Identification of Microrna in Pediatric Latent Tuberculosis: A Preliminary Study in Indonesia

by Ayling Sanjaya

RESEARCH ARTICLE

Identification of Microrna in Pediatric Latent Tuberculosis: A Preliminary Study in Indonesia

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Abstract

Background: Pediatric tuberculosis (TB) is a major health problem in the world and Indonesia. Latent tuberculosis infection (LTBI) in children can be developed in to active state. It is difficult to prevent LTBI due to the lack of specific and diagnostic biomarkers. Research of microRNA (miRNA) conducted to identify profile of miRNA expression among pediatric LTBI specifically in Indonesia. This suggests that miRNA can be one of the tools to diagnose LTBI in children that needs to be developed. Aims: To identify specific miRNA expression in Indonesian pediatric latent tuberculosis infection. Methods: This research was an observational analytic study using a cross-sectional study design. The study used whole blood of healthy control children who had never received BCG immunization and children diagnosed LTBI. Profiling and identified miRNA expression with microarray analysis. Results: Four LTBI and two healthy control children were included in this study. Extraction of RNA with whole blood were done and profiled with gene chip microarray. Fold change >2 or <-2 with p-val <0.05. Total number of gene expressed are 6631. Genes passed filter criteria are 292 miRNA with 29 (9.93%) miRNA up-regulated and 263 (90.07%) miRNA down-regulated. Conclusions: Our study suggests that there are specific miRNAs identified in LTBI pediatric in Indonesia and can be one of the tools to diagnose LTBI in children that need to be developed.

Keywords: *Identification, LTBI, MIRNA, Pediatric.*

Introduction

Latent Tuberculosis Infection (LTBI) is a state of a persistent immune response to the stimulation to *Mycobacterium tuberculosis* (M.tb) without clinical symptoms of active TB, radiological abnormalities, and bacteriological disorders. Latent TB infection is characterized by positive tuberculin or Interferon Gamma Release Assay (IGRA) test results [1, 2]. Estimated around two billion people suffer from latent TB infection and cause new TB cases in 9.2 million people and deaths in 1.7 million people in the world. About 5-10% of people who suffer from latent TB infection will become active TB in the first five years after becoming infected with M.b.

Children with LTBI are more likely to develop into a serious disease than adults [3-5]. The early diagnosis of LTBI in children is important to control LTBI and TB in the community. There are no validated biomarkers for diagnosis of LTBI and TB in childen. In recent years the role of micro ribonucleic acid (miRNA or miR) has become a concern for researchers as biomarkers of diagnosis and therapy in TB in adults and children. MicroRNA is a ribonucleic acid composed of 19-24 nucleotides, which do not encode proteins, can complement the target genes that encode messenger RNA (mRNA) and function to regulate the level of expression of mRNAs [6-9].

Research on the characteristics of miRNA in several countries and ethnic groups has been carried out. On this study, we conducted a preliminary study of miRNA profiling to identify the characteristics of specific miRNA expressed in children with LTBI in Indonesia and contribute in finding a potential biomarker for diagnosis of pediatric LTBI.

Materials and Methods

Sample Collection

Whole blood samples (2 ml) were obtained from 4 children with LTBI and 2 children healthy control aged 0-<18 years recruited from Perak Timur Primary Health Care and Surabaya in November 2019 until January 2020. Eligibility for entry into this study was based on clinical signs and symptoms of M.tb infection. Children with exclusion criteria were not included in this study. LTBI subjects were recruited from close contact with active adult TB, with positive tuberculin test, without clinical and chest radiographs of active TB. Healthy control were children who had been in close contact with TB or LTBI patients, show no clinical sign of TB or LTBI, negative tuberculin test, and never received BCG immunization.

Preparation of Whole Blood Samples

Whole blood samples were taken each of 2 ml from 6 children included in the study inclusion criteria, then stored in a vacutainer tube with K2EDTA anticoagulant (Becton Dickinson). The tubes are stored in refrigerated boxes in upright conditions and sent to a laboratory for total RNA extraction.

