Content available at: https://www.ipinnovative.com/open-access-journals

IP International Journal of Medical Microbiology and Tropical Diseases

Journal homepage: https://www.ijmmtd.org/

Review Article

Micro-RNA: A potential screening marker for latent tuberculosis

Anuradha Sharma[1,*], Priyanshu Srivastava[2], Aditi Sharma[2], Madhu Sharma[3], Pushkar[4], Manisha Sawhney[5]

[1]*Dept. of Microbiology, Faculty of Dentistry, Jamia Millia Islamia,, New Delhi, India*
[2]*Faculty of Dentistry, Jamia Millia Islamia, New Delhi, India*
[3]*Dept. of Microbiology, Pandit BD Sharma University, Rohtak, Haryana, India*
[4]*Pandit BD Sharma University, Rohtak, Haryana, India*
[5]*Jaslok Hospital, Mumbai, Maharashtra, India*

ARTICLE INFO

Article history:
Received 22-03-2023
Accepted 29-03-2023
Available online 19-04-2023

Keywords:
Biomarkers
Latent tuberculosis
miRNA
Pediatric
Diagnosis

ABSTRACT

An ancient disease, Tuberculosis is one of the most challenging infectious disease contributing to mortality and morbidity worldwide. Tuberculosis elimination globally, by 2050, is a mammoth task as Mycobacterial infections have wide range of presentation, from the clinical to the subclinical or latent and pose a diagnostic and therapeutic challenge. The virulence as well as evading property of Mycobacterium tuberculosis (Mtb) from the host's immune system confers upon it the ability to remain latent in the host cells. This forms the basis of classification of tuberculosis patient as having latent-TBI or active TB.

This review focuses on the role of miRNA as biomarkers of LTBI. The aim is to have an overview of the current knowledge about miRNA, its involvement in TB pathogenesis and its role as a reliable tool for diagnosis of latent tuberculosis.

miRNA are non-encoding endogenous RNAs which regulate gene expression by directing their target RNA for degradation or translational repression. Degraded RNA are released in the extracellular milieu, are present in various body fluids, such as blood, saliva, and urine, and are biomarkers for a number of diseases including cancer, Parkinsons' disease, CAD, liver diseases, TB and other infectious diseases. miRNAs are differentially expressed during active TB and LTBI, and therefore can be used as biomarkers of disease progression and response to anti-TB therapy. This will further permit more specific therapeutic interventions in TB management.

A thorough search of available literature resources was performed on online databases such as Google Scholar, NCBI, Nature, Research gate, PubMed, Science Direct. It was found that miRNA are promising biomarkers to identify healthy latent TB individuals for further course of action and can be reliable tools for routine use in current clinical practice for specific therapeutic interventions to limit active TB population. They meet the criteria of ideal biomarkers, such as minimally invasive, accessibility, high specificity, and sensitivity.

1. Background

Tuberculosis which is unaccompanied by symptoms and physical signs, causing no obvious disturbance, not recognised by the physician is known as latent tuberculosis, as defined by Opie and McPhedran in 1926.[1] The line between latent and active tuberculosis is often blurred and can be made more clear by using specific diagnostic tools. One such tool uses MicroRNA's, which are potential biomarkers of infections caused by a range of pathogens,

* *Corresponding author*.
E-mail address: asharma2@jmi.ac.in (A. Sharma).

https://doi.org/10.18231/j.ijmmtd.2023.001

including Hendra virus, HIV, tuberculosis and malaria.[2,3]

microRNAs (miRNAs) are a class of highly conserved, single-stranded RNA molecules (length, 18-25 nt) that regulate the expression of their target mRNAs. These are 8-10 nucleotide length stop codons, which negatively govern the post-transcriptional gene expression by binding to target messenger RNA, causing the degradation in translation or protein synthesis.[4]Present in body fluids, these can resist degradation and can be measured repeatedly and thereby are suitable as biomarkers.[5]

2. Materials and Methods

A review of literature was done , using the key words, through search engines, Google Scholar, PubMed, NLM, Google and Scopus. Various studies came to the forefront, on the use of miRNA as diagnostic markers for latent tuberculosis. These are mentioned as below:

1. Wang C et al in 2011 in Shanghai, China in a comparative study used human miRNA microarrays to probe the transcriptome of peripheral blood mononuclear cells (PBMCs) in patients with active tuberculosis (ATB), latent TB infection (LTBI), and healthy controls. 17 mi RNAs were found to be differentially expressed between the three groups with a p value < 0.01.[6]
2. Wu J et al in 2012 in State Key Laboratory of Genetic Engineering, Institute of Genetics, School of Life Sciences, Fudan University, Shanghai, China, studied the expression of specific miRNA. Two groups were included, ATB patients and healthy controls(HC) and they were induced by exposure to purified protein derivative (PPD).Microgray analysis in peripheral blood mononuclear cells from both the groups was done . There was characteristic expression of specific mi RNAs by TB-specific antigen.[7]
3. Zhang H et al in 2014, in China used Solexa sequencing to study microRNA expression in ATB patient, LTBI patients,healthy controls and those without prior BCG vaccination. 24 microRNAs were found to be up-regulated and 6 microRNAs down-regulated in patients with active TB relative to the three groups of healthy controls.[8]
4. Zhou M et al in 2016 in China, studied circulating microRNAs as biomarkers in the timely diagnosis of childhood tuberculosis where 14 miRNA's are critical. Microarray analysis and validation by RT-qPCR followed by ROC showed the diagnostic sensitivity and specificity of confirmed miRNAs. 29 miRNAs were seen to be altered with 15 upregulated and 14 downregulated.[9]
5. Ndzi EN et al in 2018, in Yaounde, Cameroon used quantitative real-time PCR and receiver operating characteristics to estimate the ability of miRNAs to discriminate between healthy controls and latent and active TB.[10]
6. Lyu L et al in 2019, in Beijing, China, performed small RNA sequencing to explore small RNA profiles of serum exosomes derived from LTBI, TB patients and healthy controls. 250 differentially expressed miRNAs were screened including 130 specifically expressed miRNAs. These specifically expressed miRNAs and differentially expressed miRNAs in different panels and patterns provide potential biomarkers for the detection/diagnosis of latent and active TB using exosomal miRNAs. Plenty of small RNAs derived from genomic repetitive sequences were also discovered which might play roles in host immune responses along with Mtb infection progresses.[11]
7. Kathirvel M, Saranya S & Mahadevan in Pondicherry, India, conducted a cross-sectional comparative study to investigate the potential of candidate circulating miRNAs, as potential blood biomarkers for the effective diagnosis of pediatric tuberculosis. The participants were 30 children with ATB (25 pulmonary TB and 5 extrapulmonary TB) and 30 healthy controls in a tertiary care hospital in Puducherry. Using SYBR green-based miScript qRT-PCR assay the expression levels of miRNAs in plasma was analysed. The receiver operating characteristic curve (ROC) was used to evaluate the diagnostic value of miRNAs. A significant upregulation was noted in miR-21, miR-29a, miR-31, miR-155, and down regulation of miR-146a in children with ATB compared to HC. The ROC analysis showed an excellent diagnostic value of miRNAs as follows: miR-31> miR-155> miR-146a with AUC of (95% CI) miRNAs 0.978, 0.953, and 0.903, respectively.[12]
8. Yareta J et al in 2020, in Laboratorio de Referencia Nacional de Biotecnología Biología Molecular, Instituto Nacional de Salud, Lima, Perú, South America, conducted an analytical study to find out the differential expression of miR-21, miR-29a, miR-99b and miR-155 in patients with LTBI, ATB and compared them to healthy controls. Small sample size was the limitation of the study.[13]
9. Korma W et al, in 2020, in Ethiopia, in the north-eastern part of Africa, also known as 'Horn of Africa', evaluated the stability and expression level of five candidate miRNAs including U6 small nuclear RNAs to normalize circulating miRNAs in the plasma of 68 participants recruited from four health centers and three hospitals in Addis Ababa, and Ethiopia. The expression levels of miRNAs isolated from plasma of culture-confirmed newly diagnosed pulmonary tuberculosis patients were compared with latently infected and non-infected healthy controls. miRNA-22-3p and miRNA-93-5p were suitable plasma reference miRNAs in the tuberculosis study.[14]

