



Gut Microbiome in Probable Intestinal Tuberculosis and Changes following Anti-Tuberculosis Treatment

Hyuk Yoon^{1,2}, Young Soo Park¹, Cheol Min Shin^{1,2}, Nayoung Kim^{1,2}, and Dong Ho Lee^{1,2}

¹Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam;

²Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, Seoul, Korea.

Purpose: Information on the gut microbiome in patients with intestinal tuberculosis (ITB) and changes therein following anti-tuberculosis treatment (ATT) is lacking. We aimed to elucidate differences in stool microbiome between ITB patients and controls and to evaluate stool microbiome changes after ATT.

Materials and Methods: Eleven patients with probable ITB underwent ATT for 6 months, with stool samples collected at 0, 2, and 6 months. We performed next-generation sequencing of 16S rRNA genes in stool bacteria and compared the gut microbiome.

Results: Initially, the relative abundance of *Verrucomicrobia* was higher (5.0% vs. <1%) and that of *Proteobacteria* was lower (<1% vs. 6.6%) in ITB patients than in controls. Higher numbers of butyrate-producing bacteria (*Blautia* and *Roseburia*) were noted in ITB patients. The alpha-diversity of stool microbiome of ITB patients was lower than that in controls ($p=0.045$). There was a significant difference in beta-diversity between the groups ($p=0.001$). At 6 months, the proportion of *Verrucomicrobia* decreased to <1%, while the proportion of *Proteobacteria* remained at <1%.

Conclusion: There were no significant differences in alpha- and beta-diversity in the stool microbiome at 0, 2, and 6 months after ATT. The stool microbiome composition of probable ITB patients was different from that of controls, and 6 months of ATT did not significantly affect it.

Key Words: Drug therapy, gastrointestinal tuberculosis, microbiota

INTRODUCTION

Mycobacterium tuberculosis (*M. tuberculosis*) infection is one of the most common infections in humans worldwide.¹ Although it usually presents as pulmonary tuberculosis, it can also involve the gastrointestinal tract. Intestinal tuberculosis usually occurs in immune-suppressed persons, such as patients with a human immunodeficiency virus infection. However, it can also develop in healthy people, especially in countries where

M. tuberculosis infection is still common. In intermediately developed countries, differentiating it from Crohn's disease is sometimes challenging.²

With the development of next-generation sequencing technology, many studies have evaluated the characteristics of the gut microbiome in various diseases. In the field of *M. tuberculosis* infections, a few studies have recently reported analyses of stool microbiome in patients with pulmonary tuberculosis and any changes after anti-tuberculosis treatment.^{1,3,4} Hu, et al.⁵ reported that pulmonary tuberculosis and anti-tuberculosis treatment cause a distinct dysbiosis of the gut microbiome. Maji, et al.⁶ showed that some butyrate-producing bacteria were significantly enriched in patients with pulmonary tuberculosis. However, little is known about the composition of the gut microbiome in patients with intestinal tuberculosis and its change following anti-tuberculosis treatment.

Therefore, we aimed to elucidate the differences in stool microbiome between patients with intestinal tuberculosis and healthy controls. In addition, we aimed to evaluate changes in the stool microbiome after anti-tuberculosis treatment.

Received: August 12, 2021 **Revised:** September 25, 2021

Accepted: October 1, 2021

Corresponding author: Young Soo Park, MD, MS, Department of Internal Medicine, Seoul National University Bundang Hospital, 82 Gumi-ro 173beon-gil, Bundang-gu, Seongnam 13620, Korea.

Tel: 82-31-787-7009, Fax: 82-31-787-4051, E-mail: dkree@snuh.org

•The authors have no potential conflicts of interest to disclose.

© Copyright: Yonsei University College of Medicine 2022

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<https://creativecommons.org/licenses/by-nc/4.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

MATERIALS AND METHODS

Subjects

Consecutive patients suspected of having intestinal tuberculosis who were referred to our clinic were prospectively enrolled. Inclusion criteria were as follows: 1) patients whose colonoscopic findings suggested intestinal tuberculosis, but a definite diagnosis could not be made because none of the following criteria were positive, including caseating granuloma on mucosal biopsy, tissue acid-fast bacilli staining, and tissue culture of *M. tuberculosis*;⁷ 2) patients who did not receive any treatment for intestinal tuberculosis; and 3) patients who were at least 18 years old. Patients who were definitively diagnosed with Crohn's disease after careful review, and patients who had taken any antibiotics, probiotics, or non-steroidal anti-inflammatory drugs within 3 months before enrollment were excluded. All patients provided informed consent, and the study was approved by the Institutional Review Board of Seoul National University Bundang Hospital (B-1608-359-301).