Total RNA Extraction from Whole Blood

Extraction of total RNA from whole blood samples using mirVana ™ PARIS ™ RNA and Native Protein Purification Kit (Thermo Fisher Scientific, Invitrogen ™). The total RNA extraction procedure is as follows: 350 µl whole blood is transferred in a 1.5 ml tube, then 350 µl Denaturing Solution and 700 µl Acid-Phenol Chloroform are added. All samples were vortexed for 1 minute and followed by centrifugation at maximum speed (≥10,000 x g) for 15 minutes at room temperature.

The upper phase formed is transferred to a new 1.5 ml tube and recorded in volume. Absolute ethanol is added to the sample in 1: 1 (v / v) volume, then tossing and turning the sample tube until it is well mixed. Move the entire sample volume into the cartridge filter. Centrifugate at maximum speed (≥10,000 x g) for 30 seconds at room temperature, then dispose of the collected fluid in the collection tube. A total of 700 µl of miRNA Wash Solution 1 was added to the filter cartridge. Centrifugate at maximum speed (≥10,000 x g) for 15 seconds at room temperature, then dispose of the collected fluid in the collection tube. A total of 500 µl Wash Solution 2/3 was added to the filter cartridge.

Centrifugate at maximum speed (≥10,000 x g) for 15 seconds at room temperature, then dispose of the collected fluid in the collection tube. Repeat the washing step with Wash Solution 2/3 again. After that, centrifugate at maximum speed (≥10,000 x g) for 1 minute at room temperature, then discard the collection tube. Attach the cartridge filter to the new collection tube.

Add 50 µl Elution Solution at ~ 95oC to the center of the filter. Centrifugate with a maximum speed (≥10,000 x g) for 30 seconds at room temperature. The total RNA is in the collection tube. Transfer the total RNA into the 1.5 ml non-stick tube for storage. The total RNA concentration was measured using a Nano Drop spectrophotometer at a wavelength of 260 nanometers.

Analysis of MicroRNA Profiles with Microarrays Techniques

A total of 6 samples were selected to be analyzed for their microRNA profile by microarray technique. The 6 samples namely the healthy control group (2 samples) and the LTBI group (4 samples).

A total of 500 nanograms of total RNA were labeled using FlashTag ™ Biotin HSR RNA Labeling Kits (Thermo Fisher Scientific, Applied Biosystems ™). The labeled RNA was then hybridized to the GeneChip ™ miRNA array 4.0 overnight in the GeneChip ™ Hybridization Oven 645 (Thermo Fisher Scientific, Applied Biosystems ™) with a temperature of 48oC and a rotation speed of 60 rpm for 16-18 hours.

Hybridization control (GeneChip ™ Hybridization Control Kit) is included in the hybridization process. After the hybridization process is complete, the GeneChip ™ miRNA array 4.0 containing the sample is washed and colored using the Gene Chip ™ Fluidics Station 450 instrument (Thermo Fisher

Scientific, Applied Biosystems™). The GeneChip ™ miRNA array 4.0 is then scanned using the GeneChip ™ Scanner 3000 7G (Thermo Fisher Scientific, Applied Biosystems ™) instrument. Each scanned array will produce CEL and CHP files, which will be further analyzed using the Transcriptome Analysis Console (TAC) software version 4.0.1.

The quality of hybridization and labeling is observed first before starting the data analysis by observing the Hybridization Control and Spike-In Control signals. If the quality meets the threshold requirements, the analysis is continued by comparing the microRNA expressions in the three sample groups. Only microRNAs that had more than twice the difference in expression in each sample group would be displayed in the final results.

Ethics Statement

This study was approved by the Ethics Committee of the Faculty of Medicine, Wijaya Kusuma University, Surabaya, Indonesia. Written informed consent was obtained from participants prior to their enrollment in this study. All legal guardians of included children gave written informed consent.

This study was observational analytic using a cross-sectional design. Whole blood from 2 healthy control children and 4 children suffering from latent TB were obtained. Profiling miRNA expression using microarray then analysed the characterization. Differential expression of miRNA categorized in downregulated and upregulated genes and miRNAs.

Results

In the LTBI group compared with healthy control, the number of LTBI subjects was 4 children while the healthy controls were 2 children. The filter criteria in the microarray program according to the detected miRNA ID, with fold change> 2 or <-2, using p-value <0.05.