Table 1: Molecular methods of miRNA assays

	Name	Type	Use	Advantage	Disadvantage
A.	Northern blot-based platforms	Semi-quantitative.	For analysis of the expression of levels of individual miRNAs	For simultaneous detection of the mature miRNAs and miRNA precursors. Only method that provides information regarding both sequence and length. Validate results obtained with other methods.	Expensive, labor-intensive, time-consuming requires radio-labeling, special training and safety measurements. Omits rare types of miRNA
B.	In-situ hybridization (ISH)	Quantitative	Used to localize a sequence of DNA or RNA in a biological sample	Can be applied to archival materials and frozen tissues. Can be combined with immunohistochemistry to detect protein as well as mIRNA of interest	Low affinity of conventional RNA or DNA probes due to the small sizes of miRNAs.
C.	Reverse transcription qPCR	Quantitative	Used for analysing gene expression and quantitating RNA.	Examination of multiple genes at once	Costly, need high technical expertise, kits are not available for all kinds of genes , high risk of contamination .
D.	Nuclease protection assays	Quantitative	Highly sensitive method for the detection, quantitation and mapping of specific RNAs in a complex mixture of total cellular RNA	Examination of multiple genes at once.	The lack of information on transcript size and the lack of probe flexibility.
E.	Fluorescent in-situ hybridization	Quantitative	Used for finding specific features in DNA, and to detect and localize specific RNA targets (mRNA, lncRNA and miRNA).	Examination of multiple genes at once. Does not require the isolation of RNA. Provides information about RNA localization within the cell or tissue	Only the alterations affecting the labeled region can be studied and elected. Precise sample preparation is required needing high technical skill.
F.	Microarray next-generation sequencing.	Quantitative	Complementary base pairing between the sample and the chip-immobilized fragments produces light through fluorescence that can be detected using a specialized machine.	Provides data of thousands of genes in real time. Different parts of DNAcan be used for study gene expression	Expensive and time exhaustive. DNA chips required do not have long shelf life.

3. RNA Technology

Molecular methods of testing have a range of efficient and cost effective methods for analyzing miRNAs and guide in therapeutics, such as Northern blot based platforms, In-Situ Hybridization(ISH), Reverse Transcription Q PCR, Nuclease Protection Assays and Microarray Next Generation sequencing.[15] These are depicted in Table 1.

4. Discussion

The early diagnosis of tuberculosis is a game changer in the control of its spread and drug resistance. Routine clinical methods for diagnosing TB, involving radiography, culture of sputum and the tuberculin skin test (TST), have had many shortcomings.

The TST (tuberculin skin test) and IGRA (interferon gamma release assay test) have limitations in sensitivity and specificity and are also poor predictors of future development of active tuberculous disease. This calls for the need to use a diagnostic method that distinguishes the disease state rapidly, with high sensitivity and specificity and also gives the probability of a person with LTBI to develop active tuberculosis or whether therapy for LTBI would be effective to decrease the risk of developing active

TB. Finding new biomarkers in tuberculosis is not only necessary for diagnosing patients with TB, but also for the staging or classification of TB, TB prognosis, and TB drug and vaccine trials. Diagnosis of LTBI remains a challenge till today by conventional diagnostic techniques based on sputum sample.

In such a scenario, the role of biomarkers such as miRNA in this arena has been explored by many researchers in China, India, Cameroon, South America and Ethiopia. These studies have highlighted the importance of studying TB infection at the RNA level.

