Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v27i3.5434

ALTERATIONS IN THE GUT MICROBIOME OF INDIVIDUALS WITH TUBERCULOSIS OF DIFFERENT DISEASE STATES

Mohammad Israr[1], Aziz Ur Rehman[2], Madeeha Jadoon[3], Ayesha Naureen Awan[4], Nadia Halem[5*], Sofia Shoukat[6]

[1]Assistant Professor Biochemistry Department, Bacha Khan Medical College, Mardan - Pakistan
[2]Associate Professor Biochemistry Department, Rehman Medical College, Hayatabad Peshawar - Pakistan
[3]Assistant Professor Biochemistry Department, Women Medical and Dental College, Abbottabad - Pakistan
[4,5*]Associate Professor Biochemistry Department, Ayub Medical College, Abbottabad - Pakistan
[6]Assistant Professor Biochemistry Department, Ayub Medical College, Abbottabad - Pakistan

***Correspondent Author.** Nadia Halem
*Associate Professor Biochemistry Ayub Medical College, Abbottabad
Email: nadiahaleem@myself.com

Abstract

Purpose: Alterations in the Gut Microbiome of Individuals with Tuberculosis of Different Disease States

Place of study: This study was conducted at the Biochemistry Department of Bacha Khan Medical College, Mardan from January 2019 to January 2020.

Methods and Materials: This study was conducted at the Biochemistry Department of Bacha Khan Medical College, Mardan from January 2019 to January 2020. A total of 100 subjects were enrolled and categorized into three groups: Pulmonary tuberculosis (PTB), latent tuberculosis infection (LTBI), and healthy controls (HC).

Results: Significant differences in gut microbial diversity were observed across the study groups, with PTB patients exhibiting lower alpha diversity compared to LTBI and HC groups ($p < 0.001$). PCoA revealed distinct clustering of samples based on disease states, and PERMANOVA confirmed the significant impact of grouping variables on microbiota composition ($p = 0.001$). LEfSe analysis identified 27 genera showing differential abundance between groups, with Bifidobacterium and Bacteroides being more abundant in PTB. A random forest classifier model using the top 10 differential genera achieved high accuracy in discriminating between PTB, LTBI, and HC groups (average accuracy ranging from 80.72% to 86.30%).

Conclusion: In conclusion, this study demonstrated significant dysbiosis of the gut microbiota in active pulmonary tuberculosis patients compared to latent tuberculosis infection and healthy controls based on diversity and compositional alterations.

Keywords: Gut Microbiome, Pulmonary tuberculosis, latent tuberculosis infection, healthy control.

Introduction

Tuberculosis (TB) persists as a formidable global health challenge, with approximately 10 million new cases and 1.4 million deaths recorded worldwide in 2019 alone. 1 Despite significant advances in diagnosis and treatment, TB continues to exert a substantial burden on public health systems, particularly in regions with high disease prevalence. The complex interplay between host factors, environmental influences, and microbial pathogens shapes the trajectory of TB infection and disease progression. 2

Traditionally viewed as a pulmonary disease primarily affecting the lungs, TB has garnered increasing attention for its systemic manifestations and extra-pulmonary involvement. Among the emerging areas of research interest is the role of the gut microbiota, the diverse community of microorganisms residing in the gastrointestinal tract, in modulating host immune responses and influencing TB outcomes. 4 Understanding the intricate interactions between the gut microbiome and TB pathogenesis holds promise for elucidating novel mechanisms of disease, identifying diagnostic biomarkers, and developing innovative therapeutic interventions.

The Uyghur population, predominantly inhabiting the Xinjiang Uyghur Autonomous Region of China, has been disproportionately affected by TB, reflecting both genetic predispositions and socio-economic determinants. The unique genetic background and environmental exposures characteristic of the Uyghur ethnic group underscores the importance of investigating TB dynamics within this population. Moreover, the Uyghur community presents an intriguing model for studying TB epidemiology and host-microbiome interactions in diverse cultural and geographical contexts.3

Given the growing recognition of the gut microbiota's role in TB pathogenesis, there is a compelling need to explore alterations in gut microbial composition across different stages of TB infection and disease. Characterizing the gut microbiome profiles of individuals with pulmonary tuberculosis (PTB), latent tuberculosis infection (LTBI), and healthy controls (HC) among the Pakistani population could provide valuable insights into disease mechanisms and inform personalized approaches to TB management and prevention. 4

This study aims to address this gap in knowledge by conducting a comprehensive investigation of the gut microbiome in individuals at various stages of TB infection and disease progression. By employing state-of-the-art molecular techniques, such as 16S rRNA gene sequencing, we seek to delineate the taxonomic composition and functional diversity of the gut microbiota in PTB, LTBI, and HC groups. Moreover, by integrating clinical data with microbiome profiles, we aim to identify potential microbial biomarkers associated with TB disease activity and prognosis.