MicroRNA in accordance with these criteria will be entered and detected in microarray profiling. In the latent TB group and healthy controls there were 6631 total gene counts and as many as 292 miRNAs that could pass filter criteria. From 292 miRNAs there were 29 (9.93%) miRNAs up-regulation and 263 (90.07%) miRNAs down-regulation (Figure 1).

Latent vs Control
- Latent: 4 samples, Control: 2 samples

Filter criteria:
- Fold Change: > 2 or < –2
- P-val: < 0.05

Total number of genes: 6631
- Genes passed filter criteria: 292 (4.4%)
  - Up-Regulated: 29 (9.93%)
 - Down-Regulated: 263 (90.07%)

Figure 1: Summaries profile of miRNA in LTBI compared with healthy control group

Figure 2 (A) illustrates scatter plot miRNA expression where red distribution is up-regulation miRNA expression and green distribution shows down-regulation miRNA expression.

Hieralchical clustering of miRNA expression (B) shows the pattern of miRNA expression where blue expresses miRNA in latent TB, red expresses miRNA in healthy controls.

(A)
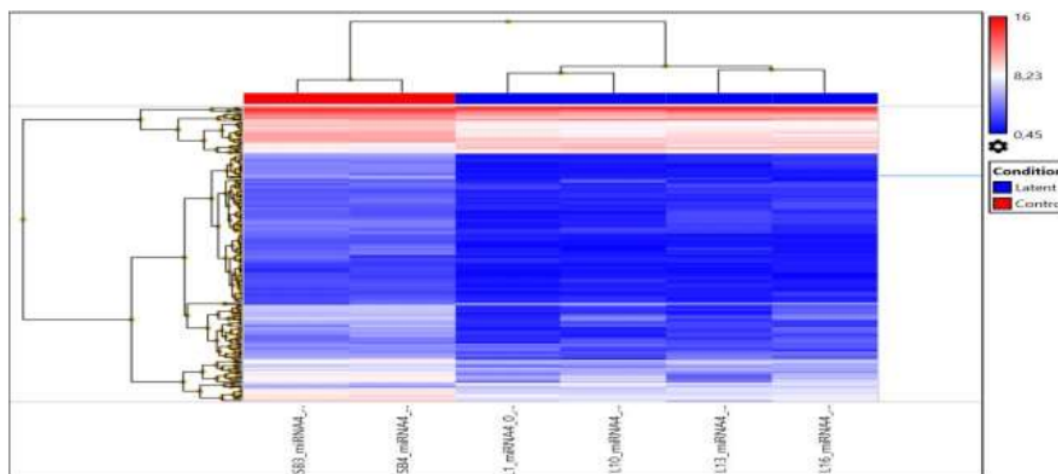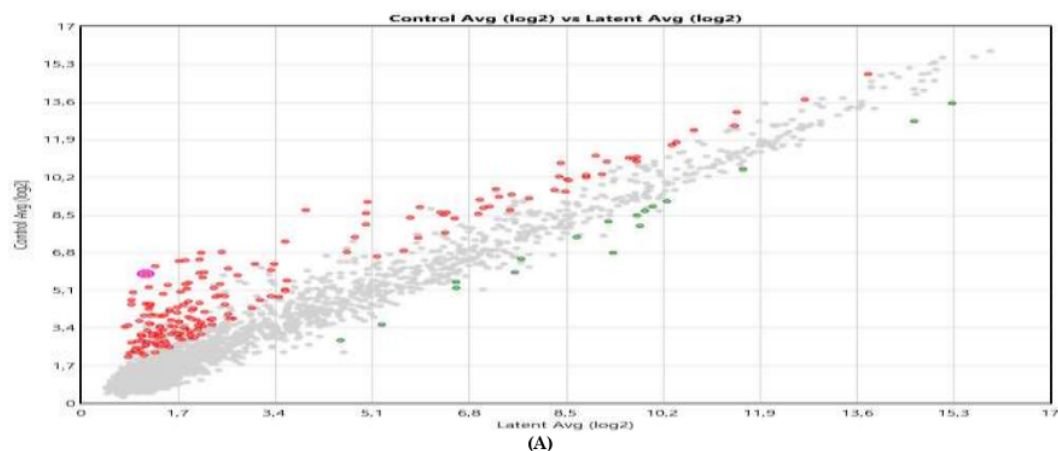
(B)
Figure 2: Scatter plot (A) and Hieralchical Clustering (B) of miRNA expressed in the LTBI Group Compared with Healthy Control

In the LTBI group and healthy controls 292 miRNA genes were expressed, 263 miRNA were down regulation with the lowest fold change miRNA-381-3p (fold change-30.92) and 29 miRNA upregulation with the highest fold change miRNA-3200-5p (fold change 5.41) (Table 1).

