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Active *versus* latent pulmonary tuberculosis: which one is the appropriate distinguishing biomarker?

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Abstract

This study tried to assess the possibility of using the estimated levels of plasma expression of microRNAs (miR-) for distinguishing healthy subjects with latent pulmonary tuberculosis (LTB) from healthy controls (HC) and patients with active tuberculosis (ATB). Study participants included 30 newly diagnosed ATB patients, 30 of the households of ATB patients who were free of clinical manifestations, had normal chest radiography but had positive results on the whole-blood QuantiFERON tuberculosis (TB) Gold In-Tube (QFT-GIT) test (LTB patients), and 30 HC who were free of clinical symptoms and showed normal chest X-rays and negative QFT-GIT tests. All participants gave blood samples for quantitation of the plasma expression levels of miR- using the reverse transcription-quantitative polymerase chain reaction. Plasma levels of miR-150-5p were significantly downregulated in ATB samples than in other samples. However, miR-155-5p and miR-378-5p were significantly overexpressed in patients' samples compared to HC's samples and in ATB samples compared to LTB samples. On the contrary, plasma miR-4523-5p showed significant upregulation in LTB samples compared to ATB and HC samples, indicating insignificant in-between differences. The receiver operating characteristic curve analysis showed the ability of the estimated levels of the four miR- to differentiate TB patients from HC. Multivariate regression analysis defined expression levels of miR-155-5p and miR-378-5p as the significant biomarkers for distinguishing TB patients and levels of miR-378-5p and miR-4523-5p for identification of LTB patients. Pulmonary TB induces deregulated expression of miR-, according to the infection severity. An estimation of the expression levels of miR-378-5p and miR-4523-5p might be a reliable combination for identifying LTB patients.

Key words: latent pulmonary TB, healthy subjects, active pulmonary TB, microRNAs.

Introduction

Despite the decreased incidence and mortality secondary to tuberculosis (TB), its global burden was still substantial and about 10 million newly infected individuals were encountered yearly [1]. Pulmonary tuberculosis (PTB) is defined as a chronic contagious disease, which is caused by the Mycobacterium tuberculosis (M. tb.), transmitted almost exclusively through cough aerosol, causing caseating granulomatous inflammation in the lungs as the main target organ but can spread to infect other organs; extra-pulmonary tuberculosis [2].

Pulmonary TB is the transmissible form of tuberculosis and presents as active (ATB) or latent (LTB) according to the WHO definition, LTB is a state of persistent immune response to stimulation by MBT antigens with no evidence of clinically manifest active disease [3]. LTB comprises infected persons who harbor the tubercle bacilli, but the absence of manifestations of active disease might be considered a huge reservoir of potential active cases in the future [4,5].

Active tuberculosis may be primary or reactivated LTB; primary TB manifests the failure of the immune system to defend against the MTB infection, while reactivation TB represents the reactivation of contained mycobacterial infection and represents 90% of cases of ATB [6].

Optimizing the reactivation of the LTB rate through diagnosis and treatment of persons at risk for TB may be an important strategy for eradication of TB [7]. However, the shortcomings of the commercially available diagnostic methods due to their insufficient sensitivities, time consumption, and weak response, especially in immune-compromised individuals hamper the differentiation between active or reactivated TB and LTB [8].

Therefore, The WHO recently identified rapid biomarker-based, non-sputum-based diagnostic testing, using an easily accessible sample as the prerequisite of the new TB diagnostics to help for differentiation between active and latent TB [9].

Objectives

This study tried to evaluate the ability of a microarray of microRNAs to identify healthy subjects with LTP from healthy subjects free of TB in comparison to LTB patients.

Materials and Methods

Design

This is a prospective case-control non-randomized comparative study carried out at the Department of Internal Medicine, Medical Biochemistry, Faculty of Medicine, Benha University.

Study participants

Patients admitted to the isolation wards of the Internal Medicine Department with provisional diagnoses of active tuberculosis, and their households were evaluated for the inclusion and exclusion criteria. A similar number of healthy volunteers with patients' matched age and accepted to participate in the study were also evaluated for the inclusion and exclusion criteria.

Exclusion criteria

Patients with ATB and maintained on antituberculosis therapy, irrespective of the response to treatment; TB patients gave undetermined QuantiFERON-TB Gold In-tube (QFT-GIT) test, newly immunized, maintained on immunotherapy, had immunological disorders or other morbidities were excluded from the study.

