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

Rizk Sayad R. Sarhan,[1] Omnia Y. Habashy,[2] Raafat R. Mohammed,[3] Yasmin M. Marei[2]

[1]Department of Internal Medicine, Faculty of Medicine, Benha University; [2]Department of Medical Biochemistry, Faculty of Medicine, Benha University; [3]Hospital Lab, Clinical Pathology Department, Faculty of Medicine, Benha University, Egypt

Correspondence: Rizk Sayad R. Sarhan, Department of Internal Medicine, Faculty of Medicine, Benha University, Benha, Egypt.
Tel.: +00201090969655.
E-mail: rizk.r.sarhan72023@gmail.com

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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 the 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.

Introduction

Despite the decreased incidence and mortality secondary to tuberculosis (TB), its global burden is still substantial, and about 10 million newly infected individuals are encountered yearly [1]. Pulmonary TB is defined as a chronic contagious disease, which is caused by the *Mycobacterium tuberculosis* (MTB), 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 TB [2].

Pulmonary TB is the transmissible form of TB and presents as active (ATB) or latent (LTB) according to the World Health Organization (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 people

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].

ATB 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 people at risk for TB may be an important strategy for the 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 immunocompromised 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 differentiate between ATB and LTB [9].

Objectives

This study tried to evaluate the ability of a microarray of microRNAs (miR) 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 ATB, and their households, were evaluated for the inclusion and exclusion criteria. A similar number of healthy volunteers with patients' matched age who accepted to participate in the study were also evaluated for the inclusion and exclusion criteria.

Exclusion criteria

Patients with ATB and maintained on anti-TB therapy, irrespective of the response to treatment; TB patients who gave an 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

The 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.

The LTB group included 30 healthy households of ATB patients who were diagnosed based on having a positive QFT-GIT test despite the absence of clinical manifestations and normal chest radiography.

The healthy control (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), which was calculated as weight in kg divided by height in m^2, smoking status; for TB patients, the presence of low-grade fever, chest pain, expectoration, and hemoptysis was also considered.

Investigations

Tuberculin skin test

An intradermal injection of 0.1 mL of purified protein derivative (PPD) was performed into the volar aspect of the forearm, and after 72 hours, the mean vertical and transverse induration diameters were measured, and a positive tuberculin skin test (TST) was indicated if the dimensions of the induration were ≥10 mm.

Whole-blood QuantiFERON TB Gold In-Tube test

The test was performed according to the manufacturer's instructions (Cellectis, Ltd., Victoria, Australia) for the measurement of the levels of interferon-γ (IFN-γ) release in the processed whole blood samples and consists of two steps:

1. 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 third tube contained TB-specific antigens. The tubes were homogenized by 10 times inversion and then incubated for 16-24 hours at 37°C.
2. enzyme-linked immunosorbent assay (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-γ release assay (IGRA) within 2 weeks after blood collection. The results obtained by the negative control were subtracted from those 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].

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

1. Blood samples were aseptically collected in ethylenediaminetetraacetic acid 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.
2. Total RNA, including miR, was isolated using the miRNeasy Mini Kit (QIAGEN, Hilden, Germany), and the complementary DNA was synthesized using the miScript II RT Kit (QIAGEN, Hilden, Germany). The recommended RNA starting amounts and buffers for reverse transcription reactions for quantization of miR, by using miScript precursor assays, were 5x miScriptHiFlex buffer, and the recommended RNA input depends on the abundance and number of target miR to be quantified; up to a maximum of 1 µg (miRNeasy Mini Kit, 2-miScript II RT Kit, QIAGEN, Hilden, Germany).
3. 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 polymerase chain reaction (PCR) for the detection of miR expression levels using QuantiTect SYBR Green PCR Kit. The PCR reaction mix was prepared in a total volume of 25 µL/tube.