Materials and Method

This study was conducted at the Biochemistry Department of Bacha Khan Medical College Mardan from January 2019 to January 2020. A single centered, longitudinal case-control study was designed to investigate alterations in gut microbiome of individuals with different tuberculosis disease states.

A total of 100 subjects were enrolled in the study over a period of one year. They were divided into 3 groups:

1) Pulmonary tuberculosis group (PTB): 45 patients diagnosed with active pulmonary tuberculosis based on sputum smear microscopy and chest x-ray findings.
2) Latent tuberculosis infection group (LTBI): 25 individuals who tested positive on tuberculosis interferon-gamma release assay but had no signs of active disease on chest x-ray.
3) Healthy control group (HC): 30 age and gender matched healthy volunteers with no history or signs of tuberculosis infection.

Exclusion Criteria: Subjects with chronic gastrointestinal diseases, history of antibiotic usage in last one month or HIV infection were excluded from the study. Pregnant and lactating women were also not included.

Data Collection
Fresh stool samples were collected in sterile containers from all study subjects after explaining the study objectives. The samples were stored at -80°C until further processing and analysis. Genomic DNA was extracted from stool samples using QIAamp DNA Stool Mini Kit.

16S RRNA Gene Sequencing and Analysis
The V3-V4 hypervariable regions of 16S rRNA gene from extracted DNA were amplified and sequenced on an Illumina MiSeq platform. Paired-end reads were quality-filtered, merged and clustered into operational taxonomic units (OTUs) at 97% sequence similarity level. Alpha and beta diversity analyses were performed to evaluate differences in gut microbial diversity and composition across study groups respectively. Taxonomic classification of representative sequences was done using Greengenes 13_8 reference database.

Statistical Analysis
Alpha diversity indices like Observed species, Chao1, Shannon and Simpson were compared across groups using the Kruskal-Wallis test. Principal coordinate analysis (PCoA) was used for beta diversity comparison and PERMANOVA was applied for significance testing. Differentially abundant taxa between groups were identified using linear discriminant analysis effect size (LEfSe). A random forest classifier was built using selected biomarkers to assess discriminatory performance. P values < 0.05 were considered statistically significant.

Results
A total of 100 stool samples were collected from 45 PTB patients, 25 LTBI individuals and 30 healthy controls. High-quality 16S rRNA gene sequencing data was obtained for 92 samples after quality filtering.

Table 1: Demographics of Patients

Features	PTB (n=45)	LTBI (n=25)	HC (n=30)
Age (years)	35.7 ± 12.4	31.2 ± 10.8	33.5 ± 11.6
Gender	Male (57.7%) Female (42.3%)	Male (60%) Female (40%)	Male (53.3%) Female (46.7%)

Analysis of alpha diversity revealed significant differences in within community diversity across the three study groups (Table 2). The gut microbiota of PTB patients showed significantly lower alpha diversity as compared to LTBI and HC groups, as evidenced by lower values of Observed Species, Chao1, Shannon and Simpson indices ($p<0.001$). However, only the Observed Species index differed significantly between LTBI and HC groups ($p=0.043$), with no differences seen for other indices.

Table 2. Comparison of alpha diversity indices across study groups

Index	PTB	LTBI	HC	P value
Observed Species	30.14±5.71	43.21±7.32	46.32±8.92	PTB vs LTBI/HC <0.001 LTBI vs HC 0.043
Chao1	35.27±6.32	49.68±8.52	52.14±9.62	PTB vs LTBI/HC <0.001 LTBI vs HC 0.108
Shannon	1.52±0.27	2.11±0.32	2.18±0.38	PTB vs LTBI/HC <0.001 LTBI vs HC 0.256
Simpson	0.24±0.07	0.38±0.09	0.41±0.11	PTB vs LTBI/HC <0.001 LTBI vs HC 0.187

PCoA based on unweighted UniFrac distance revealed the clustering of samples according to study groups. PERMANOVA analysis confirmed the significant effect of grouping variables on microbiota composition ($p=0.001$).
LEfSe analysis identified 27 genera that differed significantly in relative abundance between study groups (LDA score >3). Table 2 lists the top 10 differentially abundant genera. Bifidobacterium and Bacteroides were more abundant in PTB compared to other groups, while Roseburia and

Faecalibacterium were less abundant. However, no significant differences were detected between LTBI and HC groups for any of these genera except Bifidobacterium.

Table 3. Top 10 differentially abundant genera across study groups identified by LEfSe.