It has been found that miRNAs are involved in the regulation of several protein-coding genes. Those with previously established functions in hematopoietic cell differentiation and their target genes may be involved in the transition from latent to active TB. This implies that few miRNAs control the pathways of gene expression, regulate the immune system of the host and are important for the pathogenesis of tuberculosis.[6]mi RNAs such as hsa-miR-196b and hsa-miR-376c have the potential as markers for active TB disease.miR-155 and miR-155* have characteristic expression by TB-specific antigen, suggesting their use as potential diagnostic markers under the challenge of specific MTB antigens.[7] hsa-miR-29a-3p, hsa-miR-155-5p,and hsa-miR-361-5p are significantly upregulated in ATB as compared to LTB.[10] miR-155 overexpression in LTBI patients as compared to healthy controls can be explored as a biomarker to differentiate LTBI from ATB.[13]

BCG inoculation, an integral part of the Immunization programme in India, has also been seen to have an effect at the miRNA level.In BCG-inoculated individuals, 134 microRNAs are differentially-expressed as compared to BCG un-inocluated individuals. In LTBI individuals, there is upregulation of 75 microRNAs and 11 microRNAs down-regulated relative to BCG-inoculated individuals.[8] This provides an insight into the role of BCG in modulating the progression of the disease.[8]

For the early diagnosis of childhood TB, a combination of miRNA are required to increase the diagnostic value. Compared to uninfected children, seven miRNA's, miR-1, miR-155, miR-31, miR-146a, miR-10a, miR-125b and miR-150 have seen to be down regulated in children with TB and only one, miR-29, unregulated in children with TB and show a great deal of promise as non-invasive and effective biomarkers.[9] miR-31, miR-155 and miR-146a are effective diagnostic biomarkers for the detection of active-TB in children and their altered expression levels could be involved in the dysregulation of the host immune response to TB.[12]miR-29a-3p has a good distinguishing performance in discriminating ATB and HEC and a good diagnostic performance in discriminating ATB and LTB. Its presence in plasma, which is easy to collect compared to sputum, makes it an easy candidate marker in pediatric as well as extra pulmonary TB cases.[10]

Various molecular based technologies have come to the forefront for diagnostic assays for detection of miRNAs, reverse transcription q PCR being the gold standard.[15] Times have changed and so have healthcare facilities. It has acquired a new dimension and there is a shift of the site of testing from the laboratory to the patient's bedside. This has become possible through point of care (POC) diagnostics, where POC testing is defined as "A testing that is performed near or at the site of a patient with quicker results leading to a possible change in the patient care". mi RNA assays are not ideal POC diagnostic tools as they are time consuming, require expertise for sample preparation, and miniaturizations, making them more suitable for centralized labs.[16] They are also faced with a lot of challenges, as, being a relatively new field, certain properties of mi RNA, such as limit of detection, concentration range in body fluid , demographic and disease factors affecting their levels are yet to be uncovered.[17]

5. Conclusion

mi-RNA can be used in translational research as a biomarker of disease progression, Since treatment outcomes are not always accurate, this leads to missed diagnoses and ineffective treatment. Recent researches indicate that miRNAs are promising as a more reliable and sensitive diagnostic tool for latent TB. More research is needed to validate these findings and develop miRNA-based tests and treatments, but the potential for improved accuracy and effectiveness in the diagnosis and treatment of this condition is exciting.

6. Source of Funding

None.

7. Conflicts of interest

There are no conflicts of interest.

References

1. Opie EL, Mcphedran FM. The contagion of tuberculosis. *Am Rev Tuberculosis*. 1926;14(4):347–419.
2. Stewart CR, Marsh GA, Jenkins LA, Gantier MP, Tizard ML, Middleton D, et al. Promotion of Hendra Virus Replication by MicroRNA 146a. *J Virol*. 2013;87(7):3782–91. doi:10.1128/JVI.01342-12.
3. Zhang X, Guo J, Fan S, Li Y, Wei L, Yang X. Screening and identification of six serum microRNAs as novel potential combination biomarkers for pulmonary tuberculosis diagnosis. *PLoS One*. 2013;8(12):81076. doi:10.1371/journal.pone.0081076.
4. Li J, Huang MJ, Li W, Wang L, Li X, Zheng X, et al. Identification of potential whole blood MicroRNA biomarkers for the blood stage of adult imported falciparum malaria through integrated mRNA and miRNA expression profiling. *Biochem Biophys Res Commun*. 2018;506(3):471–7. doi:10.1016/j.bbrc.2018.10.072.
5. Zhou M, Yu G, Yang X, Zhu C, Zhang Z, Zhan X, et al. Circulating microRNAs as biomarkers for the early diagnosis of childhood tuberculosis infection. *Mol Med Rep*. 2016;13(6):4620–6. doi:10.3892/mmr.2016.5097.