Tabel 1: miRNAs expressed in the LTBI group compared with the healthy control group

miRNA	Fold Change	P-val	miRNA	Fold Change	P-val
Down Regulated					
hsa-miR-381-3p	-30.92	1.73E-07	hsa-miR-6833-5p	-3.15	4.86E-05
hsa-miR-487a-3p	-29.04	0.0002	hsa-miR-941	-3.15	0.0021
hsa-miR-99a-5p	-27.10	6.77E-07	hsa-miR-4723-5p	-3.15	0.0044
hsa-miR-487b-3p	-26.03	0.0057	hsa-miR-148a-3p	-3.13	0.0263
hsa-miR-493-3p	-22.62	0.0003	hsa-miR-34a-5p	-3.13	0.0038
hsa-miR-337-5p	-21.51	8.30E-05	hsa-miR-18a-5p	-3.13	0.0014
hsa-miR-431-5p	-19.86	0.0002	hsa-mir-423	-3.12	0.0003
hsa-miR-376c-3p	-18.8	0.0001	hsa-let-7g-3p	-3.11	0.0198

hsa-miR-379-5p	-17.45	0.0015	hsa-miR-493-5p	-3.08	0.0003
hsa-miR-3130-5p	-16.89	0.0004	hsa-miR-1273d	-3.04	0.0001
hsa-miR-483-5p	-16.41	4.41E-06	hsa-miR-6503-3p	-3.04	0.0071
hsa-miR-1185-2-3p	-15.37	3.33E-06	hsa-mir-487b	-3.02	0.0102
hsa-miR-505-3p	-15.26	4.79E-05	hsa-miR-1229-3p	-3	0.0241
hsa-miR-433-3p	-13.74	0.0042	hsa-miR-202-3p	-3	0.0236
hsa-miR-376a-3p	-13.06	2.23E-05	hsa-miR-299-5p	-2.99	0.0026
hsa-miR-1185-1-3p	-13.06	0.0025	hsa-miR-6516-5p	-2.99	0.0145
hsa-miR-487a-5p	-12.65	1.56E-07	hsa-miR-4521	-2.99	0.0144
hsa-miR-1306-5p	-12.38	0.003	hsa-miR-6804-5p	-2.96	0.0002
hsa-miR-411-5p	-12.21	2.86E-05	hsa-miR-454-3p	-2.96	0.0002
hsa-miR-641	-11.87	0.0007	hsa-miR-181b-5p	-2.95	0.0004
hsa-miR-127-3p	-11.81	6.53E-05	hsa-miR-1254	-2.95	0.0037
hsa-miR-1271-5p	-11.64	0.004	hsa-miR-106b-3p	-2.94	0.0113
hsa-miR-654-3p	-11.07	3.97E-05	hsa-miR-15a-3p	-2.91	0.0117
hsa-miR-20b-3p	-11.04	0.0007	hsa-miR-671-3p	-2.9	0.013
hsa-miR-5001-3p	-10.83	0.0005	hsa-miR-3150a-5p	-2.89	0.0053
hsa-miR-542-5p	-10.10	2.69E-06	hsa-miR-181a-5p	-2.88	5.65E-05
hsa-miR-6799-3p	-9.86	0.0141	hsa-miR-7162-3p	-2.87	0.0087
hsa-miR-1296-5p	-9.77	3.08E-05	hsa-miR-6826-5p	-2.87	0.0076
hsa-miR-485-5p	-9.6	0.011	hsa-miR-22-5p	-2.86	0.0412
hsa-miR-409-5p	-9.39	0.0011	hsa-miR-125a-3p	-2.86	0.0004
hsa-miR-1299	-8.97	0.0086	hsa-miR-659-3p	-2.86	0.0119
hsa-miR-122-5p	-8.4	0.0005	10 sa-miR-378e	-2.86	0.001
hsa-miR-543	-8.36	1.97E-05	hsa-miR-6783-3p	-2.10	0.0048
