microRNA-379 as a Candidate Biomarker for Early Diagnosis of Childhood Active and Latent Tuberculosis

Ayling Sanjaya[1], Dwi Yuni Nur Hidayati[2], Susanthy Djajalaksana[3], HMS Chandra Kusuma[4], Renny Suwarniaty[5], Sumarno[6]*

[1]Department of Pediatric, Faculty of Medicine, Universitas Wijaya Kusuma Surabaya, Indonesia. Email: ayling.sanjaya@gmail.com
[2]Department of Microbiology, Faculty of Medicine, Brawijaya University, Indonesia. Email: dwi_yuni@ub.ac.id
[3]Department of Pulmonology, Faculty of Medicine, Brawijaya University, Indonesia. Email: susanthydj@gmail.com
[4]Department of Pediatrics, Faculty of Medicine, Brawijaya University, Indonesia. Email: hmschandrak@gmail.com
[5]Department of Pediatrics, Faculty of Medicine, Brawijaya University, Indonesia. Email: renny_nus@yahoo.co.id
[6]Department of Microbiology, Faculty of Medicine, Brawijaya University, Indonesia. Email: sumarno_prof@ub.ac.id

Corresponding Email: ayling.sanjaya@gmail.com

Abstract

Background: Childhood active and latent tuberculosis (TB) often go undiagnosed due to limited symptoms and biomarkers. Recently, miRNA has been considered as a candidate biomarker for early diagnosis which can also distinguish between childhood active and latent TB. **Aim:** This study aimed to identify miRNAs that play a regulatory role in active and latent TB in children, analyzing differences in expression levels and diagnostic values of miRNA-379. **Methods:** A cross-sectional observational analytic study on children aged 0-15 years having contact history with TB patients was used in this study. Anamnesis, physical examination, tuberculin test, culture, chest X-ray and TB scoring were performed. The samples were divided into three groups: healthy control children, active TB children and latent TB children. miRNA profiling was performed using microarray and miRNA-379 validation was performed using RT-qPCR. The data was analysed to determine miRNAs characterization, expression level and diagnostic value of miRNA-379. **Results:** Children in active TB-control group expressed 251 miRNA genes comprising 221 down-regulated and 30 upregulated miRNAs.The lowest fold change was observed with miRNA-379 whereas the highest fold change was noticed with miRNA-3613. The latent TB-control group expressed 292 miRNA genes, of which263 miRNAs downregulated and 29 miRNAs up-regulated. Moreover, the lowest and the highest fold change was observed for miRNA-381 and miRNA-3200 respectively.In case of active-latent TB group, 21 miRNA genes were expressed with 2 down-regulatedand 19 up-regulated miRNAs. Amongst these, the lowest fold change was observed for miRNA-379 whereas the highest fold change was observed for miRNA-1299. Expression level of miRNA-379 decreased in controls, decreased even more in latent TB and decreased the most in active TB. The difference in the level of expression of these miRNAs was found statistically significant ($p<0.001$). **Conclusion:** miRNA has the potential of being a candidate biomarker for childhood TB. Differences in expression level of miRNA-379 can be used as candidate biomarker to distinguish between latent and active TB in children.

Keywords: Childhood tuberculosis, biomarker, expression level, miRNA-379

Introduction

In 2015, approximately 10.4 million new cases of TB were reported across the globe, of which 10% comprised children. In 2016, of all the new TB infections reported in Indonesia, an estimated 9.04% consisted of children aged between 0 to 14 years. Of the mentioned reported cases, an approximate 5-10% of latent TB cases can convert into active TB cases over a length of five years. [2-5]

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Address for Correspondence: Department of Pediatric Faculty of Medicine, Wijaya Kusuma Surabaya University Dukuh Kupang XXV No.54, Surabaya, East Java, Indonesia (60225)
Email: ayling.sanjaya@gmail.com

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Latent TB is a condition of the body with a specific persistent immune response to mycobacterium tuberculosis antigen stimulation. As a result clinical symptoms of active TB, as well as radiological and bacteriological abnormalities do not appear. Whereas, active TB is a multiorgan illness that can be caused by a primary infection or a reactivation of latent TB.[6,7]

Definite diagnosis of TB is established by finding TB bacteria through direct smear examination or culture, which is considered the gold standard examination. However, it is still not as simple as that because it is difficult for a child to cough up sputum, as the location of bacteria is in the parenchyma area which is far from the bronchi.[8–11] Despite the development of a scoring system for TB in children, there are obstacles in detecting this disease in them due to the inherent weaknesses of the scoring system parameters.[10,12,13]

The role of microRNA (miRNA or miR) as a biomarker and therapy for TB both in adults and children has captured the attention of researchers worldwide MiRNA is a ribonucleic acid composed of 19-24 nucleotides. Despite regulating the expression level of mRNAs, these miRNAscan complement the target genes which encode for the mRNAs.[14–17] In-depth studies have revealed that miRNA not only can be used as a biomarker but also as an agent for TB therapy owing to its stability in plasma and other body fluids.[12,16–19] Several types of miRNAs are immune-related genes that can regulate immune response in active and latent TB.[14,16-18]

In the present study, the researchers sought to analyze the expression of several miRNAs in children suffering from TB. In addition, the potential of miRNA for the diagnosis of both active and latent TB infection is also investigated.

Methods

This study was conducted by analytical observational design using cross-sectional approach. After obtaining ethical approval from the Ethical Committee of Wijaya Kusuma Surabaya University, Surabaya, sera from healthy children, active and latent TB children were collected from Primary Health Care in Surabaya. The sera were used to determine the characteristics of identified miRNAs and their expression levels in the study subjects. The analysis procedure was conducted in the Tropical Disease Research Centre in Surabaya Indonesia and The Applied Biosystems Research Laboratory in Singapore. The study began in October 2019 and took approximately six months to complete.