4. 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 miR as follows: i) for miR-150-5p and miR-155-5p: initial denaturation for 20 seconds at 95°C was followed by 40 cycles of annealing at 95°C for 3 seconds and extension at 60°C for 3 seconds [11]; ii) for miR-378-5p: initial activation for 10 minutes at 95°C, denaturation at 94°C for 10 seconds, annealing at 60°C for 20 seconds and extension at 72°C for 34 seconds, for 40 cycles [12]; iii) for miR-4523-5p: after initial denaturation at 95°C for 2 minutes, 45 cycles of denaturation at 95°C for 10 seconds and annealing for 1 minute at 56°C were conducted [13].
5. miR 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 miR were determined by the 2-ΔΔCT (cycle threshold) method and expressed as fold change using Step One software (Applied Biosystems, USA).

Statistical analyses

The obtained results were analyzed using IBM® SPSS® Statistics (Version 22, 2015; IBM, Armonk, NY, USA) to compare the inter-group difference by the one-way analysis of variance test. The receiver operating characteristic (ROC) curve was used to evaluate the predictability of plasma expression levels of the studied miR for differentiation between patients' samples and those of HC, and between samples of ATB and LTB subjects. The results of the ROC analysis were presented as the area under the curve (AUC), and its significance was verified *vs.* the area under the reference 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 6 patients had other chest diseases, 5 patients were maintained on anti-TB 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 the ATB group. A total of 44 of the patients' households were evaluated, but 4 gave an undetermined QFT-GIT test, 3 were diabetics, 3 were cardiac patients, 2 had autoimmune disorders, and 2 refused to participate in the study; these 14 households were excluded, and 30 were enrolled in the study as the 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 they 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' samples than HC samples, with significantly (p=0.0014) higher expression levels in ATB patients than LTB subjects. On the contrary, plasma miR-

Table 1. The data of the study participants.

Variate group		HC (n=30)	ATB (n=30)	LTB (n=30)	p
Age (years)	<40, n (%)	4 (13.3)	5 (16.7)	3 (10)	0.969
	40-49, n (%)	6 (20)	5 (16.7)	6 (20)	
	50-59, n (%)	12 (40)	13 (43.3)	11 (36.7)	
	≥60, n (%)	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, n (%)	20 (66.7)	23 (76.7)	21 (70)	0.685
	Females, n (%)	10 (33.3)	7 (23.3)	9 (30)	
BMI (kg/m2)	Underweight, n (%)	0	5 (16.7)	2 (6.7)	0.192
	Average, n (%)	5 (16.7)	8 (26.7)	7 (23.3)	
	Overweight, n (%)	11 (36.7)	10 (33.3)	9 (30)	
	Obese, n (%)	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, n (%)	6 (20)	12 (40)	9 (30)	0.355
	Ex-smoker, n (%)	9 (30)	10 (33.3)	8 (26.7)	
	Non-smoker, n (%)	15 (50)	8 (26.7)	13 (43.3)	
Clinical data	Low-grade fever, n (%)	-	6 (20)	-	-
	Hemoptysis, n (%)	-	3 (10)	-	-
	Chest pain, n (%)	-	5 (16.7)	-	-
	Expectoration, n (%)	-	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

ATB, active tuberculosis; BMI, body mass index; HC, healthy controls; LTB, latent pulmonary tuberculosis; SD, standard deviation. The p-value indicates the significance of the intra-group differences.

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 4 miR 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 miR 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% confidence interval (CI): 0.016-0.238] in favor of miR-378-5p. On the contrary, the AUC 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 miR 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 the identification of LTB subjects and also showed the ability to estimate levels of miR-150-5p as a screening variable for LTB, but with lower significance (Table 3).

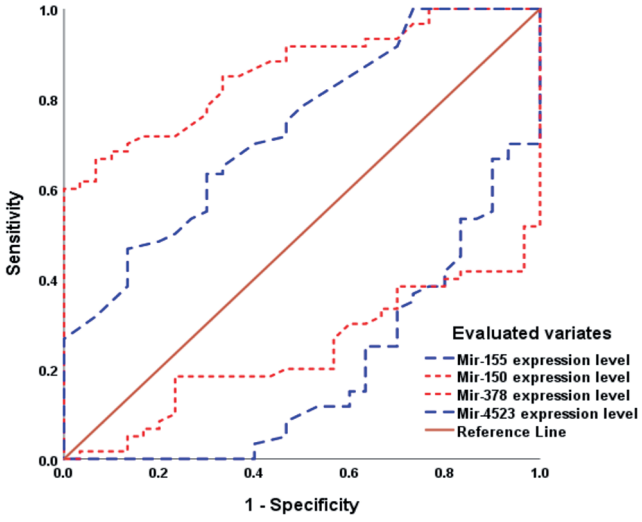

Figure 1. Receiver operating characteristic curve for evaluation of the ability of expression levels of the studied microRNAs to differentiate between samples of tuberculosis patients and healthy control subjects. Mir, microRNA.

