



Up-regulation of Serum microRNA-21 in Patients with Pulmonary Tuberculosis (PTB) as Compare to Healthy Individuals

Aditya Upadhyay¹, Anmol Kulshrestha¹, R.R.K. Niraj², Deepika Gupta⁴, Arvind Jat⁴, Mrituanjai Singh¹, Archana Tiwari¹, Shubhra Jain³, and Sandeep K. Shrivastava^{4*}

¹School of Biotechnology – Rajiv Gandhi Proudhyogiki Vishwavidyalaya, Airport Road, Bhopal, 462033, India

²Dr. B. Lal Institute of Biotechnology, Jaipur, India

³Department of Respiratory Medicine, Sawai Man Singh (SMS) Medical College, Jaipur, 302004, India

⁴Centre for Innovation Research and Development (CIRD), Dr. B. Lal Institute of Biotechnology, Dr. B. Lal Clinical Laboratories Pvt. Ltd., Jaipur, 302017, India

*Corresponding Author E-mail: ssk.cird1@gmail.com

Received: 10.09.2019 | Revised: 17.10.2019 | Accepted: 24.10.2019

ABSTRACT

Tuberculosis is a lethal disease worldwide due to absences of early diagnostic processes. microRNAs have been denoted their functional and diagnostic potential in various diseases like cancer and tuberculosis. In the present study, we performed the miRNA isolation from the clinical confirm pulmonary tuberculosis (PTB) and healthy control cases. We have observed the up-regulation of serum microRNA-21 in serum sample of pulmonary tuberculosis (PTB) patients. Our results based on qRT-PCR confirmation and receiver operational curve (ROC) analysis indicate that the serum microRNA-21 can be explored as potential non invasive biomarker for early detection of pulmonary tuberculosis infection in human.

Keywords: Pulmonary Tuberculosis, microRNA-21, Non-Invasive Biomarker, Up-regulation, ROC.

INTRODUCTION

Tuberculosis is a major health problem in developing countries with 10.4 million new TB cases and 1.7 million death from TB in 2016-2017. Seven countries have been notified for 64 % of new TB case: India, China, Philippines, Nigeria, Pakistan and South Africa [WHO Tuberculosis Global Report 2017]. WHO has set target of decrease the number of TB death by 95% and also reducing number of new TB case by 90% by 2035.

Early diagnosis of tuberculosis is the utmost need of the time to achieve this goal in time.

Tuberculosis is an infectious disease caused by *Mycobacterium Tuberculosis*. At the current days, early diagnostic of TB is very important for controlling the disease in developing nations (Harapan et al., 2013). At current scenario, Rapid TB culture test is being considered gold standard test for tuberculosis diagnosis. But it is very time consuming process.

Cite this article: Upadhyay, A., Kulshrestha, A., Niraj, R.R.K., Gupta, D., Jat, A., Singh, M., Tiwari, A., Jain, S., & Shrivastava, S.K. (2019). Up-regulation of Serum microRNA-21 in Patients with Pulmonary Tuberculosis (PTB) as Compare to Healthy Individuals, *Ind. J. Pure App. Biosci.* 7(5), 499-503. doi: <http://dx.doi.org/10.18782/2320-7051.7839>

New diagnostic approaches at molecular diagnostics are also operational for tuberculosis. However, their diagnostic potential depend on the suitable and optimum sample type availability along with presence of detectable bacterial count in the sample specially in children, old age people and very weak patients who cannot give the sputum sample for test procedure. Due to poor sample and non-optimum sample, molecular diagnostic techniques targeting the mycobacterium in the sample comes out with false negative outcome (Wagh et al., 2017; O' Connell et al., 2010; Sabir et al., 2018). The available diagnostic methods for PTB are dependent on presence of Mtb bacteria pathogen only. However, availability of any potential diagnostic host biomarker would be a great achievement for early and non-invasive diagnosis of PTB. The early diagnostic of pulmonary tuberculosis is very important for containment of disease in

the community. Serum biomarkers may be an alternative approach for pulmonary tuberculosis.