hsa-miR-224-3p	-8.29	0.0006	hsa-miR-636	-2.10	0.0053
hsa-miR-2276-3p	-7.72	0.0061	hsa-miR-874-3p	-2.83	0.0395
hsa-miR-589-3p	-7.66	0.009	hsa-miR-874-5p	-2.82	0.0005
hsa-miR-206	-7.56	0.0159	hsa-miR-4290	-2.8	0.0144
hsa-miR-412-5p	-7.39	2.51E-06	hsa-miR-625-5p	-2.79	0.001
hsa-miR-409-3p	-7.33	0.0211	hsa-miR-210-5p	-10 4	0.0022
hsa-miR-769-3p	-7.33	0.0022	hsa-miR-27b-3p	-2.73	0.0185
hsa-miR-154-5p	-7.32	0.0002	hsa-miR-3195	-2.7	0.0025
hsa-miR-495-3p	-7.11	4.00E-05	hsa-miR-192-5p	-2.69	0.0086
hsa-miR-654-5p	-7.06	0.0001	hsa-miR-574-3p	-2.69	0.0001
hsa-miR-485-3p	-6.97	0.0121	hsa-miR-4485	-2.66	0.0311
hsa-miR-192-3p	-6.72	0.0002	hsa-miR-361-3p	-2.66	0.0099
hsa-miR-329-3p	-6.43	5.13E-06	hsa-miR-4747-3p	-2.65	0.0005
hsa-miR-539-5p	-6.32	9.83E-06	hsa-miR-6852-5p	-2.65	0.025
hsa-miR-125b-5p	-6.31	0.0162	hsa-let-7b-3p	-2.6	0.0178
hsa-miR-3158-3p	-6.31	0.0031	hsa-miR-425-3p	-2.6	0.0148
hsa-miR-301a-3p	-6.25	0.001	hsa-miR-758-5p	-2.6	0.001
hsa-miR-1303	-6.22	0.0026	hsa-miR-4435	-2.59	0.0044
hsa-miR-589-5p	-6.2	0.0342	hsa-miR-197-3p	-2.58	0.0004
hsa-miR-4746-5p	-6.17	8.34E-05	hsa-miR-340-5p	-2.58	0.0446
hsa-miR-370-3p	-6.14	0.0068	hsa-miR-744-5p	-2.55	0.0011
hsa-miR-377-5p	-6.13	3.00E-05	hsa-miR-501-5p	-2.54	0.0016
hsa-miR-214-3p	-6.1	0.002	hsa 10 iR-3615	-2.54	0.0023
hsa-miR-1343-3p	-6.09	0.0002	hsa-miR-331-3p	-2.54	0.0002
hsa-miR-584-3p	-6.06	0.0003	hsa-miR-323a-3p	-2.54	0.0008
5 sa-miR-2277-3p	-5.99	0.0009	hsa-miR-550a-3p	-2.53	0.0062
hsa-miR-6783-5p	-5.95	9.86E-05	5 sa-miR-3175	-2.53	0.0373
hsa-miR-3909	-5.77	1.35E-05	hsa-miR-17-3p	-2.53	7.98E-05
hsa-miR-1273c	-5.63	0.0009	hsa-miR-187-5p	-2.52	0.0319
hsa-miR-7-1-3p	-5.54	0.0095	hsa-miR-584-5p	-2.51	0.0112
hsa-miR-2278	-5.51	0.0211	hsa-let-7i-3p	-2.49	0.0371
hsa-miR-3157-5p	-5.43	0.0002	hsa-miR-215-5p	-2.47	0.009
hsa-miR-624-5p	-5.37	0.0002	hsa-miR-188-5p	-2.47	5.24E-05
hsa-miR-3074-3p	-5.33	0.0037	hsa-miR-7113-5p	-2.46	0.0436
hsa-miR-210-3p	-5.23	9.51E-05	hsa-miR-23b-5p	-2.45	0.0133
hsa-miR-660-3p	-5.22	5.30E-05	hsa-mir-224	-2.45	0.001