Inclusion criteria and grouping

1. ATB group included 30 newly diagnosed ATB patients depending on typical clinical presentation, chest radiography, acid-fast stain of sputum smear, and positive sputum culture for MBT, and fulfilled the exclusion criteria.
2. The LTB group included 30 healthy households of ATB patients who were diagnosed depending on having a positive QFT-GIT test despite the absence of clinical manifestations and normal chest radiography.
3. HC group included 30 healthy volunteers of cross-matched age to the enrolled patients who were free of clinical symptoms, had normal chest X-rays, and showed negative QFT-GIT test.

Participants' data

Clinical data of the enrolled 90 participants were determined and included age, gender, body mass index (BMI) that was calculated as weight in kg divided by height in meter square, smoking status, TB patients the presence of low-grade fever, chest pain, expectoration, and hemoptysis.

Investigations

1. Tuberculin Skin Test (TST): intradermal injection of 0.1 ml of purified protein derivative was performed into the volar aspect of the forearm, and after 72-h, the mean vertical and transverse induration diameters were measured and positive TST was indicated if dimensions of the induration were 10 mm.
2. The whole-blood QuantiFERON TB Gold In-Tube (QFT-GIT) Test: The test was performed according to the manufacturer's instructions (Cellectis, Ltd., Victoria, Australia) for the measurement of the levels of interferon-gamma (IFN-γ) release in the processed whole blood samples and consists of two steps:
   - Incubation of the whole blood with antigens: peripheral venous blood was withdrawn and immediately transferred as 1 ml in each of the three QFT-GIT tubes; a tube contained only heparin as a negative control, a tube contained T-cell mitogen as a

positive control, and the 3rd tube containing TB-specific antigens. The tubes were homogenized by 10-time inversion and then were incubated for 16-24 h at 37 °C.

- ELISA measurement of IFN-γ production: after the end of the incubation period, the tubes were centrifuged, and the separated plasma was stored at 4 °C until being ELISA assayed to measure IFN-γ release using interferon-gamma release assay (IGRA) within 2 weeks after blood collection. The results obtained by the negative control were subtracted from that of the positive control and the antigen-stimulated samples and the cutoff point for the positive result is 0.35 IU/ml. The result was considered negative if the IGRA measures were <0.35 IU/ml. In case of a negative result for the antigen-stimulated sample with the positive control reading <0.5 IU/ml or the negative control reading >8 IU/ml, the result was considered undetermined [10].

3. Total RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) (*Supplementary Table 1*).

- Blood samples were aseptically collected in EDTA tubes and preserved at –80°C for the relative quantification of the plasma expression levels of miR-155-5p, miR-150-5p, miR-31-5p and miR-4523-5p.
- Total RNA including microRNA was isolated using the miRNeasy Mini Kit (QIAGEN, Germany) and the complementary DNA was synthesized using the miScript II RT Kit (QIAGEN, Germany. The recommended RNA starting amounts and buffers for reverse transcription reactions for quantization of miRNAs, by using miScript precursor assays were 5x miScriptHiFlex buffer and the recommended RNA input depends on the abundance and number of target miRNAs to be quantified; up to a maximum of 1 µg (miRNeasy Mini Kit, 2- miScript II RT Kit:QIAGEN).
- The mixture was incubated at 37°C for 60 minutes and at 95°C for 5 minutes to inactivate the miScript Reverse Transcriptase. Then, the mixture was placed on ice and diluted with 40 µl RNase-free water to the 10-µl reverse transcription reaction, mixed gently then briefly centrifuged and continued with quantitative real-time PCR for detection of microRNAs expression levels using QuantiTect SYBR Green PCR Kit. The PCR reaction mix was prepared in a total volume of 25µl / tube.
- The reaction conditions: the real-time cycler was programmed using ABI 7900HT Fast Real-Time PCR System, (Applied Biosystem, Singapore) according to the instructions of the manufacturer for each microRNA as follows:

- For miR-150-5p and miR-155-5p: initial denaturation for 20-sec at 95°C was followed by 40 cycles of annealing at 95°C for 3-sec and extension at 60°C for 3-sec [11].
- For miR-378-5p: initial activation for 10-min at 95°C, denaturation at 94°C for 10-sec, annealing at 60°C for 20-sec and extension at 72°C for 34-sec, for 40 cycles [12].
- For miR-4523-5p: after initial denaturation at 95°C for 2-min, 45 cycles of denaturation at 95°C for 10-sec and annealing for 1-min at 56°C were conducted [13].