6. Wang C, Yang S, Sun G, Tang X, Lu S, Neyrolles O, et al. Comparative miRNA expression profiles in individuals with latent and active tuberculosis. *PLoS One*. 2011;6(10):25832. doi:10.1371/journal.pone.0025832.
7. Wu J, Lu C, Diao N, Zhang S, Wang S, Wang F, 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(1):31–7.
8. Zhang H, Sun Z, Wei W, Liu Z, Fleming J, Zhang S, et al. Identification of Serum microRNA Biomarkers for Tuberculosis Using RNA-seq. *PLoS ONE* . 2014;9(2):88909. doi:10.1371/journal.pone.0088909.
9. Ndzi EN, Nkenfou CN, Mekue LM, Zentilin L, Tamgue O, Pefura EW, et al. MicroRNA hsa-miR-29a-3p is a plasma biomarker for the differential diagnosis and monitoring of tuberculosis. *Tuberculosis*. 2019;114:69–76. doi:10.1016/j.tube.2018.12.001.
10. Ndzi EN, Nkenfou CN, Mekue LM, Zentilin L, Tamgue O, Pefura EW, et al. MicroRNA hsa-miR-29a-3p is a plasma biomarker for the differential diagnosis and monitoring of tuberculosis. *Tuberculosis*. 2019;114:69–76. doi:10.1016/j.tube.2018.12.001.
11. Lyu L, Zhang X, Li C, Yang T, Wang J, Pan L. Small RNA profiles of serum exosomes derived from individuals with latent and active tuberculosis. *Frontiers in microbiology*. 2019;10:1174–1174.
12. Kathirvel M, Saranya S, Mahadevan S. Expression levels of candidate circulating microRNAs in pediatric tuberculosis. *Pathog Glob Health*. 2020;114(5):262–70.
13. Yareta J, Galarza M, Capristano S, Pellón O, Sánchez C, Ballon J, et al. Differential expression of circulating micro- rnas in patients with active and latent tuberculosis. *Rev Peru Med Exp Salud Publica*. 2020;37(1):51–6. doi:10.17843/rpmesp.2020.371.4468.
14. Korma W, Mihret A, Tarekegn A, Chang Y, Hwang D, Tessema TS, et al. Identification of circulating miR-22-3p and miR-93-5p as stable endogenous control in tuberculosis study. *Diagnostics (Basel)*. 2020;10(11):868. doi:10.3390/diagnostics10110868.
15. Condrat CE, Thompson DC, Barbu MG, Bugnar OL, Boboc A, Cretoiu D, et al. miRNAs as Biomarkers in Disease: Latest Findings Regarding Their Role in Diagnosis and Prognosis. *Cells*. 2020;9(2):276. doi:10.3390/cells9020276.
16. Dave V, Ngo A, Pernestig AK, Tilevik D, Kant K, Nguyen T, et al. MicroRNA amplification and detection technologies: opportunities and challenges for point of care diagnostics. *Lab Invest*. 2019;99(4):452–69. doi:10.1038/s41374-018-0143-3.
17. Zhang J, Zhao H, Gao Y, Zhang W. Secretory miRNAs as novel cancer biomarkers. *Biochim Biophys Acta*. 2012;1826(1):32–43. doi:10.1016/j.bbcan.2012.03.001.

Author biography

Anuradha Sharma, Professor https://orcid.org/0000-0002-7336-9103

Priyanshu Srivastava, Final year BDS Student https://orcid.org/0000-0003-0078-3757

Aditi Sharma, 3rd Year BDS Student https://orcid.org/0000-0003-2329-0162

Madhu Sharma, Professor

Pushkar, Senior Resident

Manisha Sawhney, Ph.D

Cite this article: Sharma A, Srivastava P, Sharma A, Sharma M, Pushkar, Sawhney M. Micro-RNA: A potential screening marker for latent tuberculosis. *IP Int J Med Microbiol Trop Dis* 2023;9(1):1-5.