Study Subjects

Children of age 0-15 years, diagnosed with active TB, latent TB and who had history contact with adult TB were included in this study. However, children with any congenital or chronic disease, such as diabetes, malignancies, allergies, malaria, immunocompromised diseases such as HIV and with diseases of lung, kidney, liver and blood were excluded from this study. Moreover, children who had history of atopy, heart and kidney failure and who were receiving therapy of tuberculosis were also excluded. The control group of the study comprised healthy children of 1-14 years of age and who had negative tuberculin result and never received BCG immunization, and the study subject were divided into three groups: the healthy control group, the active TB group and the latent TB group. There were 7 children in the control group whereas the active and latent TB groups contained 16 and 9 children respectively.

Clinical Procedure

Profiling of miRNAs using microarray technique

A total of 10 samples were selected for miRNA profiling using the microarray technique. These samples were divided into three groups: the control group having two samples, the active TB group and the latent TB group each comprising four samples. A total of 500 ng of RNA was labelled using FlashTag ™ Biotin HSR RNA Labelling Kit (Thermo Fisher Scientific, Applied Biosystems™). The labelled RNA was then hybridized on the GeneChip ™ miRNA array 4.0 by keeping them overnight at 48°Cin a GeneChip ™ Hybridization Oven 645 (Thermo Fisher Scientific, Applied Biosystems ™) at 60 rpm speed for 16-18 hours. Hybridization control (GeneChip ™ Hybridization Control Kit) was included in the whole process of hybridization. After the completion of the process, the samples in the GeneChip ™ miRNA array 4.0 were washed and stained using the GeneChip ™ Fluidics Station 450 instrument (Thermo Fisher Scientific, Applied Biosystems ™).

Validation of miRNA expression using Real-Time PCR

The components of the Real-Time PCR reaction consisted of 10 µl of TaqMan® Fast Advanced Master Mix (2X), 1 µl of TaqMan® microRNA Assay (20X), 1.33 µl of cDNA sample and 7.67 µl of nuclease-free water. The total reaction volume was 20 µl. The QuantStudio ™ 5 Real-Time PCR System (Thermo Fisher Scientific, Applied Biosystems ™) was used to carry out the reaction with the following program: incubation for 2 minutes at 50°C, pre-denaturation for 20 seconds at 95°C, denaturation for 3 seconds at 95°C and annealing / extension for 30 seconds at 60°C. The denaturation, annealing and extension stages were repeated for 40 cycles. After the reaction was completed,

data was collected and analysed using the Comparative CT ($2-\Delta\Delta Ct$) method with the help of Expression Suite Software v1.3 (Thermo Fisher Scientific).

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) v22 for Windows (IBM Inc., Chicago, IL) was used for the statistical analysis of the data. Kolmogorov-Smirnov test was used to assess the normal distribution of data. After that, the Shapiro-Wilk test was used to carry out the normality test for data with a ratio scale and small sample size. Comparison between two sample groups was done with the independent sample t test. In addition, One Way Anova test (F test) was used for comparison between more than two sample groups. On getting the normal distribution of the data, Pearson correlation test was performed. The diagnostic values were analyzed using the ROC and AUC curves. The P-value of less than 0.05 was considered statistically significant.

Ethical Considerations

Ethical approval from the Ethics Committee of the Faculty of Medicine, Wijaya Kusuma University, Surabaya, Indonesia was obtained on 30th September 2019 (No. 98/SLE/FK/UWKS/2019).

Results

General Characteristics

A total of 32 children were recruited in the study of which 17 were males and 15 were females. Amongst the female patients, 68.8% (n=11) were suffering from active TB, 11.1 % (n=1) had latent TB whereas 31.3% (n=5) and 88.9% (n=8) males had active and latent TB respectively. The healthy control group comprised 57.1% (n=4) males and the rest were females. Group wise distribution of the subjects and their socio-demographic characteristics are shown in Table 1

Characterization of miRNA Expression in Active TB and Control Group

In the active TB and control groups, the former group contained four children and the latter group comprised two children. In both groups, a total of 6631 genes were found to be expressed of which 251 genes were in accordance with the initial filter criteria. Out of these, 30 (11.95%) were up-regulated miRNAs with miRNA-3613 showing the highest fold change i.e., 7.14 whereas the remaining 221 (88.05%) miRNAs were down-regulated with the lowest fold change observed for miRNA-379 i.e. -81.92 (Figure 1).

Table 1: Sociodemographic Characteristics of study subjects

Characteristics	Active Tuberculosis		Latent Tuberculosis		Healthy Control	
	n	%	n	%	n	%
Gender						
Male	5	31.3%	8	88.9%	4	57.1%
Female	11	68.8%	1	11.1%	3	42.9%
Mother's Education						
Primary school	3	18.8%	2	22.2%	5	71.4%
Secondary School	13	81.3%	7	77.8%	2	28.6%
Father's Education						
Primary school	4	25.0%	3	33.3%	4	57.1%
Secondary School	12	75.0%	6	66.7%	3	42.9%
Immunization of BCG						
No	1	6.3%	0	.0%	7	100%
Yes	15	93.8%	9	100.0%	0	0%
Contacting with TB Patients						
Yes	15	93.8%	9	100%	0	0%
No	1	6.3%	0	0%	7	100%
Tuberculin Test						
Positive	16	100%	9	100%	0	0%
Negative	0	0%	0	0%	7	100%
Weight/ Nutritional Status						
Less	3	18.8%	3	33.3%	3	42.9%
Good	13	81.3%	6	66.7%	4	57.1%
Fever >2 weeks						
Yes	4	25%	0	0%	0	0%
No	12	75%	9	100%	7	100%
Cough >3 Weeks						
Yes	8	50.0%	1	11.1%	0	0%
No	8	50.0%	8	88.9%	7	100%
Swollen Lymph Nodes						
Yes	16	100%	0	0%	0	0%
No	0	0%	9	100%	7	100%
Swelling of Bones / Joints						
Yes	0	0%	0	0%	0	0%
No	16	100%	9	100%	7	100%
Chest X-ray						
Suggestive TB	15	93.8%	0	0%	0	0%
Normal	1	6.3%	9	100%	7	100%