- MicroRNA expression levels in each sample were determined after correction with the U6 expression level. Controls were chosen as the reference samples, and fold changes in the plasma expression levels of microRNAs were determined by the 2- CT (cycle threshold) method and expressed as fold change (FC) using Step One software (Applied Biosystems, USA).

Statistical analyses

The obtained results were analyzed using IBM® SPSS® Statistics (Version 22, 2015; Armonk, USA) to compare the inter-group difference by One-way ANOVA test. The Receiver Operating Characteristic (ROC) Curve was conducted to evaluate the predictability of plasma expression levels of the studied microRNAs for differentiation between patients' samples and that of HC and between samples of ATB and LTB subjects . The results of the ROC analysis were presented as the area under the curve and its significance was verified versus the area under the references line (AUC=0.5). These predictors were verified using the Multivariate Regression analysis to define the best predictor for the identification of TB patients and LTB subjects . The optimum cutoff point for the significance of a result is P=0.05.

Results

Through the duration of the study, 51 ATB were evaluated for enrolment, but six patients had other chest diseases, five patients were maintained on antituberculosis therapy, 4 patients were maintained on immunosuppressive drugs, 3 patients had diabetes mellitus and 3 patients had impaired liver functions; these 21 patients were excluded and 30 patients were enrolled in the study as ATB group. Forty-four of the patients' households were evaluated, but four gave an undetermined QFT-GIT test, three were diabetics, 3 were cardiac patients,

two had autoimmune disorders and two refused to participate in the study; these 14 households were excluded and 30 were enrolled in the study as LTB group.

Patients showed significantly (P=0.0051) lower BMI in comparison to HCs despite the insignificant differences as regards participants' distribution according to BMI grades. Other participants' enrolment data showed insignificant intra-group differences (Table 1).

Estimated plasma levels of miR-150-5p showed significant downregulation in samples of ATB in comparison to levels estimated in samples of HC (P<0.001) and LTB subjects (P=0.0025), while were insignificantly (P=0.072) lower in samples of LTB subjects than in samples of HC. However, miR-155-5p levels were overexpressed in samples of ATB (P<0.001) and LTB (P=0.036) subjects in comparison to samples of HC with significantly (P=0.0003) lower expression levels in samples of LTB subjects than ATB patients. Moreover, plasma expression levels of miR-378 were significantly (P<0.001) upregulated in patients' than HC samples with significantly (P=0.0014) higher expression levels in ATB patients than LTB subjects . On contrary, plasma miR-4523-5p showed significant upregulation in samples of LTB subjects in comparison to HC samples (P<0.001) and ATB samples (P=0.00003) with insignificantly upregulated expression levels in samples of ATB patients than HC samples (Table 2).

Statistical analysis using ROC defined the ability of the estimated levels of the four microRNAs to differentiate TB patients from HC. Further, miR-150-5p and miR-155-5p expression levels might be used as screening biomarkers, while estimated levels of miR-378-5p and miR-4523-5p might be used as specific predictors for the presence of TB infection (Table 3). Verification of the levels of these microRNAs according to the significance of the AUC-difference showed a significant (P=0.025) difference between AUCs for miR-378-5p and miR-4523-5p (AUC difference= 0.127±0.301, 95% CI: 0.016-0.238) in favor of miR-378-5p. On the contrary, the AUCs difference for miR-150-5p and miR-155-5p (AUC difference= 0.042±0.314, 95% CI: [-0.095]-0.179) was insignificant (P=0.545) but in favor of miR-150-5p. Multivariate Regression analysis defined expression levels of miR-155-5p and miR-378-5p as the significant biomarkers for distinguishing TB patients with β=0.426 and 0.445, respectively (Figure 1).

Differentiation of the ability of TB diagnostic procedures and estimated levels of microRNAs to distinguish LTB subjects from ATB patients defined estimated levels of miR-378-5p and miR-4523-5p as screening and diagnostic variates, respectively with significant AUC, while excluding the remaining biomarkers (Figure 2). Regression analysis assured the significant ability of miR-378-5p and miR-4523-5p for identification of LTB subjects and also showed

the ability estimated levels of miR-150-5p as screening variate for LTB but with lower significance (Table 3).

