discriminating PTB, LTBI, and LNTB. (A) LNTB vs LTBI, model 1, single miRNA; model 3, adjusted for age; model 4, combination of hsa-mir-425-5p and hsa-mir-4523; model 5, combination of hsa-mir-425-5p and hsa-mir-4523, adjusted for age. (B) PTB vs LTBI, model 1, single miRNA; model 2, adjusted for sex. (C) LTBI vs HC, model 1, single miRNA. All ROC curve analyses included were significant ($p < 0.05$).

been discovered that the expression of miR-425-5p is markedly up-regulated in the tissues and cells of ovarian cancer [20]. In addition, miR-4523 overexpression can inhibit phosphoglycerate kinase 1 (PGK1), which reduces the amount of cell death brought on by dexamethasone (DEX) [21].

miR-425 has been identified previously as one of the top three miRNAs (besides miR-484 and miR-96-3p), modulating an important pathway in serum exosomes of TB patients. According to Alipoor et al., M. bovis bacillus Calmette-Guerin infection may cause control host metabolic pathways and energy production [22]. Furthermore, Alipoor et al. observed the overexpression of miR-425 in serum exosomal miRNAs of patients with active TB compared with HCs [23]. This study, miR-425-5p was also found to be significantly increased in patients with active TB compared with LTBI but not significantly different when

compared with HCs. The results differed because the HCs were not individuals who were free from exposure to TB but were contacts of patients with TB and negative QuantiFERON results. We hypothesized that the TB exposure factor might have confounded the results. However, owing to data limitations such as the duration of interaction or number of exposures that were not obtained in this study, we could not determine further whether exposure could be a confounding factor in this study.

Conversely, the biological roles of miR-4523 must be better understood. It is found in chromosome 17 (Chr17). Previous studies have shown that miR-4523 is a new and precise PGK1-targeting miRNA. Experiments using RNA-FISH, RNA pull-down, and Ago2 RNA-IP showed that miR-4523 directly correlated with PGK1 in hFOB1 and primary human osteoblasts, which showed that miR-4523 expression levels were lower in necrotic femoral head tissues than in healthy bone tissues. Meanwhile, in TB studies, only a few researchers have analyzed the expression of this miRNA type in active TB, LTBI, and EPTB. Chakrabarty et al. (2019) only analyzed the expression level of mir-4523 in EPTB and active TB and compared it with healthy asymptomatic patients; however, they did not further investigate the TB infection status of these patients [7]. They found that mir-4523 regulation declined by approximately 5.05 (log) in EPTB compared with HCs. In addition, our study showed that mir-4523 experienced downregulation in EPTB. Furthermore, the Kyoto Encyclopedia of Genes and Genomes stated a significant correlation of hsa-miR-4523 with the signaling pathway of NF-kappa B (NF-kB) [24,25].

A recent study identified a new binding site of hsa-miR-452 to TLR-2. It showed a probable involvement of mir-4523 in the dysregulation and expression changes in TLR-2 [26]. TLR-2 is crucial enough for immunoprotection against *M. tuberculosis*. TLR2 disruption or blocking could cancel the translocation of NF-kB and tumor necrosis factor (TNF)-α. Through TLR2, the synthesis of TNF-α is induced by *M. tuberculosis* EsxL. Moreover, *M. tuberculosis* EsxL induces TNF-α through the phosphorylation of p38 and the p38 and ERK1/2 MAPK- and NF-kB-dependent pathways [27].

The results of the analysis were significantly different in the Mann–Whitney test between the two groups, followed by the ROC analysis. The ROC analysis of a single miRNA (model 1) revealed that mir-425-5p had an AUC of >0.5, showing significance ($p = 0.013$). However, the specificity was only 47.6%. In addition, the ROC analysis of a single miRNA (model 1) showed that only the comparison of LTBI with HC could be differentiated by hsa-mir-4523 (AUC >0.5), whereas the comparisons of PTB with LTBI and LNTB with LTBI could not be differentiated by hsa-mir-4523 (AUC <0.5). We further analyzed the ROC curve with a single miRNA and a combination of mir-425-5p and mir-4523 after performing logistic regression by adjusting for significant variables (sex or age, models 2–5). The results showed that the best model was model 5, i.e., by combining these variables and adjusting for age, its AUC was 0.907 ($p < 0.001$). The findings demonstrated that a single serum miRNA may not be a single biomarker for diagnosing LTBI, but a combination, and taking into account other variables such as sex and age, may provide better results.

This study has several limitations. The study sample was not homogeneous based on age and sex. However, we adjusted the analysis for age and other variables to minimize bias. In addition, we did not further analyze the relationship between mir-425-5p and mir-4523 with the degree of active TB. Nevertheless, this study provides evidence that mir-425-5p and mir-4523 have a potential in diagnosing LNTB and LTBI and that mir-4523 has the potential to differentiate PTB from LTBI after adjusting for sex.

Studies on mir-425-5p and mir-4523 in TB are still very limited. To the best of our knowledge, this study is the first to combine mir-425-5p and mir-4523 in differentiating LNTB from LTBI. In a clinical setting, it might be difficult to diagnose LNTB because traditional approaches, such as AFB smear and culture, are not always effective and the majority of LNTB samples, such as cerebrospinal fluid and aspirates, are paucibacillary, which also contributes to the milder type of infection. Further miRNA investigations are expected to be beneficial, particularly in the development of miRNA-based vaccines, biomarkers, and host-directed therapeutics.

5. Conclusion

The expression of miRNA has-mir-425-5p was upregulated in active PTB and LNTB than in LTBI. In addition, the expression of miRNA hsa-mir-4523 was downregulated in active TB and LNTB than in LTBI. Therefore, the expression levels of miRNA hsa-mir-425-5p and hsa-mir-4523 represent potential biomarkers for LTBI.

Ethical consideration

The study protocol complies with the Declaration of Helsinki and has been approved by Health Research Ethics Committee of Faculty of Medicine, Universitas Hasanuddin (No. 258/UN4.6.4.5.3.1/PP36/2022, May 28, 2022). Each participant gave written informed consent and agreed to participate in the study.

Author Contributions

M.N.Masi: Conception and design of study, Data analysis and/or interpretation, Drafting of manuscript and/or critical revision. N. Hidayah: Acquisition of data (laboratory or clinical), Drafting of manuscript and/or critical revision. I. Handayani: Drafting of manuscript and/or critical revision. I.W. Iskandar: Acquisition of data (laboratory or clinical), Drafting of manuscript and/or critical revision. F. Djannah : Conception and design of study, Data analysis and/or interpretation. N. Angria: Acquisition of data (laboratory or clinical). H. Halik: Conception and design of study, Acquisition of data (laboratory or clinical), Data analysis and/or interpretation.

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Conflict of interest

All authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ncrna.2023.07.001.

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