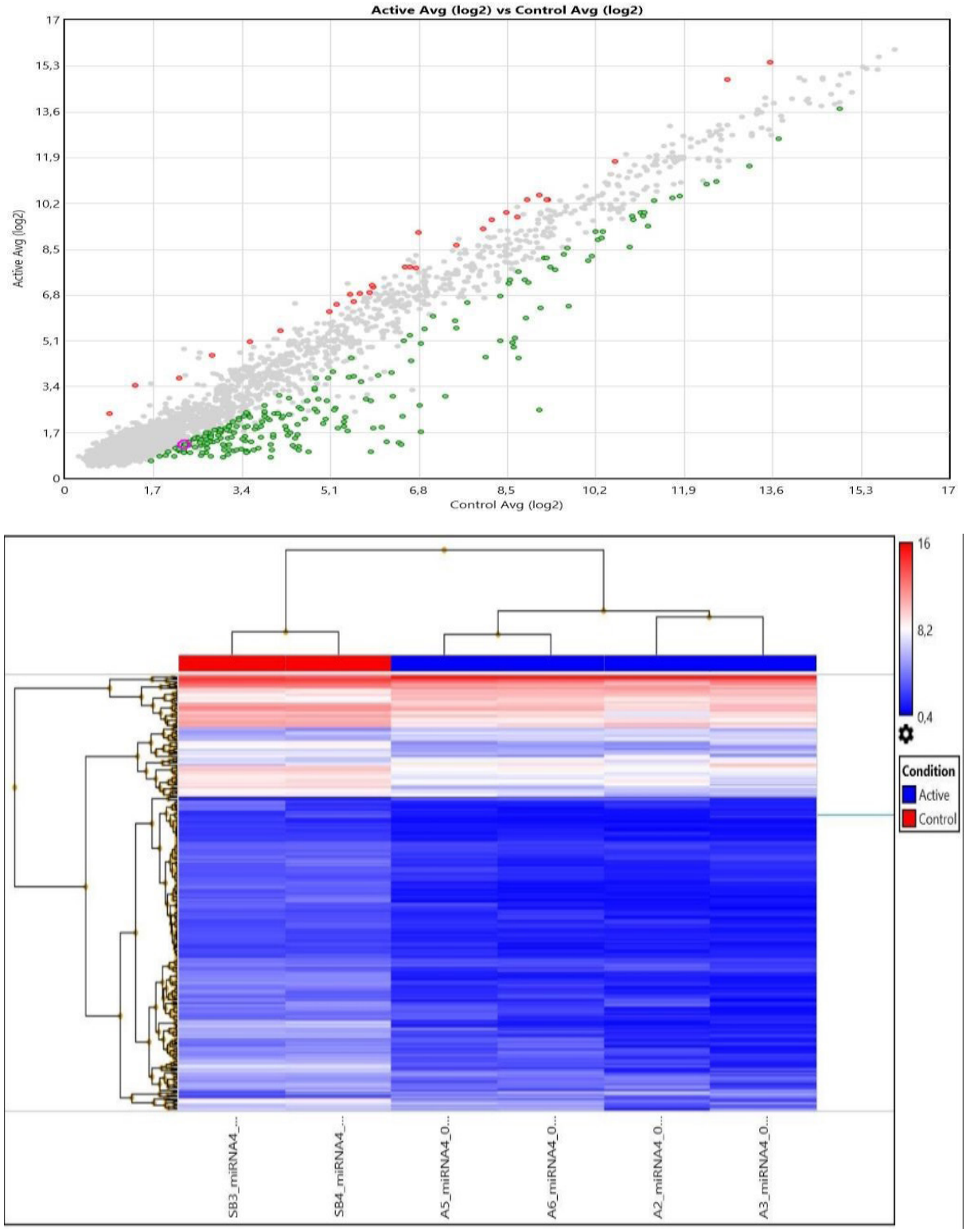

Figure 1. Overview of miRNA Expression in Active TB Children Versus Control Group

Characterization of miRNA Expression in Latent TB and Control Group

A total of four children were included in the latent TB group and two in the control group. In total, 6631 genes were found to be expressed in both group of which 292 genes passed the filter criteria. Out of these 292 miRNAs, 29 (9.93%) were up-regulated with the highest fold change observed for miRNA-3200 (5.41) and the remaining 263 miRNAs (90.07%) were down-regulated with the lowest fold change of miRNA-381 was observed (-30.92) (Figure 2).