For each patient, clinical data, including endoscopic findings and histologic results, were collected using a standardized case report form. An additional colonoscopy was performed in cases where the image quality of the endoscopic pictures was poor, such that adequate evaluation could not be performed. Baseline stool samples were collected at least 2 weeks after performing the colonoscopy procedures. We used a uniform protocol for stool collection, wherein a research nurse educated patients on how to collect their stool and submit it. Specifically, the day before patients were supposed to visit the outpatient clinic, they were instructed to collect 5 grams of stool sample in a small plastic container and to immediately freeze it at -20°C in a freezer. On the day of the hospital visit, they were instructed to bring the sample in a bag with an ice pack, which was provided at enrollment.

Intestinal tuberculosis is no longer a common disease in South Korea. Therefore, we expected that it would not be easy to enroll many patients in this study. Given that roughly one patient per month is referred to our clinic for suspicion of intestinal tuberculosis and that the minimum number of samples suitable for non-parametric analysis is about 20–30 samples, we determined the sample size of our study group to be 20 patients. Data of age- and sex-matched healthy subjects at a ratio of 1:1 were randomly extracted from the Korean gut microbiome bank study (B-1701-380-304), whose purpose is to collect stool samples from healthy Korean adults and analyze their gut microbiome. Their microbiome data were used as controls in this study.

Anti-tuberculosis treatment and follow-up

Anti-tuberculosis medication (isoniazid 300 mg/d, rifampicin 600 mg/d, ethambutol 800 mg/d, and pyrazinamide 1500 mg/d) was administered to patients for 2 months. Compliance was checked by a physician at each visit to the clinic. Afterward,

we collected follow-up stool samples and performed an endoscopy. Patients who showed endoscopic healing were tentatively diagnosed with intestinal tuberculosis and continued anti-tuberculosis medication for another 4 months, with the exception of pyrazinamide.⁸ The final stool samples were collected at the end of the treatment. The final diagnosis of probable intestinal tuberculosis was made when a patient fulfilled the criteria of clinical and endoscopic remission after empiric anti-tuberculosis treatment with the following findings: characteristic colonoscopy findings, suspected histology of tuberculosis, positive polymerase chain reaction (PCR) test results for *M. tuberculosis* DNA in tissue samples, positive interferon gamma release assay, chest X-ray finding suggestive of active or inactive tuberculosis, and previous history of tuberculosis.⁷

Microbiome analysis

Total DNA was extracted from stool samples, and PCR amplification was performed for the V3–V4 regions of the 16S rRNA gene. Sequencing was performed using an Illumina MiSeq Sequencing system (Illumina, San Diego, CA, USA). Basic microbiome analyses were conducted according to previously described procedures.^{9–11} Since the 16S rRNA copy number varies greatly among bacteria, the abundance of operational taxonomic units was normalized to a read count of 10000. For analysis of alpha-diversity, the Simpson diversity index was calculated. For analysis of beta-diversity, the overall phylogenetic distance between communities was estimated and visualized using Jensen–Shannon-based principal coordinates analysis. PERMANOVA was used for evaluating set differences between groups. We performed Linear discriminant analysis effect size (LEfSe) was conducted to determine the features that most likely explained the differences between groups by coupling standard tests for statistical significance with additional tests encoding biological consistency and effect relevance.¹² All microbiome analyses were performed using non-parametric methods, and results were considered statistically significant when *p* values were <0.05.