hsa-miR-550b-3p	-5.2	0.0003	hsa-miR-4538	-2.44	0.0005
hsa-miR-3620-3p	-5.18	0.0009	hsa-miR-532-5p	-2.44	0.0018
hsa-miR-432-5p	-5.16	0.0182	hsa-miR-7155-3p	-2.43	0.0183
hsa-mir-381	-5.14	4.10E-06	hsa-miR-186-5p	-2.43	0.0006
hsa-miR-140-5p	-5.11	0.0426	hsa-miR-130a-3p	-2.43	0.0003
hsa-miR-194-3p	-4.95	0.0001	hsa-mir-501	-2.42	0.0001
hsa-miR-494-3p	-4.92	0.0043	hsa-miR-7846-3p	-2.42	0.0038
hsa-miR-382-5p	-4.91	0.0153	hsa-miR-3165	-2.41	0.0247
hsa-miR-330-5p	-4.83	0.0002	hsa-miR-503-5p	-2.4	0.0006
hsa-miR-193b-3p	-4.79	0.0006	hsa-miR-193b-5p	-2.4	0.0019
hsa-miR-378a-5p	-4.75	0.0104	hsa-mir-210	-2.4	0.0102
hsa-miR-3120-3p	-4.69	8.67E-05	hsa-miR-6849-3p	-2.39	0.0147
hsa-miR-431-3p	-4.6	1.26E-06	hsa-let-7f-1-3p	-2.38	0.0262
hsa-miR-21-3p	-4.55	0.0121	hsa-miR-3177-3p	-2.36	0.0027
hsa-miR-134-5p	-4.52	0.027	hsa-mir-484	-2.35	0.0008
hsa-miR-502-5p	-4.5	0.0042	hsa-miR-27b-5p	-2.34	0.0079
hsa-miR-4482-5p	-4.5	3.53E-05	hsa-miR-4714-3p	-2.33	0.0071
hsa-miR-423-3p	-4.49	6.11E-05	hsa-miR-491-5p	-2.33	0.0091
hsa-miR-374c-5p	-4.48	0.0012	hsa-miR-381-5p	-2.3	0.0035
hsa-miR-185-3p	-4.47	0.0015	hsa-miR-601	-2.3	0.0055
hsa-miR-3605-3p	-4.45	0.0015	hsa-miR-6832-5p	-2.28	0.0302
hsa-miR-181d-5p	-4.45	0.0128	hsa-miR-484	-2.28	0.0012
hsa-miR-3198	-4.41	0.0429	hsa-miR-128-1-5p	-2.28	0.0412
hsa-miR-483-3p	-4.39	0.0018	hsa-miR-1292-5p	-2.26	0.002
hsa-miR-424-5p	-4.36	0.0095	hsa-miR-5088-5p	-2.25	0.0183
hsa-miR-6770-5p	-4.35	0.0017	hsa-miR-4665-3p	-2.25	0.0026
hsa-miR-18a-3p	-4.34	1.21E-05	ACA48	-2.24	0.008
hsa-miR-374a-5p	-4.31	0.0055	hsa-miR-6837-5p	-2.24	0.0071
hsa-miR-326	-4.3	0.0318	hsa-mir-4746	-2.24	0.0094
hsa-miR-335-5p	-4.23	0.0016	hsa-miR-3135a	-2.23	0.0124
hsa-mir-299	-4.21	3.86E-05	hsa-mir-7156	-2.22	0.0007
hsa-miR-138-5p	-4.2	4.88E-05	hsa-miR-6759-5p	-2.21	0.0007
hsa-miR-4310	-4.2	0.0021	hsa-miR-548w	-2.21	0.0115
hsa-miR-5187-5p	-4.1	0.0449	hsa-miR-345-3p	-2.2	0.0131
hsa-miR-3158-5p	-4.09	0.0028	hsa-miR-362-5p	-2.2	0.0003
hsa-miR-374b-5p	-4.07	0.0134	hsa-miR-532-3p	-2.2	0.0008
hsa-miR-629-3p	-4.05	0.0004	hsa-miR-4655-3p	-2.19	0.0032
hsa-miR-33b-3p	-3.98	0.0023	hsa-miR-680-3p	-2.19	0.014
hsa-miR-574-5p	-3.97	0.0216	hsa-miR-22-3p	-2.18	0.0088
hsa-mir-487a	-3.93	0.0025	hsa-miR-6074	-2.18	0.0286
hsa-miR-7706	-3.85	0.0108	hsa-mir-494	-2.18	0.0016
hsa-miR-7855-5p	-3.83	0.0007	hsa-mir-93	-2.16	0.0028
hsa-miR-3939	-3.83	0.0002	hsa-miR-766-3p	-2.16	0.0044
hsa-miR-6741-3p	-3.81	0.002	hsa-miR-342-3p	-2.15	0.0327
hsa-miR-6513-3p	-3.76	0.0034	hsa-mir-377	-2.15	0.0069
hsa-miR-421	-3.72	5.70E-05	hsa-miR-1471	-2.15	0.0098
hsa-miR-1307-3p	-3.67	0.0038	hsa-miR-3148	-2.15	0.0395
hsa-miR-579-5p	-3.61	0.0055	hsa-miR-424-3p	-2.14	0.0009
hsa-miR-466	-3.6	0.0206	hsa-miR-4713-3p	-2.14	0.0039
hsa-miR-219b-5p	-3.59	0.0066	hsa-miR-4726-5p	-2.14	0.0451
hsa-miR-6876-5p	-3.57	0.0133	hsa-miR-7-5p	-2.13	0.004
hsa-miR-148b-3p	-3.52	0.0009	hsa-miR-219a-1-3p	-2.12	0.0324
hsa-miR-93-3p	-3.49	0.0002	hsa-miR-6877-3p	-2.12	0.0009
hsa-miR-452-5p	-3.47	6.08E-05	hsa-miR-500a-5p	-2.12	0.0013
hsa-miR-128-3p	-3.46	0.0012	hsa-miR-93-5p	-2.11	0.007
hsa-miR-146b-3p	-3.44	0.0011	hsa-miR-541-5p	-2.11	0.0103
hsa-mir-487b	-3.38	0.0003	hsa-miR-345-5p	-2.1	0.0062
hsa-miR-6764-5p	-3.38	2.21E-05	hsa-miR-4754	-2.08	0.0122
hsa-miR-324-5p	-3.34	0.0003	hsa-miR-378a-3p	-2.05	0.0068
hsa-miR-3163	-3.34	0.0024	hsa-miR-196b-5p	-2.05	0.0427
hsa-miR-6882-5p	-3.28	0.0025	hsa-miR-4672	-2.05	0.0343