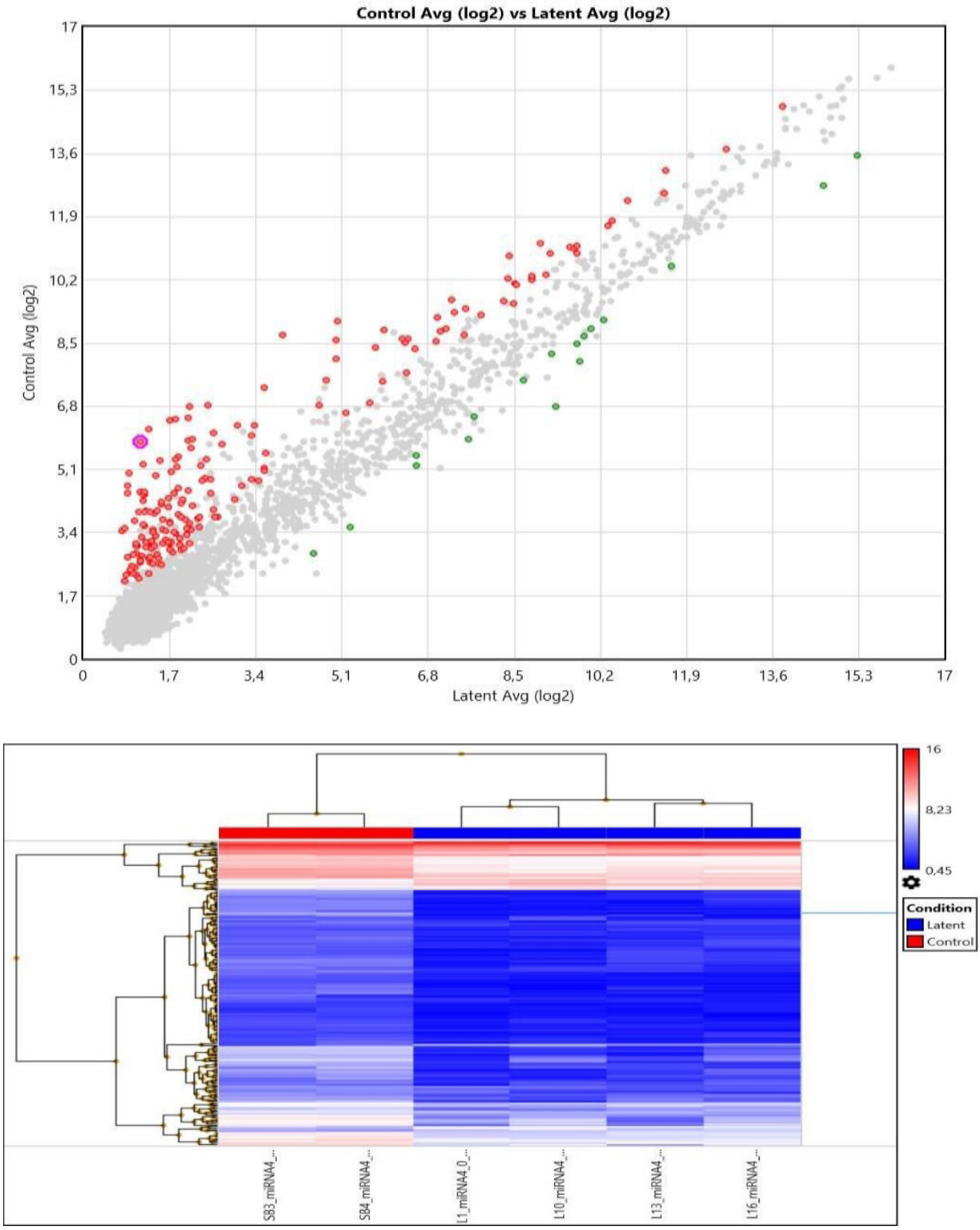

Figure 2. Overview of miRNA Expression in Latent TB Children Versus Control Group.

Characterization of miRNA Expression in Active TB and Latent TB Group

In both the active TB and latent TB groups, the number of subjects was four. Both groups expressed 6631 genes in total out of which 21 genes passed the filter criteria. Total 19 (90.48%) miRNAs were found to be up-regulated with the highest fold change of miRNA-1299 (fold change 7.89) and remaining 2 (9.52%) miRNAs were down-regulated with the lowest fold change of miRNA-379 was observed (fold change -4.05) (Figure 3).