Discussion

Recently, latent TB (LTB) was considered a huge reservoir of potential active cases for the future [4,5], but unfortunately, the differentiation of LTB subjects was still a dilemma because of the dependence on clinical manifestations and chest x-rays a fake for being normal in healthy controls (HCs) and LTB subjects . Moreover, the shortcomings of the available investigations as documented in the current results where both TST and IGRA showed insignificant differences between ATB and LTB subjects and statistical analyses showed the weak differentiating ability of TST and IGRA for LTB from ATB.

These data point to the necessity for getting more accurate distinguishing methods, thus the current study tried to elucidate the adequacy of estimated plasma expression levels of certain microRNAs using the quantitative PCR as reliable biomarkers for defining LTB subjects from HC. In line with this hypothesis, multiple recent studies documented that microRNAs represent potential biomarkers for LTB and ATB from HC [14-17].

The expression levels of miR-150 were significantly downregulated in samples of ATB patients than in samples of HCs and LTB subjects who showed insignificant differences versus HC levels. Thus, miR-150 might be used as a single biomarker for the identification of ATB, but not for differentiating cases of LTB from HC. In support of this, statistical analyses excluded expression levels of miR-150 on the evaluation of the differentiating ability for LTB. Moreover, Zhou et al. and Chen et al. detected downregulation of miR-150 in children and adults, respectively [11,18], with TB compared with uninfected cross-matched HCs and assured the high diagnostic value for ATB over other microRNAs as a single biomarker.

The reported decreased expression levels of miR-150 that were significant in ATB and insignificant in LTB than HC samples might illustrate the inverse relation between disease activity and severity and the release of the anti-inflammatory miR-150-5p. In line with this assumption, using an animal model of sepsis, the expression of miR-150 was downregulated and replenishing miR-150 reduced the immunosuppressing function of myeloid-derived suppressor cells by down-regulating arginine-1 gene expression [19]. Clinically, miR-150-5p expression was downregulated in the serum of septic acute kidney injury patients and animal model of sepsis-induced acute kidney injury, miR-150-5p was found to exert its protective effects by regulating the mitogen-activated protein kinase 3/c-Jun NH2-terminal kinase pathway [20]. Recently, it was documented that the anti-inflammatory miR-150-5p was

significantly upregulated in serum and cerebrospinal fluid-derived exosomes of relapsing-remitting multiple sclerosis patients than HCs [21].

On the other hand, miR-155 expression levels were upregulated in samples of ATB patients and LTB subjects than in samples of HCs, and ATB patients than LTB subjects . These results supported the preliminary experimental study that found miR-155 exhibited 1.4-fold change in HC and 3.7 in ATB peripheral blood mononuclear cells upon MTB purified protein derivative (PPD) stimulation [22]. Recently, another recent study found the fold change in expression of miR-155 on PPD stimulation was significantly higher in ATB samples than in samples of HC and LTB [16]. Clinically, Shepelkova et al. detected overexpression of miR-155 in samples of patients had tuberculoma with decay than in samples of patients had tuberculoma without decay [23].

The increased expression levels of miR-155 in TB patients especially those who had active disease illustrated a positive relation between expression levels of miR-155 and disease activity. Such relation was attributed to the proinflammatory function of miR-155 through suppressing the nuclear-factor-E2-related factor-2 (Nrf2) cascade, which functions to suppress pyroptosis in MTB-infected macrophages with subsequent flaring up of infection [24].

Furthermore, ROC curve analysis found miR-155 could differentiate samples of TB from those of HC and Regression analysis assured its significant predictability for the presence of TB infection but excluded miR-155 as a predictor for LTB. These data point to the inadequacy of reliance on miR-155 as solo differentiating procedure and this assumption was supported by review of literature that showed the presence of some inconsistency in the reported expression levels of miR-155 in case of TB where Kathirvel et al. and Alijani et al. detected upregulated expression [16,25], while Zhou et al. and Abdalla et al. reported downregulation of expression of miR-155 in TB patients [18,26].

Regarding the expression levels of miR-378, the present study found miR-378 was overexpressed in samples of TB patients than HCs and in samples of ATB than in samples of LTB subjects and statistical analyses showed the ability of miR-378 to distinguish TB patients and identify LTB subjects . These results go in hand with Sun et al. [27], who found miR-378 was more highly expressed in TB patients than HC and in the active than the latent group and detected downregulated miR-378 expression in treated TB patients than untreated and in responders to those showed drug resistance.