miRNA	Fold Change	P-val	miRNA	Fold Change	P-val
hsa-miR-500b-3p	-3.28	6.24E-05	hsa-miR-18b-5p	-2.05	0.0045
hsa-miR-299-3p	-3.28	0.0008	hsa-miR-6862-5p	-2.04	0.0032
hsa-miR-92a-1-5p	-3.28	0.0077	hsa-miR-376b-3p	-2.04	0.0126
hsa-miR-590-5p	-3.25	9.58E-05	hsa-miR-3191-3p	-2.10	0.0169
hsa-miR-652-5p	-3.24	0.0014	hsa-miR-6875-5p	-2.03	0.0208
hsa-miR-6893-3p	-3.22	0.0012	hsa-mir-421	-2.02	0.0008
hsa-miR-6886-5p	-3.21	0.0015	hsa-miR-411-3p	-2.02	0.0088
hsa-miR-1180-3p	-3.19	0.0001	hsa-miR-25-5p	-2.01	0.0061
hsa-mir-185	-3.19	0.0006	hsa-mir-6743	-2.01	0.0224
hsa-mir-3158-1	-3.19	0.0253	hsa-miR-20b-5p	-2.01	0.0058
hsa-mir-3158-2	-3.19	0.0253	hsa-miR-30d-5p	-2	0.0007
UP REGULATED					
miRNA	Fold Change	P-val	miRNA	Fold Change	P-val
U23	2.04	0.0295	hsa-miR-92b-5p	2.34	0.0035
hsa-miR-4505	2.07	0.0069	hsa-miR-6085	2.37	0.008
hsa-miR-4534	2.09	0.0282	ENSG00000238388	2.68	0.0287
hsa-miR-1207-5p	2.09	0.0148	hsa-miR-6754-5p	2.7	0.0381
ENSG00000239080	2.1	0.0283	ENSG00000238388	2.84	0.0265
hsa-miR-6820-5p	2.12	0.0007	hsa-miR-486-3p	3.01	0.005
hsa-miR-4467	2.13	0.0162	hsa-miR-6813-5p	3.04	0.0151
hsa-miR-4492	2.17	0.003	ENSG00000239154	3.09	0.0221
hsa-miR-1587	2.18	0.003	hsa-miR-8075	3.26	0.0488
hsa-miR-183-5p	2.25	0.0163	hsa-miR-6132	3.29	0.003
hsa-miR-6779-5p	2.27	0.001	hsa-let-7b-5p	3.3	7.42E-05
U91	2.28	0.0454	hsa-let-7c-5p	3.59	5.44E-05
mgU12-22-U4-8	2.28	0.0454	hsa-miR-4487	3.96	0.0199
hsa-miR-6726-5p	2.33	0.0155	hsa-miR-3200-5p	5.41	0.0006
hsa-miR-99b	2.34	0.0155			