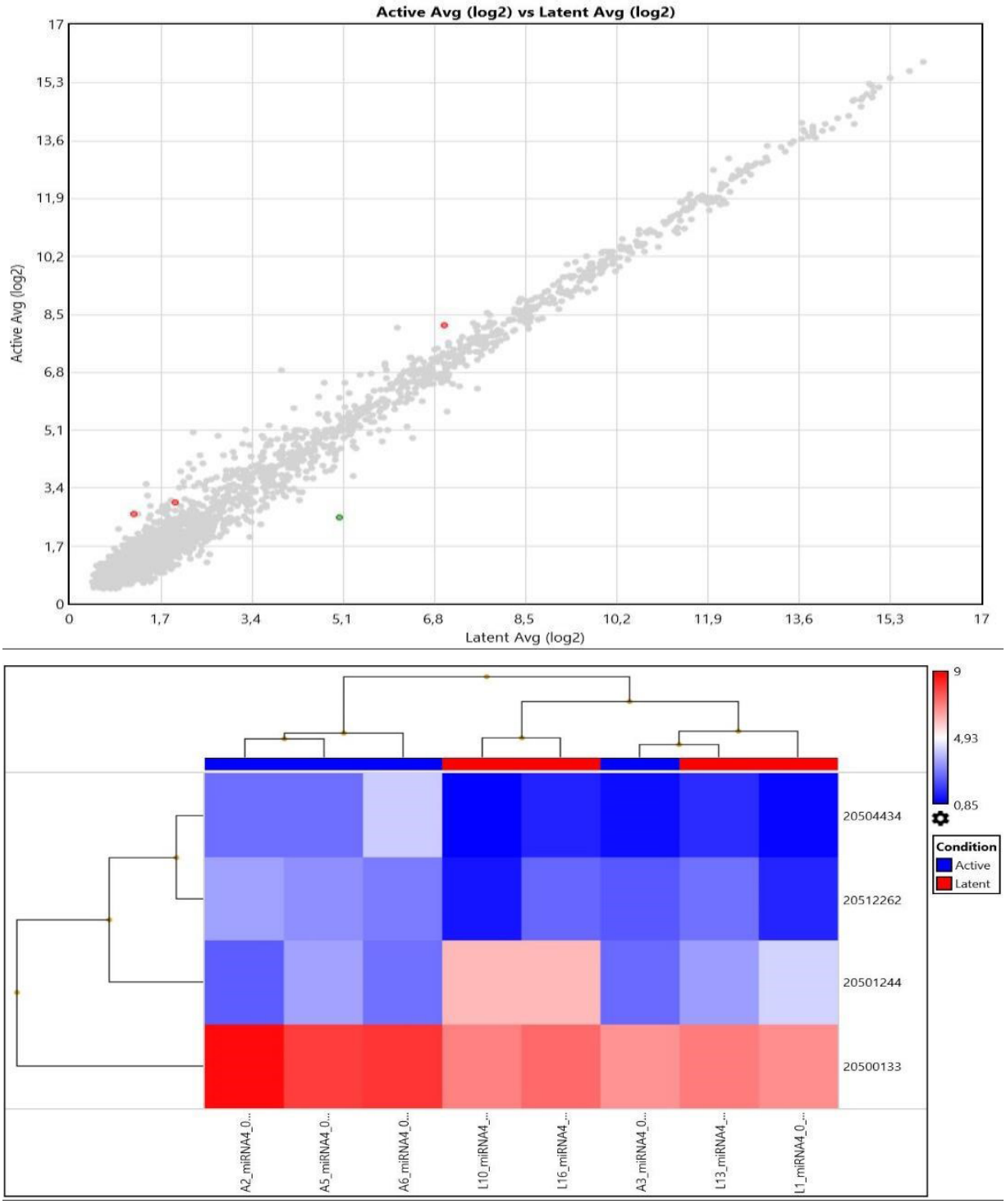

Figure 3. Overview of miRNA Expression in Active TB and Latent TB Children

Overlapping microRNAs in Active TB, Latent TB and Control Group

Characterization of the miRNAs in all groups showed a number of genes which were expressed in them. Figure 4 shows details of all the genes differentially expressed in each group. Of all the miRNAs which were found to be expressed in all the three groups of children, four miRNAs were overlapping. These were miRNA-18a, miRNA-379, miRNA-542 and miRNA-2277. Amongst these, miRNA-379 showed down-regulation in all study subjects with the lowest fold change of -4.05. Hence, miRNA-379 was selected for further investigation i.e., validation and measurement of the level of expression. Table 2 summarizes the expression pattern (fold change) of overlapping miRNAs in all the groups.

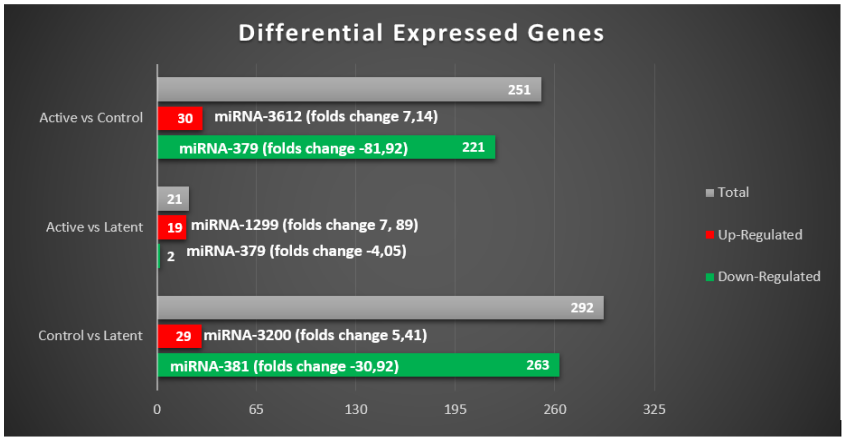

Figure 4: miRNA Genes Expressed in the Three Groups and the MiRNA Venn Diagram in the Research Subjects

Table 2: Overlapping miRNAs in All Groups

miRNA	Active TB-control (*fold change*)	Latent TB-control (*fold change*)	Active TB-Latent TB (*fold change*)
miRNA-18a	-2.05	-4.63	2.26
miRNA-379	-95.43	-17.03	-5.6
miRNA-542	-3.6	-9.76	2.71
miRNA-2277	-2.48	-5.08	2.05

Differences in the Expression Level of miRNA-379 Between Children with Active TB, Latent TB and Controls.

Based on preliminary analysis with microarray profiling, miRNA-379 was found to be differentially expressed in all groups i.e., active TB, latent TB and control group. (Figure 5). Validation and quantification of miRNA-379 expression was carried out using the RT-qPCR method. Tests for differences in miRNA-379 expression levels were carried out for each group. This was preceded by the Kolmogorov-Smirnov test to assess the normal distribution of miRNA-379 data. After that, the data normality test was carried out with the Shapiro-Wilk test. (Table 3).

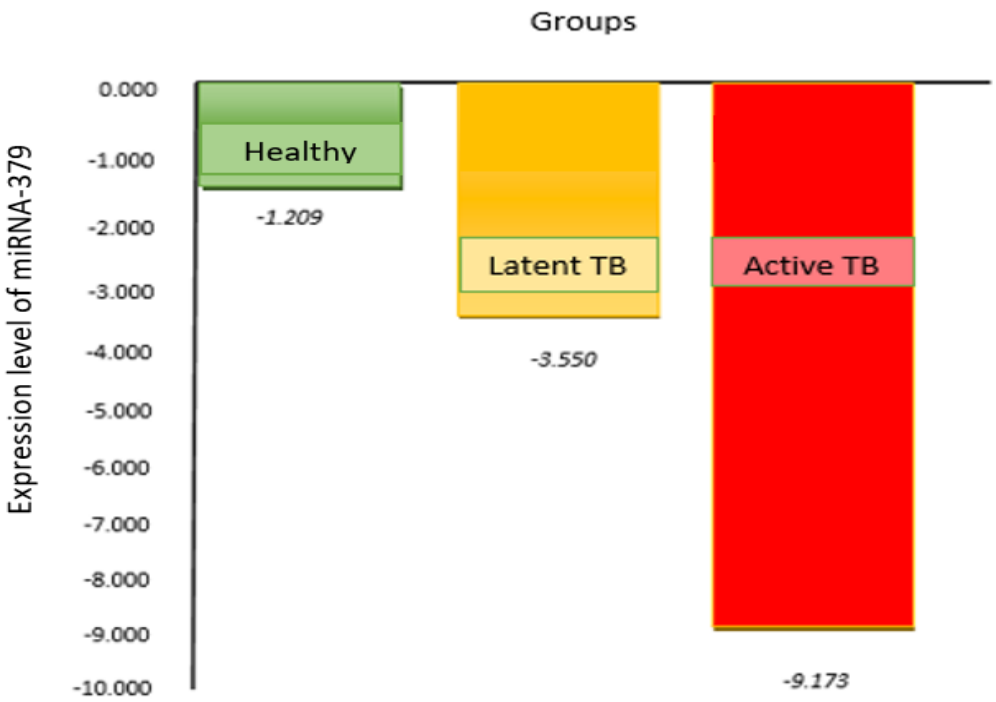

Figure 5: Expression Levels of miRNA-379 in Children with Active TB, Latent TB and Healthy Controls.