These findings indicated a positive relation between expression levels of miR-378 and disease activity and adverse outcomes. Similarly, Soonthornchai et al. and Diotallevi et al. detected overexpression of miR-378 in psoriatic lesions in parallel with increased

inflammatory cytokines than in non-psoriatic skin biopsies and its expression levels were decreased with methotrexate therapy [28,29]. The role of miR-378 in lesion flaring up might occur through causing cell cycle arrest via suppressing cyclin D1 that controls G1 exit [30], miR378-a/Gli3/p53 axis independently of cyclin D1 or directly targeted bone morphogenetic protein-2 which is an essential element for activating TGF-β signaling cascade [28].

The expression of miR-4523-5p was significantly upregulated in samples of LTB than in samples of HCs and ATB patients and thus can distinguish LTB subjects from both HCs and ATB patients. Similarly, Massi et al. found the expression of miR-4523 was significantly higher in LTB than in ATB ($P<0.00001$) and lymph node TB ($P<0.015$) and ROC analysis showed that only miR-4523 could discriminate LTB and HCs with significant high AUC [31].

The overexpression of miR-4523 might be a defense mechanism against infection-induced cell injury and development of complications of infection, in support of this assumption, an experimental study using cell culture found overexpression of miR-4523 significantly attenuated the production of reactive oxygen species, oxidative stress and cell apoptosis through activation of the Nrf2 cascade mostly via silencing of phosphoglycerate kinase-1 leading to its depletion which efficiently activates Nrf2 signaling [32].

These data indicated the unreliability of dependence on one biomarker for diagnosis and/or differentiation of cases had LTB. In support of this assumption, statistical analyses for the ability of TB diagnostic procedures and estimated levels of microRNAs to distinguish LTB subjects from ATB patients showed the complementary diagnostic yield of combined estimation of the expression levels of miR-378-5p and miR-4523-5p as screening and diagnostic variates, respectively, while other procedures and microRNAs were excluded.

Conclusions

Pulmonary TB induced deregulated expression of microRNAs with contradictory roles in immune response according to the severity of tuberculous infection. Single microRNAs could not be a reliable solo biomarker for the differentiation of LTB subjects from HC, so combined estimations of the expression levels of miR-378-5p and miR-4523-5p might be reliable biomarkers for identifying LTB patients.

Limitations

Measurements of serum levels of cytokines associated with TB infection to interpret the mechanisms of action of the studied microRNAs were the limitations of this study. Also, the study being a single-center study is another limitation of the study. Another limitation of the

current study, is the need for well-equipped lab and the test being an expensive as screening test

Recommendations

Multicenter large scale studies are mandatory to establish the obtained results. The implementation of these tests for subjects in contact with ATB patients as a preliminary step to reduce the number of those have LTB because the cost of the test might be equalized for the consumption of hospital resource for admission and treatment of these subjects.

References

1. Dheda K, Perumal T, Moultrie H, et al. The intersecting pandemics of tuberculosis and COVID-19: population-level and patient-level impact, clinical presentation, and corrective interventions. Lancet Respir Med 2022;10:603-22.
2. Furin J, Cox H, Pai M. Tuberculosis. Lancet 2019;393:1642-56.
3. World Health Organization. Guidelines on the management of latent tuberculosis infection. 2015. Available from: https://iris.who.int/bitstream/handle/10665/136471/9789241548908_eng.pdf?sequence=1.
4. Kiazyk S, Ball TB. Latent tuberculosis infection: an overview. Can Commun Dis Rep 2017;43:62-6.
5. Palanivel J, Sounderrajan V, Thangam T, et al. Latent tuberculosis: challenges in diagnosis and treatment, perspectives, and the crucial role of biomarkers. Curr Microbiol 2023;80:392.
6. MacPherson P, Lebina L, Motsomi K, et al. Prevalence and risk factors for latent tuberculosis infection among household contacts of index cases in two South African provinces: Analysis of baseline data from a cluster-randomised trial. PLoS One 2020;15:e0230376.
7. Vonnahme L, Raykin J, Jones M, et al. Using electronic health record data to measure the latent tuberculosis infection care cascade in safety-net primary care clinics. AJPM Focus 2023;2:100148.
8. Bi D, Liang C, Huang X, et al. Clinical utilization of multiple antibodies of Mycobacterium tuberculosis for serodiagnosis evaluation of tuberculosis: a retrospective observational cohort study. Ann Med 2023;55:2238186.