Table 2. Mean expression levels of the studied microRNAs in samples of tuberculosis patients compared to samples of healthy controls.

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

ATB, active tuberculosis; HC, healthy controls; LTB, latent pulmonary tuberculosis; miR, microRNA; SD, standard deviation. P1 signifies the difference in expression levels in patients' samples vs. samples of HC subjects; P2 signifies the difference in expression levels in samples of ATB vs. 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
	TST	0.629	0.073	0.085	0.486-0.773	0.198	0.070
Between LTB and ATB patients	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

AUC, area under the curve; ATB, active tuberculosis; CI, confidence interval; HC, healthy controls; IGRA, interferon-γ release assay; LTB, latent pulmonary tuberculosis; miR, microRNA; ROC, receiver operating characteristic; TST, tuberculin skin test.

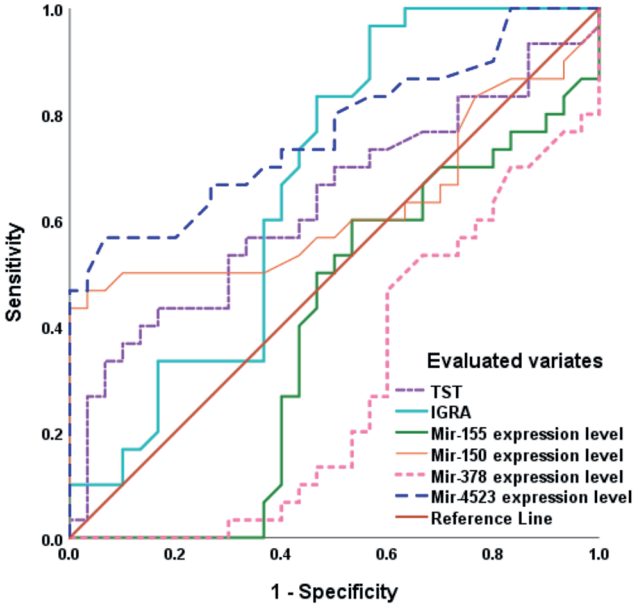

Figure 2. Receiver operating characteristic curve for evaluation of the ability of tuberculosis diagnostic tests and expression levels of the studied microRNAs to differentiate between samples of active tuberculosis and latent pulmonary tuberculosis patients. Mir-, microRNA; TST, tuberculin skin test; IGRA, interferon-γ release assay.

Discussion

Recently, 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 interpreted as normal in healthy controls (HCs) and LTB subjects. Moreover, the shortcomings of the available investigations, as documented in the current results, were that 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 miR using the quantitative PCR as reliable biomarkers for defining LTB subjects from HC. In line with this hypothesis, multiple recent studies documented that miR 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 *vs.* 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 in 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 miR 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 an 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 in ATB patients than LTB subjects. These results supported the preliminary experimental study that found miR-155 exhibited a 1.4-fold change in HC and 3.7 in ATB peripheral blood mononuclear cells upon MTB PPD stimulation [22]. Recently, another 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 who had tuberculoma with decay than in samples of patients who 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 a 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 the sole differentiating procedure, and this assumption was supported by a review of the literature that showed the presence of some inconsistency in the reported expression levels of miR-155 in the 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 that 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 hand in hand with those of 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 who 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 targeting bone morphogenetic protein-2, which is an essential element for activating transforming growth factor-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 significantly 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 miR 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 miR were excluded.

Conclusions

Pulmonary TB induced deregulated expression of miR with contradictory roles in immune response according to the severity of tuberculous infection. Single miR 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 miR were a limitation 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 a well-equipped lab, and the test being as expensive as a 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 who have LTB, because the cost of the test might be equalized for the consumption of hospital resources for admission and treatment of these subjects.

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Online supplementary material:

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