MicroRNAs is small non coding RNA molecules that play important functions at cellular level. miRNAs are also detected in circulating fluid of healthy individuals. miRNAs are present and stable in body fluid that is most important conditions for developing the new novel diagnostic tool as earlier diagnostic biomarker. Recent study has shown that microRNAs work as useful biomarker in cancer disease (Upadhyay et al., 2019; Miotto et al., 2013). Alterations in miRNAs expression have also been reported in serum of TB patients. In the present study we aimed to evaluate the variation in the level of microRNA-21 in serum of TB patients with comparison of healthy individual in the population of Rajasthan.



Fig. 1: Flow chart of the work pipeline

MATERIALS AND METHODS

A total of 34 serum samples were taken for the study including 23 serum samples which were diagnosed with the pulmonary TB and 11 were healthy ones. The samples utilized in this investigation were collected from the collaborative research partners – Dr. B. Lal Clinical Laboratory Pvt. Ltd., Jaipur and TB Hospital, Jaipur during the year 2017-19.

2.1. miRNA EXTRACTION:

Isolation of miRNA from the serum samples of pulmonary patients and healthy individuals was done by using Qiagen's miRNeasy Serum/Plasma Kit. 200 µl serum sample was taken for extraction according to the manufacturer's protocol. The RNA were extracted out by using Nucleospin silica membrane supplied by the manufacturer and stored at -20°C till further use.

2.2. cDNA SYNTHESIS:

For the cDNA synthesis of the extracted miRNA was done by Qiagen's miScript

Reverse Transcription Kit. According to manufacturer's protocol, 12 µl of miRNA sample was used. The cDNA synthesis occurred at 37°C for 60 minutes followed by incubation at 95°C for 5 minutes.

2.3. QUANTITATIVE REALTIME PCR:

Qiagen's miScript SYBRGreen RTPCR Kit was used for the RTPCR. The temperature profile for RTPCR reactions was as follow: 95°C for 15 minutes followed by 94°C for 15 seconds, 55°C for 30 seconds and 70°C for 30 seconds and 40 cycles were run. QauntStudio 3.0 [Thermo Fischer] was used for the RTPCR. Data analysis was done by using the $2^{-\Delta\Delta C_T}$ method. qRT-PCR and miRNA expression data was normalized by using miRNA-39.

2.4. STATISTICAL ANALYSIS:

The GraphPad Prism 8 was used for the statistical analysis. The data was tested against the Student's t-Test which gives the significant difference in the data at $p < 0.05$. The Receiver

Operating Characteristics (ROC) curve analysis reveals the diagnostic potential. The area under curve (AUC) value signifies the degree of separatability of the data i.e, how much the two groups Healthy Control and infected are separated from each other. Results were considered statistically significant when the p value less than 0.05 was observed.

RESULTS

In this study, serum samples were collected from 34 subjects out of which 23 samples were

pulmonary Tuberculosis patients and the remaining 11 were Healthy controls. The expression of miRNA-21 was significantly altered in case of pulmonary TB cases as compared to Healthy ones. The expression of miRNA-21 was up-regulated (2 to 6 fold) in the serum of pulmonary Tuberculosis patients as compared to the Healthy ones. MiRNA-21 was 2 to 6 fold upregulated on basis of Statistical analysis.

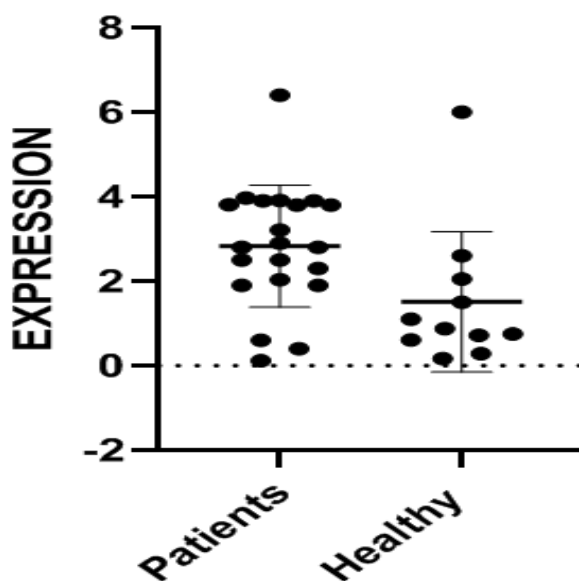


Fig. 2: Expression level of miRNA-21 in pulmonary & healthy serum samples

The ROC curve analysis gave significant and distinguishing efficiency with a good diagnostic potential having an AUC value of 0.7619 which statistically significant and having P-value of

less than 0.05. The t-Test was also applied on data which showed that the data was significant at $p < 0.05$.