9. Wu X, Tan G, Ma J, et al. Assessment of the Cepheid 3-gene host response fingerstick blood test (MTB-HR) on rapid diagnosis of tuberculosis. Emerg Microbes Infect 2023;12:2261561.
10. Siegel SAR, Cavanaugh M, Ku JH, et al. Specificity of QuantiFERON-TB Plus, a new-generation interferon gamma release assay. J Clin Microbiol 2018;56:e00629-18.
11. Chen Y, Lee C, Hsiao C, et al. MicroRNA-23a-3p down-regulation in active pulmonary tuberculosis patients with high bacterial burden inhibits mononuclear cell function and phagocytosis through TLR4/TNF-α/TGF-β1/IL-10 signaling via targeting IRF1/SP1. Int J Mol Sci 2020;21:8587.
12. Zhou R, Jia Y, Wang Y, et al. Elevating miR-378 strengthens the isoflurane-mediated effects on myocardial ischemia-reperfusion injury in mice via suppression of MAPK1. Am J Transl Res 2021;13:2350-64
13. Chakrabarty S, Kumar A, Raviprasad K, et al. Host and MTB genome encoded miRNA markers for diagnosis of tuberculosis. Tuberculosis 2019;116:37-43.
14. Angria N, Massi M, Bukhari A, et al. Expression of miRNA-29a-3p and IFN-γ as biomarkers in active and latent pulmonary tuberculosis. Ann Med Surg (Lond) 2022;83:104786.
15. Ann Daniel E, Sathiyamani B, Thiruvengadam K, et al. MicroRNAs as diagnostic biomarkers for Tuberculosis: a systematic review and meta-analysis. Front Immunol 2022;13:954396.
16. Alijani E, Rad F, Katebi A, Ajdary S. Differential expression of miR-146 and miR-155 in active and latent tuberculosis infection. Iran J Public Health 2023;52:1749-57.
17. Sheng Y, Hua H, Yong Y, Zhou L. Identification of hub genes and typing of tuberculosis infections based on autophagy-related genes. Pol J Microbiol 2023;72:223-38.
18. Zhou M, Yu G, Yang X, et al. Circulating microRNAs as biomarkers for the early diagnosis of childhood tuberculosis infection. Mol Med Rep 2016;13:4620-6.
19. Liu Q, Wang Y, Zheng Q, et al. MicroRNA-150 inhibits myeloid-derived suppressor cells proliferation and function through negative regulation of ARG-1 in sepsis. Life Sci 2021;278:119626.
20. Shi L, Zhang Y, Xia Y, et al. MiR-150-5p protects against septic acute kidney injury via repressing the MEKK3/JNK pathway. Cell Signal 2021;86:110101.
21. Mohammadinasr M, Montazersaheb S, Molavi O, et al. Multiplex analysis of cerebrospinal fluid and serum exosomes micrornas of untreated relapsing remitting multiple sclerosis (RRMS) and proposing noninvasive diagnostic biomarkers. Neuromolecular Med 2023;25:402-14.

22. Wu J, Lu C, Diao N, et al. Analysis of microRNA expression profiling identifies miR-155 and miR-155* as potential diagnostic markers for active tuberculosis: a preliminary study. Hum Immunol 2012;73:31-7.
23. Shepelkova G, Evstifeev V, Tarasov R, et al. MicroRNAs as biomarkers of active pulmonary TB course. Microorganisms 2023;11:626.
24. Fu Y, Shen J, Liu F, et al. Andrographolide suppresses pyroptosis in Mycobacterium tuberculosis-infected macrophages via the microRNA-155/Nrf2 axis. Oxid Med Cell Longev 2022;2022:1885066.
25. Kathirvel M, Saranya S, Mahadevan S. Expression levels of candidate circulating microRNAs in pediatric tuberculosis. Pathog Glob Health 2020;114:262-70.
26. Abdalla A, Alanazi A, Abosalif K, et al. MicroRNA-155, a double-blade sword regulator of innate tuberculosis immunity. Microb Pathog 2023;185:106438.
27. Sun X, Liu K, Zhao Y, Zhang T. High miRNA-378 expression has high diagnostic values for pulmonary tuberculosis and predicts adverse outcomes. BMC Mol Cell Biol 2022;23:14.
28. Soonthornchai W, Tangtanatakul P, Meesilpavikkai K, et al. MicroRNA-378a-3p is overexpressed in psoriasis and modulates cell cycle arrest in keratinocytes via targeting BMP2 gene. Sci Rep 2021;11:14186.
29. Diotallevi F, Matacchione G, d'Agostino G, et al. Inflamma miR-146a and -155 Plasma Levels are Associated with Clinical Efficacy of Risankizumab Treatment in Psoriatic Patients: Pilot Study. Dermatol Ther (Heidelb) 2023;13:1377-87.
30. Feng M, Li Z, Aau M, et al. Myc/ miR-378/TOB2/cyclin D1 functional module regulates oncogenic transformation. Oncogene 2011;30:2242-51.
31. Massi M, Hidayah N, Handayani I, et al. 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 2023;8:527-33.
32. Liang J, Zhou Z, Bo L, et al. Phosphoglycerate kinase 1 silencing by a novel microRNA microRNA-4523 protects human osteoblasts from dexamethasone through activation of Nrf2 signaling cascade. Cell Death Dis 2021;12:964.