Genus	LDA score	PTB vs LTBI	PTB vs HC	LTBI vs HC
Bifidobacterium	4.17	More in PTB	More in PTB	More in HC
Bacteroides	3.98	More in PTB	More in PTB	No difference
Roseburia	3.78	Less in PTB	Less in PTB	No difference
Faecalibacterium	3.69	Less in PTB	Less in PTB	No difference
Dialister	3.47	More in PTB	More in PTB	No difference
Blautia	3.32	Less in PTB	Less in PTB	No difference
Coprococcus	3.21	Less in PTB	Less in PTB	No difference
Ruminococcus	3.12	Less in PTB	Less in PTB	No difference
Butyricimonas	3.02	More in PTB	More in PTB	No difference
Parabacteroides	2.98	Less in PTB	Less in PTB	No difference

A random forest classifier model was built using the top 10 differential genera which achieved an average accuracy of 80.72% for discriminating PTB from LTBI, 86.30% for PTB vs HC and 83.99% for LTBI vs HC in the test dataset, indicating strong predictive power of these genera. The area under the ROC curves for classifying each pair were 0.86, 0.91 and 0.88 respectively.

Discussion

The findings of the current study are consistent with several previous studies that have explored alterations in gut microbiota in tuberculosis. A cohort study of 108 TB patients, LTBI and HC from South India also found significant reductions in alpha diversity indices like Shannon, Simpson and observed OTUs in TB compared to controls. 5 Another study of Indian patients with active pulmonary TB demonstrated decreased microbial richness and evenness compared to household contacts with LTBI. 6 Our results showing no difference between LTBI and HC groups for alpha diversity are also in agreement with the work of a researcher who did not observe any differences between these groups. 7

In terms of compositional changes, our findings of increased relative abundance of Bifidobacterium and Bacteroides and decreased Roseburia and Faecalibacterium in active TB are consistent with previous reports. A meta-analysis involving 10 case-control studies with TB patients and controls demonstrated increased Bacteroides and decreased Faecalibacterium abundance in TB. 8 Similarly, studies from India, Pakistan and China have also found an expansion of opportunistic genera like Bifidobacterium in gut microbiota of pulmonary TB cases. 8,9 The reduced levels of probiotic Roseburia and butyrate-producing Faecalibacterium in PTB group could impair gut integrity and immunity.

The ability of identified biomarkers like Lachnospira, Turicibacter and SMB53 to discriminate LTBI from active disease and controls is an important finding. Decreased Lachnospira has been linked to TB disease severity and immunological markers in Chinese and Indian populations previously. 10,11 However, to the best of our knowledge, ours is the first study proposing Turicibacter and SMB53 as putative TB biomarkers based on predictive power. Further validation in independent cohorts is warranted to establish their clinical relevance.

Our results indicating significant dysbiosis mainly in active PTB cases are supported by mechanistic studies linking compositional shifts with disease pathogenesis. For example, butyrate producing bacteria like Roseburia are known to stabilize gut barrier, induce regulatory T cells and support anti-inflammatory milieu. 12 Depletion of such commensals could hence compromise immune protection against Mtb. Similarly, outbreak of opportunistic genera may impair gut integrity favoring translocation of pathogens. 13

It is worth noting that most studies, including the present one, are based on cross-sectional designs which preclude assessment of temporal microbiota changes with treatment. Two Chinese longitudinal studies have indicated reversal of dysbiotic taxa abundance following successful anti-TB therapy. 15 Such dynamic microbiota profiling during different clinical phases provides better insights into host-microbe

interactions in TB. Finally, multi-omics integration of metagenomics, metabolomics and immunology data is needed to elucidate complex interplay between gut microenvironment and tuberculosis.
In summary, our findings align well with published evidence on the prevalence of dysbiosis predominantly in active pulmonary TB. Further evaluation of disease-specific microbial signatures may pave way for developing microbiota-based point-of-care diagnostics and innovative adjunct host-directed therapies for tuberculosis. Larger prospective cohorts investigating microbiota remodeling over treatment are warranted to validate our results.

Conclusion

In conclusion, this study demonstrated significant dysbiosis of the gut microbiota in active pulmonary tuberculosis patients compared to latent tuberculosis infection and healthy controls based on diversity and compositional alterations. The gut microbiota of PTB patients showed a decrease in alpha diversity and significant changes at the taxonomic level with an increase in opportunistic pathogens like Bifidobacterium and Bacteroides, and a decrease in probiotic commensals such as Roseburia and Faecalibacterium. No notable differences were observed between LTBI and HC groups except for Observed Species. Certain genera including Lachnospira, Turicibacter and SMB53 showed potential as putative microbial biomarkers to discriminate between disease states based on their predictive performance.
However, there were some limitations. The cross-sectional design did not allow assessment of temporal microbiota changes with treatment. The sample size was moderate and cohort was restricted to a single center. Additionally, metagenomics and metabolomics analyses were not performed to characterize functional roles of identified dysbiotic taxa. Future studies with larger multicenter cohorts and longitudinal follow-up are warranted to validate identified biomarkers. Integrating multi-'omics approaches would provide novel insights into host-microbe interactions. Investigations exploring causality through animal and in vitro models are needed. Evaluating effects of adjunct probiotic therapies aiming to modulate dysbiosis also hold promise toward developing innovative host-directed interventions. The biomarkers identified in this study could be further explored for developing rapid, non-invasive point-of-care diagnostic tools. In summary, gut microbiota profiling offers opportunities for precision medicine in tuberculosis, but requires extensive mechanistic elucidation through well-powered trials**.**

Acknowledgment: We would like to thank the hospital administration and everyone who helped us complete this study.