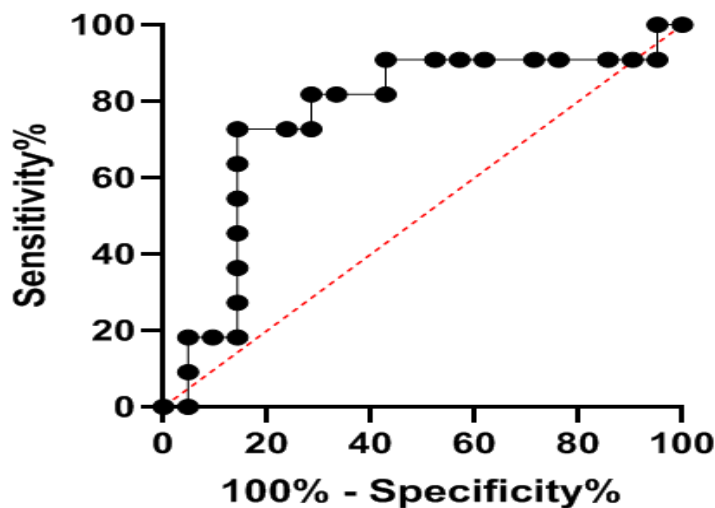


Fig. 3: ROC curve analysis of miRNA-21 in pulmonary TB & healthy ones

DISCUSSION

Mycobacterium Tuberculosis is transmitted through the droplet infection generated from TB patients and also transmitted by asymptomatic undiagnosed TB patients or latent TB cases in society. At the current scenario rapid diagnosis is very essential for controlling TB infections. At the present time, various diagnostic approaches are available in clinical diagnosis of pulmonary tuberculosis like Sputum smear, culture method and imaging methods. Sputum Method is very common method use for pulmonary tuberculosis in clinical labs, but its negative predictive value (NPV) and positive predictive value (PPV) is almost depends on person's efficiency. Hence there are much chances to miss the case. Rapid culture method is gold standard method, which is based on the growth of bacteria. But it requires the long incubation time around 4 to 6 weeks. At the current time only imaging technology used for earlier diagnosis in case of pulmonary tuberculosis. But imaging appearances are not good method for diagnosis of pulmonary tuberculosis because it is nonspecific process and it may be difficult to differentiated pulmonary TB from other lung disease. Thus we urgent need of new diagnostic approach in pulmonary tuberculosis at earlier stage. To the date various studies have been done on various microRNAs in case of pulmonary tuberculosis but it is not clear whether such microRNAs are useful for pulmonary TB diagnosis yet. Various study have been conducted on serum of pulmonary TB patients (Wagh et al., 2017; Upadhyay et al., 2019, Zheng et al., 2013). These studies showed the valuable results and conclusion in field of pulmonary tuberculosis diagnosis approach but needs further validation to establish significant biomarkers. The present study was performed with serum sample of pulmonary tuberculosis cases and in this study we observed significant alterations in the expression level (2 to 6) of microRNA-21 in pulmonary sample as compare then serum of healthy individuals.

microRNA-21 play the important role in pulmonary tuberculosis infections. microRNA-21 are involved in various

biological process during the pulmonary tuberculosis infections like apoptosis. microRNA-21 can inhibit the apoptosis process of macrophages in pulmonary tuberculosis infection. *Mycobacterium Tuberculosis* releases a protein known as MPT64 during the infection. MPT64 is a secreted protein. MPT64 is indirectly responsible for up-regulation of bcl-2. This mechanisms is responsible for increase expression level of microRNA-21. Up-regulated microRNA-21 is responsible for inhibiting the apoptosis during infection. Which provide the favourable ecosystem for bacterial survival and growth (Qingmin et al., 2014; Wang et al., 2014; Mehta et al., 2016).