Table 3: Normality Test of miRNA-379 in Active TB, Latent TB and Healthy Controls

		Normality Test						Level Difference Test	
		Kolmogorov-	Smirn	ov	Shapir	o-Wilk		N	Mean + SD	p-value
	Group	Statistic	df	Sig.	Statistic	df	Sig.			<0.001
MiR-379	TB active	.121	16	.200*	.974	16	.899	9	-3.550 ± 1.868	
	TB latent	.140	9	.200*	.957	9	.762	16	-9.173 ± 4.860	
	Control	.173	7	.200*	.908	7	.384	7	-1.209 ± 0.447	

Differences in Diagnostic Value of miRNA-379 Between Active TB, Latent TB and Control Group.

In the ROC of the active TB group,the AUC value of 1.000 was obtained with a p value <0.001, which showed that the AUC value was included in the significant category in the diagnosis of active TB when compared to the controls.

Similar results were observed in the latent TB group when compared with the control group i.e., the AUC value obtained was 0.952 with a p value <0.001. When both these grops were compared with each other, the AUC value of 0.917 was obtained with a p value <0.001 which showed its significance in the diagnosis of active TB as compared to the latent TB (Figure 6).

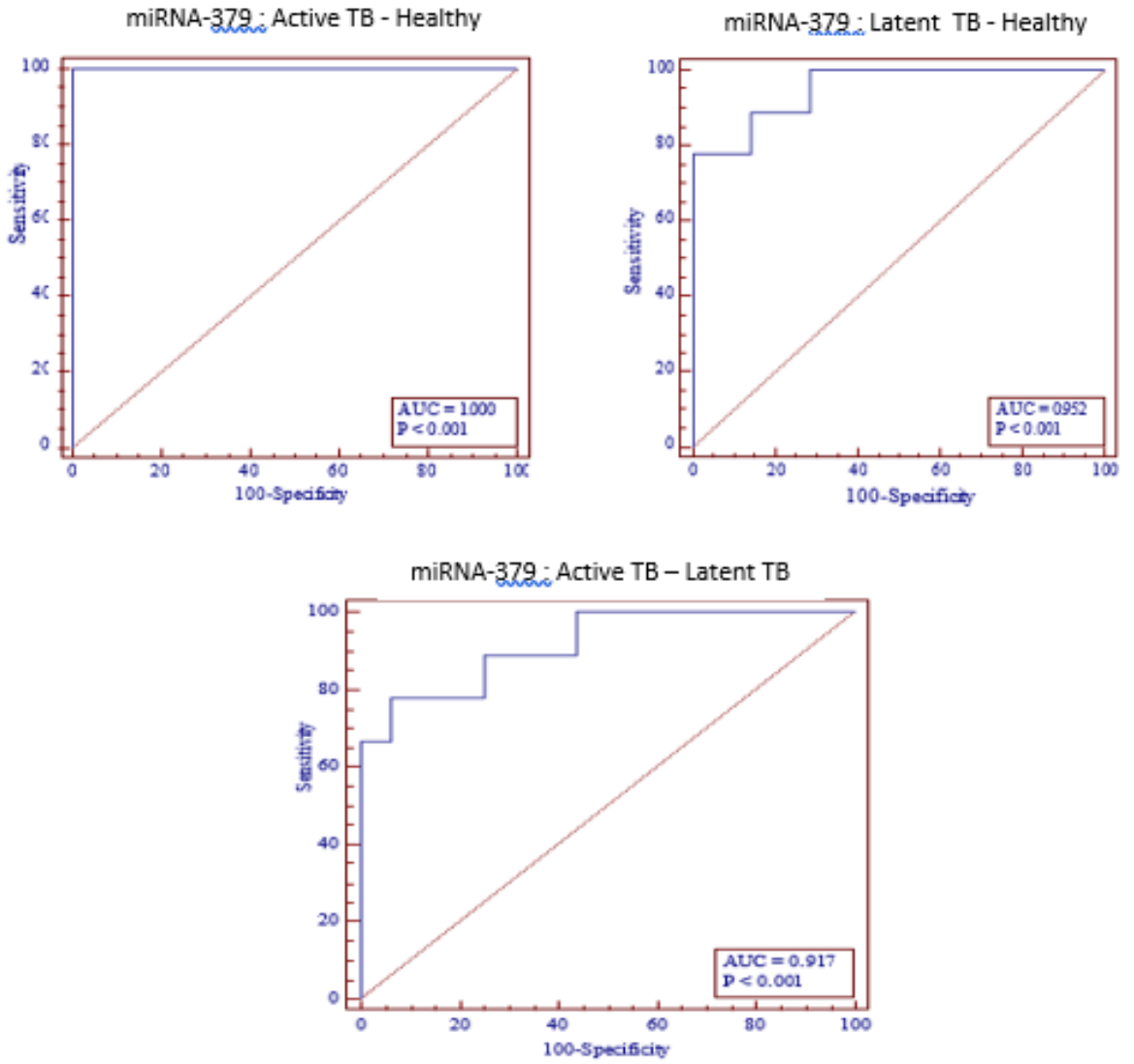

Figure 6. Diagnostic Value of miRNA-379 in Active TB, Latent TB and Healthy Control Groups

From the ROC results, the cut-off value of miRNA-379 in the active TB-control group was found to be -2.020. Whereas, in the latent TB-control group and the latent-active TB group, the cut-off values obtained were -2.020 and -4.401 respectively. Based on these values, a difference test was carried out on the diagnostic value of miRNA-379 among groups of children with active TB, latent TB and controls (Table 4). The results of this test showed significant difference in the diagnostic value of miRNA-379 in all groups of children with a p value <0.001. This showed that miRNA-379 has a significant diagnostic value for diagnosing active TB and latent TB.