RESULTS

Baseline characteristics of the patients with intestinal tuberculosis

The schematic flow of the study is outlined in Fig. 1. Between October 2016 and December 2018, 20 patients were enrolled. After the initial evaluation, three patients definitively diagnosed with Crohn's disease were excluded, and the remaining 17 were administered anti-tuberculosis drugs. Among 15 patients who underwent follow-up colonoscopy after 2 months, three patients did not show endoscopic improvement. Given the fact that multi-drug resistant tuberculosis in extrapulmonary tuberculosis is very low, these patients were classified as isolated terminal ileitis,¹³ and anti-tuberculosis treatment was

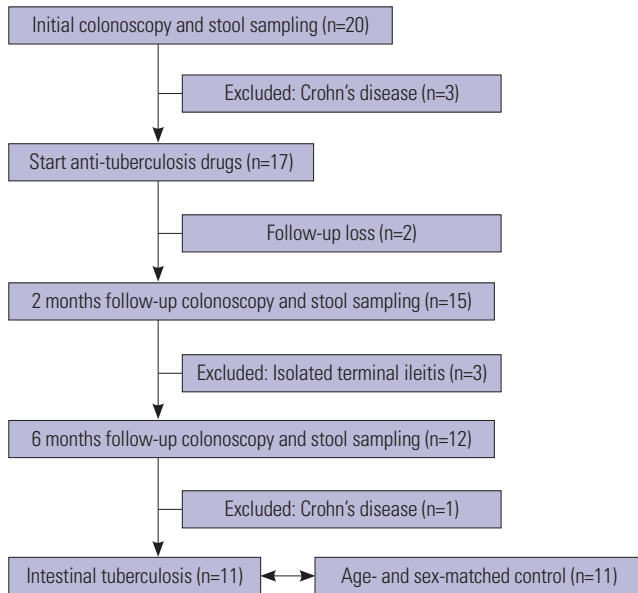


Fig. 1. Schematic flow of the study.

stopped. Twelve patients completed anti-tuberculosis treatment for 6 months. In one patient, the final diagnosis was changed to Crohn’s disease since mucosal healing was not achieved at 6 months upon follow-up endoscopy. Other information, including cross-sectional images, also contributed to the change of diagnosis. Finally, 11 patients were confirmed as having probable intestinal tuberculosis, and their stool microbiomes were compared with that of age- and sex- matched controls.

The clinical and endoscopic characteristics of patients with intestinal tuberculosis are presented in Table 1. The median age was 47 years, and 55% patients were asymptomatic. Twenty-seven percent of the patients showed inactive tuberculosis on chest X-ray, and 91% were seropositive by the interferon gamma release assay. Fecal calprotectin levels were a median of 55 mcg/g (range 29–308) at baseline and decreased during and after treatment to a median of 19.3 mcg/g (range 11.5–51.4) at 2 months and a median of 18.5 mcg/g (range 11.5–129.7) at 6 months. Regarding endoscopic findings, the most commonly involved segment was the ascending colon, including the cecum. All patients had ileocecal involvement. In contrast, the left colon or rectum was not involved in any of the patients. Transverse ulcers, which are characteristic findings of intestinal tuberculosis, were found in 45% of patients. Granuloma was found in 36%, but without accompanying caseous necrosis. PCR for *M. tuberculosis* DNA in tissue samples was positive in 18% of cases.

Comparison of stool microbiomes between patients with intestinal tuberculosis and healthy controls

At enrollment, the relative abundances of the phyla *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* were 62.4%, 22.5%, and 9.1%, respectively (Fig. 2). The relative abundance of *Verrucomicrobia* was higher (5.0% vs. <1%) and that of *Proteobacteria* was

Table 1. Baseline Characteristics of the Patients with Intestinal Tuberculosis

Variable	Value
Sex, male	6 (55)
Age (yr), median (range)	47 (30–65)
Symptom	
None	6 (55)
Hematochezia	2 (18)
Urgency for defecation	1 (9)
Constipation	1 (9)
Weight loss and anorexia	1 (9)
Involved segments on endoscopy*	
Terminal ileum	4 (36)
Ascending colon (including cecum)	7 (64)
Transverse colon	2 (18)
Transverse ulcers on endoscopy	5 (45)
Patulous ileocecal valve on endoscopy	2 (18)
Scar or pseudopolyps on endoscopy	6 (55)
Non-caseating granuloma on histology	4 (36)
Positive PCR for <i>Mycobacteria tuberculosis</i> in tissue samples	2 (18)
History of pulmonary tuberculosis	1 (9)
Inactive tuberculosis on chest X-ray scan	3 (27)
Positive interferon gamma release assay in serum	10 (91)
Positive anti- <i>Saccharomyces cerevisiae</i> antibody in serum	1 (9)
Fecal calprotectin (microgram/g), median (range)	55 (29–308)

PCR, polymerase chain reaction.