The ROC Curve analysis was done to see the diagnostic potential of serum biomarkers with AUC values. ROC curve showed good diagnostic potential of microRNAs -21 with AUC Value 0.76. The data also found significant with P value less than 0.05 and we also applied student t-Test on the data and got the statically significant p value. In this study we observed that microRNAs-21 is 2 to 6 fold up-regulated in pulmonary tuberculosis patients as compare to healthy individual. In this study, microRNA-21 was specific for pulmonary tuberculosis patients. Thus microRNA-21 might act as biomarker in case of pulmonary tuberculosis infections. Our results provide the useful information to the understanding of diagnosis of pulmonary TB at earlier stage. However, the expression pattern of the miRNA-21 need to be evaluated with larger sample size of Pulmonary tuberculosis patient population covering deferent geography and ethnicity.

CONCLUSION

In the present study, we manifested a potential of serum microRNAs-21 as diagnostic marker for pulmonary tuberculosis infection. microRNA-21 was up-regulated (2 to 6 fold) in pulmonary tuberculosis patients as compare then healthy individual. If this study conducted and validated in large population, we believe the microRNA-21 could act as valuable diagnostic tool for pulmonary tuberculosis infections in the host.

Acknowledgement

This study is a part of the research project funded by Department of Science & Technology, Government of Rajasthan, Jaipur, India. We highly acknowledge their financial support.

REFERENCES

- Harapan, Fitra, Ichsan, Mulyadi, Paolo Miotto, Nabeeh, A., Hasan, Marta C., & Daniela, M. Cirillo (2013). The Role of microRNAs on tuberculosis infection: Meaning or Myth?. *Tuberculosis (Edinburgh)* 93, 596-605.
- Mehta, A., & Baltimore, D. (2016). MicroRNAs as regulatory elements in immune system logic. *Nature Reviews. Immunology* 16, 279–294.
- Miotto, P., Mwangoka, G., Valente, I. C., Norbis, L., Sotgiu, G., & Bosu, R., (2013). MiRNA signatures in sera of patients with active pulmonary tuberculosis. *PLoS One* 8, e80149.
- Sabir, N., Hussain, T., Shah, S.Z.A., Peramo, A., Zhao, D., & Zhou, X. (2018). miRNAs in Tuberculosis: New Avenues for Diagnosis and Host-Directed Therapy. *Frontiers in microbiology* 9, 602.
- O'Connell, R.M., Rao, D.S., Chaudhuri, A.A., & Baltimore, D. (2010). Physiological and pathological roles for microRNAs in the immune system. *Nat Rev Immunol* 10, 111e22.
- Upadhyay, A., Kulshrestha, A., Patel, G., Singh, M., Gupta, D., & Shrivastava, S.K. (2019). microRNA: An Advance Diagnostic Approach in Tuberculosis, *Ind. J. Pure App. Biosci.* 7(4), 298-309. doi: <http://dx.doi.org/10.18782/2320-7051.7725>
- Wagh, V., Urhekar, A., & Modi, D. (2017). Level of microRNA miR-16 and miR-155 are altered in serum of patients with tuberculosis and associate with responses to therapy. *Tuberculosis* 102, 24-30.
- Wang, Q., Liu, S., Tang, Y., Liu, Q., & Yao, Y. (2014). MPT64 protein from Mycobacterium tuberculosis inhibits apoptosis of macrophages through NF-kB-miRNA21-Bcl-2 pathway. *PLoS One.* 9(7), 1–8.
- Wang, Q., Tang, Y., Liu, Q., & Yao, Y. (2014). MPT64 Protein from Mycobacterium tuberculosis Inhibits Apoptosis of Macrophages through NF-kB-miRNA21- Bcl-2 Pathway. *PLoS One* 9(7), e100949.
- WHO Tuberculosis Global Report 2017.
- Zhang, X., Guo, J., Fan, S., Li, Y., Wei, L., Yang, X., Jiang, T., Chen, Z., Wang, C., Liu, J., Ping, Z., Xu, D., Wang, J., Li, Z., Qiu, Y., & Li, J. C. (2013). Screening and identification of six serum microRNAs as novel potential combination biomarkers for pulmonary tuberculosis diagnosis. *PloS One* 8(12), e81076.