Discussion

M.TB infection can cause active TB and most latent TB. About 5 to 10% of LTBI can develop into active TB. The mechanism of LTBI and its reactivation is still uncertain and it is assumed that the innate and adaptive immunity systems play a role. Different studies confirm the role of miRNA as an important regulator for the survival of M.tb in host cells [10-12]. In this study, the LTBI group and healthy controls obtained 6631 total gene counts and as many as 292 miRNAs that could pass filter criteria.

From 292 expressed miRNAs, 29 (9.93%) miRNAs were up-regulation and 263 (90.07%) had down-regulation miRNAs. From 263 miRNAs that were down-regulation with the lowest fold change, miRNA-381-3p (fold change -30.92) and 29 miRNA up-regulation with the highest fold change was miRNA-3200-5p (fold change 5.41).This is supported by research by Yang who wrote the role of miRNA-381-3p in TB infection in relation to innate and adaptive immunity of the body.

The researchers stated that the differential expression of miRNA in TB patients can help distinguish between TB patients and healthy people or LTBI [13]. A study comparing LTBI and control by Yurong Fu found 33 miRNA expressed up-regulation and 46 miRNA down-regulation, including miRNA-451a, miRNA-340-5p, miR-136-5p, miR-29b down regulation [14]. A study by Wang found 5 miRNA namely hsa-miR-130a, hsa-miR-296-5p, hsamiR-493, hsa-miR-520d-3p, hsa-miR-661 had different levels of expression between LTBI compared to controls [15].

The difference in miRNA expression in LTBI group compared to control shows that there are different miRNA characteristics in each situation, country or ethnicity. This latency may depend on the Mtb strain and host immune response. This requires further studies to be used as a basis for the characterization of specific miRNA in Indonesia, so that reactivation of latent TB can be minimized [12, 16].

Important regulations post transcription of gene expression are mediated by intracellular miRNA and immune processes. Several studies have shown changes in the expression of miRNA genes in macrophages and NKC in active TB and LTBI patients [17-19]. In the pathogenesis of TB, the host cellular immune response determines whether the infection becomes latent TB infection or develops into active TB where miRNA extensively regulates cell differentiation, development and disease.

Research has found genes that alter the expression profile in macrophage cells and NK cells from individuals with active or latent TB [20].Other studies encourage further research into miRNA as a biomarker that is appropriate and statistically meaningful by using larger groups and validating potential biomarkers in larger samples [12].

Conclusion

In this study, the LTBI group and healthy controls obtained 292 expressed miRNAs, 29

(9.93%) miRNAs were up-regulation and 263 (90.07%) had down-regulation miRNAs.

From 263 miRNAs that were down-regulation with the lowest fold change, miRNA-381-3p (fold change -30.92) and 29 miRNA up-regulation with the highest fold change was miRNA-3200-5p (fold change 5.41). Our study suggests that there are a specific miRNAs identified in LTBI pediatric in Indonesia and can be one of the tools to diagnose LTBI in children that needs to be developed.

Acknowledgment

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