Data are presented as n (%).

*Each segment was independently calculated.

lower (<1% vs. 6.6%) in patients with intestinal tuberculosis than in controls. *M. tuberculosis* was not found in any sample. The alpha-diversity of stool microbiomes in patients with intestinal tuberculosis was lower than that in controls (Simpson index: $p=0.045$) (Fig. 3A). There was a significant difference in the beta-diversity of species between patients with intestinal tuberculosis and controls (PERMANOVA: $p=0.001$) (Fig. 3B). LEfSe analysis showed that the abundance of the *Blautia* and *Roseburia* genus was significantly increased in patients with intestinal tuberculosis (Fig. 4A). Cladogram data extracted from LEfSe analysis showed a relatively lower abundance of the *Megasphaera* genus, which was the main determinant differentiating patients with intestinal tuberculosis from controls (Fig. 4B).

Changes in stool microbiome in patients with intestinal tuberculosis after anti-tuberculosis treatment

Six months after starting anti-tuberculosis treatment, the relative abundances of the phyla *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* were 58.4%, 27.3%, and 12.4%, respectively (Fig. 5). The proportion of *Verrucomicrobia* decreased to <1%, while the proportion of *Proteobacteria* was still <1%. There were no significant differences in alpha- and beta-diversity among stool microbiomes at 0, 2, and 6 months after anti-tuberculosis treatment (Fig. 6).



Fig. 2. Taxonomic composition of stool microbiome of patients with intestinal tuberculosis and age- and sex-matched healthy controls (outer circle: genus level, inner circle: phylum level).

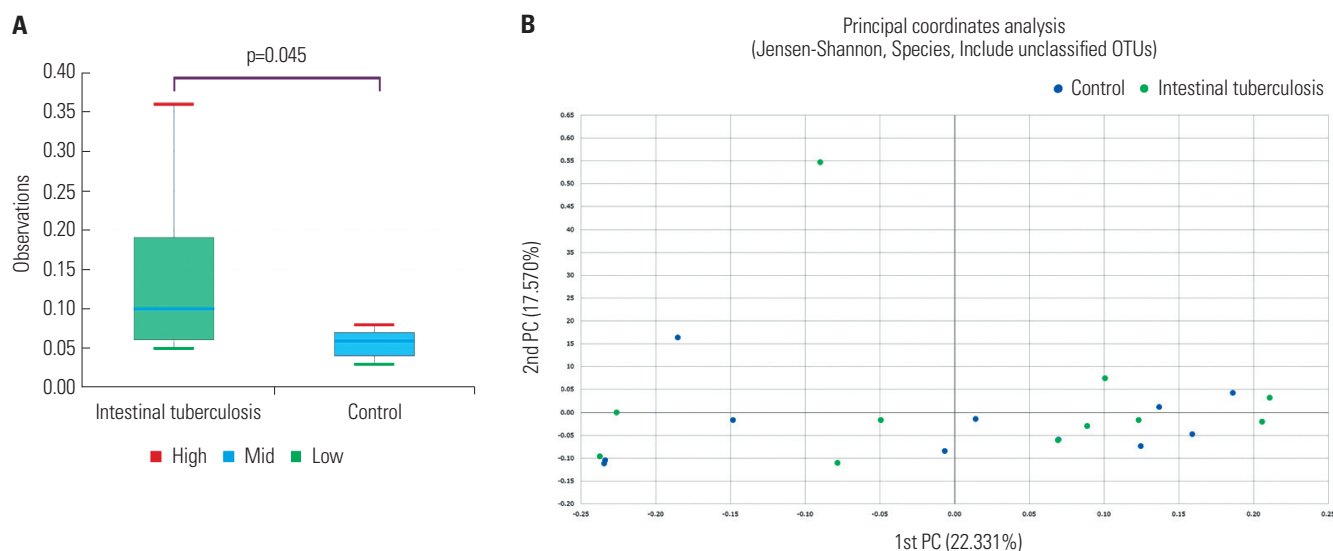


Fig. 3. Alpha- and beta-diversity of stool microbiome of patients with intestinal tuberculosis and age- and sex-matched healthy controls. (A) Simpson index. (B) Jensen-Shannon-based principal coordinates analysis at the species level. OTU, operational taxonomic unit; PC, principal component.