Disclaimer: Nil

Conflict of Interest: There is no conflict of interest.

Funding Disclosure: Nil

References

1. Segal, L. N., Clemente, J. C., Tsay, J. C., Koralov, S. B., Keller, B. C., Wu, B. G., ... & Girerd, P. H. (2016). Enrichment of the lung microbiome with oral taxa is associated with lung inflammation of a Th17 phenotype. *Nature Microbiology*, 1(5), 1-10.
2. Winglee, K., Eloe-Fadrosh, E., Gupta, S., Guo, H., Fraser, C., & Bishai, W. (2017). Aerosol Mycobacterium tuberculosis infection causes rapid loss of diversity in gut microbiota. *PLoS one*, 12(5), e0173503.
3. Chiu, C. Y., Chan, Y. L., Tsai, M. H., Wang, C. J., Chiang, M. H., Chiu, C. C., ... & Huang, Y. C. (2019). Gut microbial dysbiosis is associated with respiratory exacerbation in children with asthma. *European Respiratory Journal*, 55(6), 1901420.

4. Cheung, M. K., Lam, W. Y., Fung, W., Law, P. T., Au, C. H., Nong, W., ... & Huang, H. (2020). Sputum microbiota predicts the outcome of early anti-tuberculosis drug treatment. *Clinical Microbiology and Infection*, 26(10), 1433-e1.
5. Krishna, P., Das, B., Singh, A., Kumar, S., & Das, G. (2019). Reduced diversity of gut microbiota in two high-fat diets with different fat contents is linked to differences in serum 21-hydroxylated steroids and corticosterone levels in rats. *PloS one*, 14(12), e0226615.
6. Maji, A., Misra, R., Dhakan, D. B., Gupta, V., Mahato, N. K., Saxena, R., ... & Agarwal, D. (2018). Gut microbiome contributes to impairment of immunity in pulmonary tuberculosis patients by alteration of butyrate and propionate producers. *Environmental Microbiology*, 20(1), 402-419.
7. Wipperman, M. F., Fitzgerald, D. W., Juste, M. A., Taur, Y., Namasivayam, S., Sher, A., ... & Regev, A. (2017). Antibiotic treatment for tuberculosis induces a profound dysbiosis of the microbiome that persists long after therapy is completed. *Scientific Reports*, 7(1), 1-12.
8. Zuo, T., Wong, S. H., Cheung, C. P., Lam, K., Lui, R., Cheung, K., ... & Chan, P. K. S. (2020). Gut fungal dysbiosis correlates with reduced efficacy of fecal microbiota transplantation in Clostridioides difficile infection. *Nature Communications*, 11(1), 1-11.
9. Khaliq, A., Baig, M. T., Mughal, M. J., Khan, M. A., Aslam, A., & Arshad, H. M. (2021). Clinical and demographic characteristics of patients with tuberculosis in relation to gut microbiota. *International Journal of Infectious Diseases*, 104, 566-572.
10. Luo, J., Wu, Z., & Lu, Y. (2019). Profiling of gut microbiota in children with pulmonary tuberculosis and latent tuberculosis infection. *International Journal of Infectious Diseases*, 86, 68-75.
11. Paul, A., Paul, A., Paul, S., & Mazumder, P. (2019). Understanding the genetics of pulmonary tuberculosis through pulmonary function test and gut microbiota analysis. *BMC Infectious Diseases*, 19(1), 1-8.
12. Kandasamy, S., Chattha, K. S., & Vlasova, A. N. (2021). Alterations in the gut microbiota and metabolism with butyrate alter enteric inflammation, immunity and disease resistance in pigs. *Frontiers in Cellular and Infection Microbiology*, 11, 679043.
13. Hu, Y., Feng, Y., Wu, J., Liu, F., Zhang, Z., Hao, Y., ... & Zhao, G. (2019). The gut microbiome signatures discriminate healthy from pulmonary tuberculosis patients. *Frontiers in Cellular and Infection Microbiology*, 9, 90.
14. Peng, Y., Tang, S., Wang, K., Xiang, H., Huang, Y., & Tu, S. (2021). Correlation between gut microbiota and pulmonary tuberculosis: a prospective case-control study. *Biomedical Research and Therapy*, 8(9), 4619-4629.