Online supplementary material:
Supplementary Table 1. The sequences of the primers used for detection of the expression levels of each miR.

Table 1. The data of the study participants.

Variate	Group	HC (n=30)	ATB (n=30)	LTB (n=30)	P-value
Age (years)	<40	4 (13.3%)	5 (16.7%)	3 (10%)	0.969
	40-49	6 (20%)	5 (16.7%)	6 (20%)	
	50-59	12 (40%)	13 (43.3%)	11 (36.7%)	
	60	8 (26.7%)	7 (23.3%)	10 (33.3%)	
	Mean (±SD)	52.3 (±9.5)	51 (±10.7)	53.3 (±11.2)	0.696
Gender	Males	20 (66.7%)	23 (76.7%)	21 (70%)	0.685
	Females	10 (33.3%)	7 (23.3%)	9 (30%)	
BMI (kg/m^2)	Underweight	0	5 (16.7%)	2 (6.7%)	0.192
	Average	5 (16.7%)	8 (26.7%)	7 (23.3%)	
	Overweight	11 (36.7%)	10 (33.3%)	9 (30%)	
	Obese	14 (46.6%)	7 (23.3%)	12 (40%)	
	Mean (±SD)	29.63 (±3.6)	25.96 (±5)	27.21 (±4.2)	0.0051
Smoking	Current	6 (20%)	12 (40%)	9 (30%)	0.355
	Ex-smoker	9 (30%)	10 (33.3%)	8 (26.7%)	
	Non-smoker	15 (50%)	8 (26.7%)	13 (43.3%)	
Clinical data	Low-grade fever	-	6 (20%)	-	-
	Hemoptysis	-	3 (10%)	-	-
	Chest pain	-	5 (16.7%)	-	-
	Expectoration	-	5 (16.7%)	-	-
Tuberculin Skin Test (mm)		-	13.99 (±3.9)	16.3 (±5.1)	0.055
Interferon-γ Release Assay (IU/ml)			1.34 (±0.94)	1.8 (±1.21)	0.106

The P-value indicates the significance of the intra-group differences.

Table 2. Mean expression levels of the studied microRNAs in samples of TB patients compared to samples of HC subjects.

Variate / Group		HC (n=30)	ATB (n=30)	LTB (n=30)
miR-150-5p	Mean (±SD)	0.96 (0.43)	0.35 (0.27)	0.71 (0.61)
	P1		<0.001	0.072
	P2			0.0025
miR-155-5p	Mean (±SD)	0.53 (0.33)	1.29 (0.64)	0.74 (0.43)
	P1		<0.001	0.036
	P2			0.0003
miR-378-5p	Mean (±SD)	1.17 (0.34)	2.15 (0.59)	1.68 (0.48)
	P1		<0.001	<0.001
	P2			0.0014
miR-4523-5p	Mean (±SD)	0.18 (0.11)	0.23 (0.11)	0.48 (0.28)
	P1		0.091	<0.001
	P2			0.00003

P1 signifies the difference in expression levels in patients' samples versus samples of HC subjects; P2 signifies the difference in expression levels in samples of ATB versus LTB patients.

Table 3. Statistical analyses for the estimated expression levels of the studied microRNAs as differentiating variates.