Table 4 Test for Differences in Diagnostic Value of miRNA-379

	Group		p- value
TB Active – Control miR-397 *cut-off*	TB Active n (%)	Control n (%)	< 0.001
≤-2.020	16(100)	0	
> -2.020	0	7 (100)	
TB Latent – Control miR-379 *cut-off*	TB Latent n (%)	Control n (%)	< 0.001
≤-2.020	7 (77.8)	0	
> -2.020	2 (22.2)	7 (100)	
TB Active – TB Latent miR-379 *cut-off*	TB Active n (%)	Latent n (%)	< 0.001
≤-4.401	15 (93.8)	2 (22.2)	
> -4.401	1 (6.3)	7 (77.8)	

Discussion

In this study, it was found that, in the active TB-control group, 251 miRNAs were expressed. Amongst these, miRNA-379 was down-regulated with the lowest fold change (fold change 81.92) whereas miRNA-3613 was up-regulated with the highest fold change -(fold change 7.14). A similar study observed that out of 29 miRNAs expressed, 15 were up-regulated and 14 were down-regulated. Their results of validation with RT-PCR showed 14 essential miRNAs, namely miRNA-1, miRNA155, miRNA-31, miRNA-146a, miRNA-10a and miRNA-125b. miRNA-150 was downregulated while miRNA-29 was upregulated in children with active TB.[17,22] Another study also showed that miR-31 was significantly reduced in children with TB as compared to healthy children. The study showed 15 types of miRNAs among TB children and controls, of which miR-192 was the only candidate whose measurement results showed significant differences in adults and children.[12,23] These studies suggest that there are different miRNA characteristics in each country or ethnicity. This is supported by various studies in European and African ethnicities as well.[21]

In the latent TB- control group investigated in this study, 263 miRNAs were down-regulated with the lowest fold change being observed for miRNA-381 (fold change -30.92) and 29 miRNAs were up-regulated with the highest fold change being noticed for miRNA-3200 (fold change 5.41). Another study also reported the role of miRNA-381 in TB infection being related to the body's innate and adaptive immunity. Differential expression of miRNA in TB patients can help differentiate between TB patients and healthy people.[24] One study also recorded 33 miRNAs whose expression was up-regulating and 46 miRNAs were found to be down-regulated, including miR-451a, miR-340-5p, miR-136-5p and miR-29b[2]. In Wang's study, it was found that five miRNAs, namely, hsa-miR130a, hsa-miR-296-5p, hsa-miR-493, hsa-miR-520d-3p and hsa-miR-661, had different expression levels between latent TB and healthy controls[23]. This difference in miRNA expression shows that miRNA characteristics varies with each state, country or ethnicity. This latency may depends on the Mycobacterium tuberculosis (M.tb) strain and the host immune response. Data regarding latent TB in children have not been fully assessed, as well as research on miRNA against latent TB in Indonesia has not been widely carried out. This requires further research which can be used as a basis for specific miRNA characterization in order to minimize reactivation of latent TB.[12,25–27]

Profiling of miRNAs in the active TB-latent TB group was also conducted in this study which depicted that out of total 21 miRNAs, 2 were down-regulated with the lowest fold change of miRNA-379 (fold change -4.05) and 19 were found up-regulated with the highest fold change of miRNA-1299 (fold change 7.89). This is in line with studies which profiled miRNA expression in TB and found that miRNA-1299 was up-regulated with a fold change of 2.0797. There were differences in miRNA expression in both active and latent TB patients and showed the expression of 17 miRNAs of which 12 miRNAs were up-regulated.[23,28] Another study found up-regulation of miRNA-19 and the induction of miR-29 in T cells during infection to increase bacterial virulence. Inhibition of interferon-gamma expression by the upregulation of miR-29 has been reported by

another study. They also showed that overexpression of miR-29 converts latent TB to active TB. miRNA-29 was found to be elevated in the T cells of TB patients when compared with latent TB and controls. However, contrasting results have also been reported in a study by Kleinsteuber.[2,29,30] Another study, with the help of array examination, observed a decrease in miRNA-144 expression in CD4 T cells in TB patients as compared to latent TB patients.[21] Important post-transcription regulation of gene expression is mediated by intracellular miRNA and immunity processes. Several studies have shown changes in miRNA gene expression in macrophages and NKC in both active and latent TB patients.[31–33] In the pathogenesis of TB, the host cellular immune response determines whether the infection will become latent TB or it will progress to become active TB where miRNAs extensively regulate cell differentiation and development. Various studies have found genes that change the expression profile of miRNAs in macrophage cells and NK cells from individuals with active or latent TB.[28]

In the present study, expression of four microRNAs was found to be overlapping in the three groups of study subjects. These were: miRNA-18a, miRNA-379, miRNA-542 and miRNA-2277. In the active TB-control group, the miRNA-18a had a fold change of -2.05 whereas in the latent TB-control group and active TB-latent TB group, its fold change came out to be -4.63 and 2.26 respectively. The second common miRNA-379 was found to have a fold change of -95.43 in the active TB-control group and a fold change of -17.03 and 5.6 in the latent TB-control group and the latent-active TB group respectively. Fold change in the third common miRNA-542 in active TB-control group was -3.6 whereas in the latent TB-control group, the fold change was -9.76 and in the active TB-latent TB group it was found to be 2.71. The fourth common miRNA-2277 had a fold change of -2.48 in active TB-control group. Moreover, its fold change in latent TB-control group and latent-active TB group came out to be -5.08 and 2.05 respectively. The existence of these overlapping miRNAs in all the groups is in line with the study conducted by Lyu which described the Venn diagram of three overlapping miRNAs, namely, miRNA-140, miRNA-423 and miRNA-3184[34] and about one third of the world's population has a latent TB infection (LTBI. Another study found five overlapping miRNAs expression in the active TB-control group and the latent TB-control group. These miRNAs were: miR-21, miR-7f-1, miR-423, miR-1275 and miR-505.[35]

Expression of mir-379 was found to be decreased in both active and latent TB patients as compared to healthy controls. However, this decrease in the expression level was observed as the most dominant in active TB patients with a fold change of -9.173. On the other hand, its expression level in latent TB patients was decreased with a fold change of -3.550 and that in the control group, it was -1.209. Tests for differences in levels of expression were carried out which depicted a significant difference between the expression levels of miRNA-379 among children with active TB, latent TB and those who were healthy. This is in line with several studies on miRNAs in various countries which identified, validated and measured their level of expression. A study showed a significantly different trend of expression between the expression levels of miRNA-140, miRNA-423 and miRNA-3184 in the control group, latent TB group and active TB group. Validation with RT-qPCR obtained the expression of miRNA-2110, miRNA-1246, miRNA-370 and miRNA-193 in the healthy, latent TB and active TB groups. Their results revealed the lowest expression level of these miRNAs in control group whereas slightly increased and highest levels in latent TB and active TB groups. Another study found miRNA-29a as a potential biomarker because it has a significantly different level of expression in active TB compared to controls[18,34]and about one third of the world's population has a latent TB infection (LTBI. The mechanism of miRNA-379 in gene regulation at the time of M.tb infection is still not clear. However, expression of miRNAs is explained in literature especially in diseases like cancer.[36]

The effect of miR-379 on FAK/AKT signaling in the germinal center (GC) cells suggests that miR-379 is under-regulated in GC and reducing miR-379 is associated with poor prognosis leading to poor condition of GC patients. miR-379 can also inhibit GC migration, invasion and the epithelial–mesenchymal transition (EMT) phenotypes by targeting FAK/AKT signaling in vitro and in vivo. These data identified the underlying mechanism by which miR-379 inhibited GC migration and invasion and demonstrated miR-379 as a new prognostic biomarker for GC patients.[36] miR-379 inhibits activation of the PTEN/AKT pathway in gliomas. These results suggest that miR-379 attenuates glioma development by directly targeting MTDH and indirectly regulating the PTEN/AKT pathway. Moreover, deregulated expression of miR-379 contributes to the initiation and development of several types of cancer as well. For example, manipulation of miR-379 levels suppresses cell metastasis and EMT in gastric cancer by regulating the FAK/AKT signaling pathway. Expression of miR-379 reduces cell migration, invasion and EMT of hepatocellular carcinoma also by directly targeting FAK and regulating the AKT signaling pathway.[37] A study on the patients with active TB showed up-regulation of miR-379 in their serum as compared to controls with a 4.62 fold change observed in them. However, the regulatory mechanism is not clearly defined.[18] In the future, it is necessary to conduct research on the patho-mechanism of miRNA-379 regulation against M.Tb infection in relation to immune response and other

molecular pathways. Whether decreased miRNA379 affects IL-11, IL-8, FAK/AKT, PTEN/AKT, and Cyclin B1 in TB or not, is still an unanswered question in the literature and can be explored in future research.

In this study, AUC-ROC curve in all groups showed excellent diagnostic value in diagnosing both active and latent TB. Based on these results, it can be considered that miRNA can be used as a candidate biomarker for TB diagnosis. The miRNA-379 can also be considered as a potential candidate biomarker for TB diagnosis and is expected to differentiate between TB infection (latent TB) and TB disease (active TB). Studies that intend to evaluate the diagnostic value of miRNA in TB using the ROC and AUC curves found that the ROC analysis showed excellent diagnostic values for miR-31, miR-155 and miR-146a with AUC of 0.978, 0.953 and 0.903, respectively. By using ROC, Qi analyzed the diagnostic value of the combination of three miRNAs: miR-361, miR-889 and miR-576 and obtained an AUC value of 0.863, which means that it has a good and significant diagnostic value.[38,39]

The mechanism behind the different expression levels of miRNA-379 in children with TB has not been identified yet, however, this might happen through the regulation of the FAK/AKT signaling pathway by targeting FAK/AKT, inhibiting cell proliferation by regulating cyclin B1 expression which is proven to regulate the production of IL-11 and IL-8. This might be because miRNA characteristics differs with different ethnicities.[12] Differences in miRNA expression in each individual can also be influenced by several factors including genetics, immunity status, differences in the tissue or cell types targeted, the type of M.tb germ strain, the level of virulence and the severity of TB. This can affect differences in miRNA expression and consequently impacts the immune response. Several miRNAs have been shown to play a role in the regulation of inflammatory responses and innate and adaptive immunity in TB.[40,41] In addition, they can reflect disease progression and differentiate active TB from latent TB through their level of expression.[40,41] Further research on miRNAs as significant biomarkers and their validation is required using a larger group of study subjects.[25]

This study has certain limitations also which are: [1] This study required several meetings on different stages in the diagnosis of latent TB and active TB. Moreover, controls which have never received BCG immunization were also recruited, as a result the risk of drop-out was quite high. This can be overcome with honest communication / education in building trust and adapting to local culture. [2] The mechanism of how miRNA-379 regulates the host immune system has not been studied yet. Thus, further research is needed to answer questions about the pathological mechanism of miRNA-379 against TB. [3] The sample size in the current study was very small i.e.,32 hence a larger sample size is needed to further refine the research results.

Conclusions

This study found a significant difference in the diagnostic value of miRNA-379 between children with active TB, latent TB and healthy controls. This difference indicates that miRNA-379 can be used as a candidate biomarker to distinguish between latent and active TB in children.

Competing Interests

All authors declare that no competing interests were disclosed.

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Ethics and Consents

Ethical clearance was approved by the Ethics Committee of the Faculty of Medicine, Wijaya Kusuma University, Surabaya, Indonesia (No. 98/SLE/FK/UWKS/2019) on 30 September 2019.

Data Availability Statement

All data underlying the results are available as part of the article and no additional source data are required.

Abbreviations

AKT: Activating protein kinase B
AUC: Area under Curve
FAK: Focal Adhesion Kinase
M.tb: Mycobacterium Tuberculosis
TB: Tuberculosis
PTEN: Phosphatase and Tensin Homolog
ROC: Receiving Operating Characteristic

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