	Analyses	ROC Curve analysis				Regression analysis	
	Biomarkers	AUC	Std.	P	95% CI	β	P
Between TB patients and HC subjects	Mir-150-5p	0.237	0.049	<0.001	0.098-0.291	0.324	0.002
	Mir-155-5p	0.195	0.049	<0.001	0.141-0.333	0.426	<0.001
	Mir-378-5p	0.861	0.037	<0.001	0.788-0.934	0.445	<0.001
	Mir-4523-5p	0.734	0.054	<0.001	0.627-0.840	0.335	0.001
Between LTB and ATB patients	TST	0.629	0.073	0.085	0.486-0.773	0.198	0.070
	IGRA	0.671	0.072	0.072	0.530-0.812	-0.036	0.788
	Mir-150-5p	0.627	0.077	0.090	0.477-0.777	0.281	0.033
	Mir-155-5p	0.392	0.075	0.152	0.245-0.539	-0.223	0.093
	Mir-378-5p	0.288	0.067	0.005	0.157-0.419	-0.339	0.002
	Mir-4523-5p	0.768	0.062	<0.001	0.647-0.889	0.464	<0.001

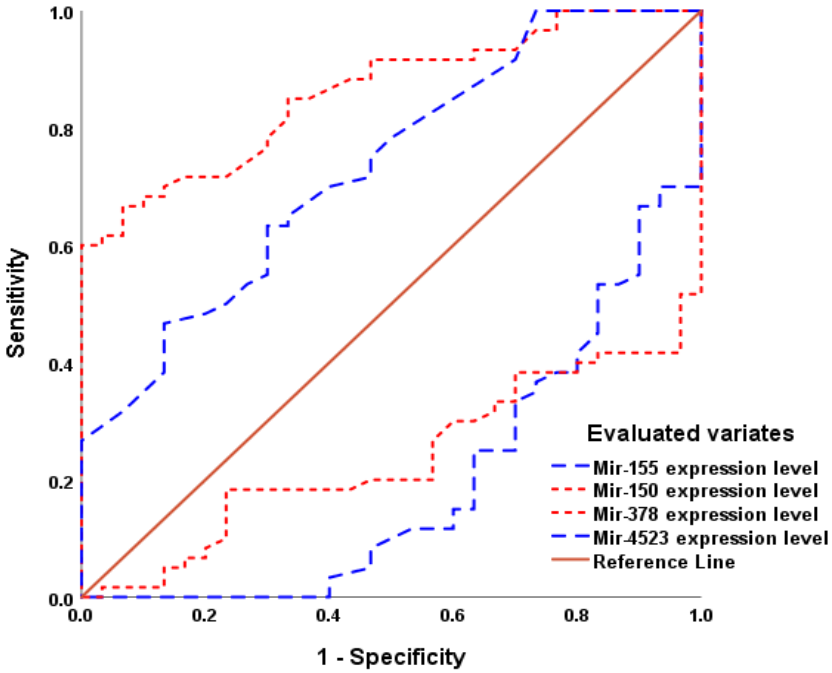

Figure 1. ROC curve for evaluation of the ability of expression levels of the studied microRNAs to differentiate between samples of TB patients and HC subjects.

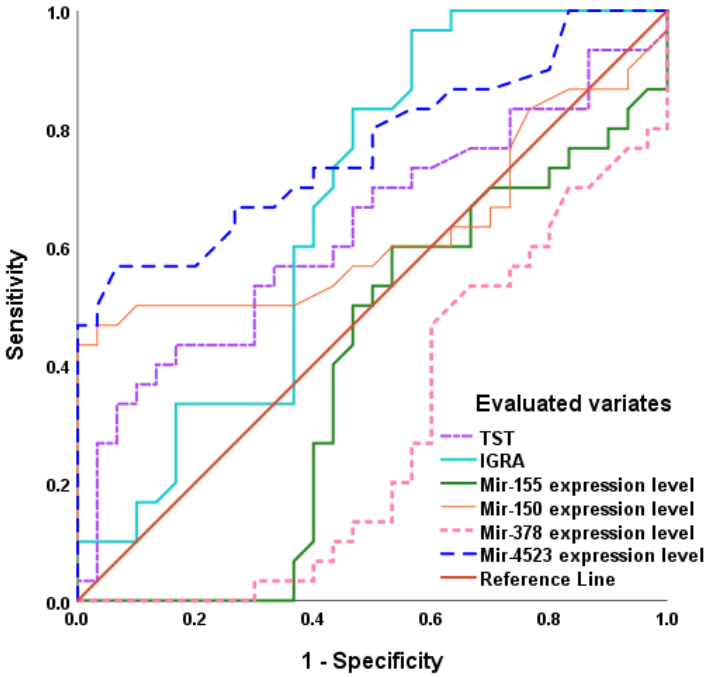

Figure 2. ROC curve for evaluation of the ability of TB diagnostic tests and expression levels of the studied microRNAs to differentiate between samples of ATB and LTB patients.