DISCUSSION

In this study, we found that the alpha-diversity of stool microbiomes in patients with intestinal tuberculosis decreased relative to that in healthy controls. The beta-diversity of the stool microbiomes also differed from that in controls. The decreased diversity of the stool microbiomes in patients with intestinal tuberculosis corresponds with results in previous studies performed in animal models and patients with pulmonary tuberculosis.^{5,14,15} The fact that the relative abundances of *Blautia* and *Roseburia*, which belong to the family *Lachnospiraceae* and are typical butyrate-producing bacteria, were higher in patients

with intestinal tuberculosis is especially interesting. A few studies have reported that butyrate-producing bacteria are increased in patients with pulmonary tuberculosis.^{6,16} Butyrate is known to induce the anti-inflammatory cytokine interleukin-10 and to activate regulatory T cells.^{17,18} In fact, butyrate increases the production of interleukin-10 and decreases *M. tuberculosis*-induced proinflammatory cytokine responses.¹⁹ These findings suggest that crosstalk between microbiota and the mucosal immune system is modified by an *M. tuberculosis* infection and that butyrate might play a role in the pathogenesis of pulmonary and intestinal tuberculosis.

In contrary to patients with intestinal tuberculosis in our

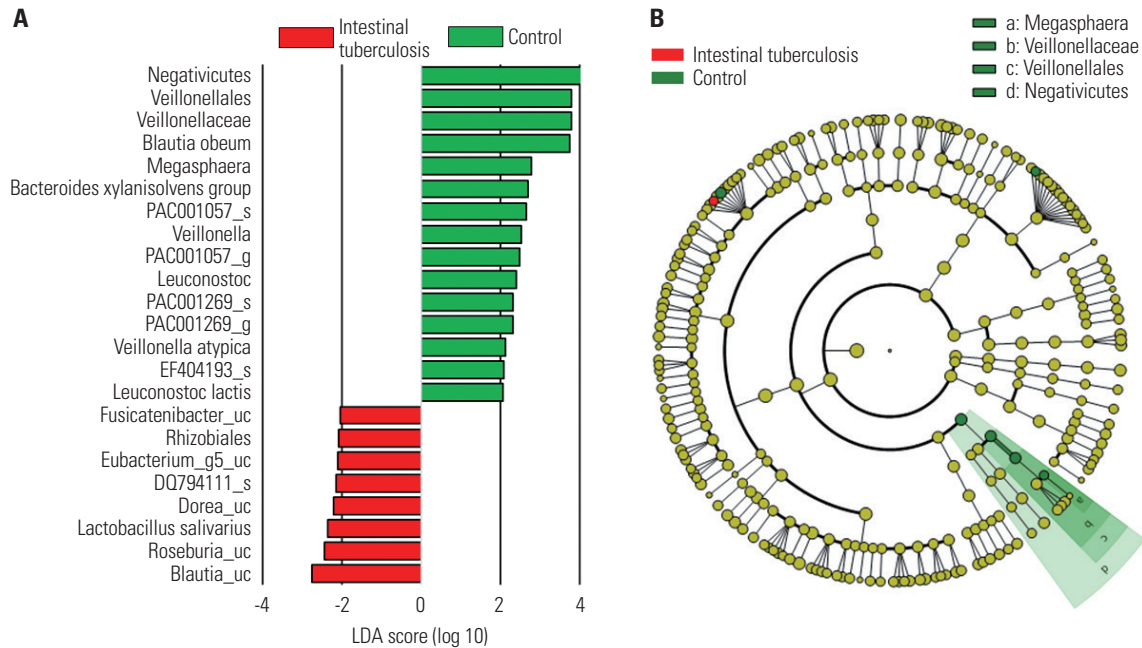


Fig. 4. (A) Linear discriminant analysis effect size and (B) cladogram of the stool microbiome of patients with intestinal tuberculosis and age- and sex-matched healthy controls. LDA, linear discriminant analysis.

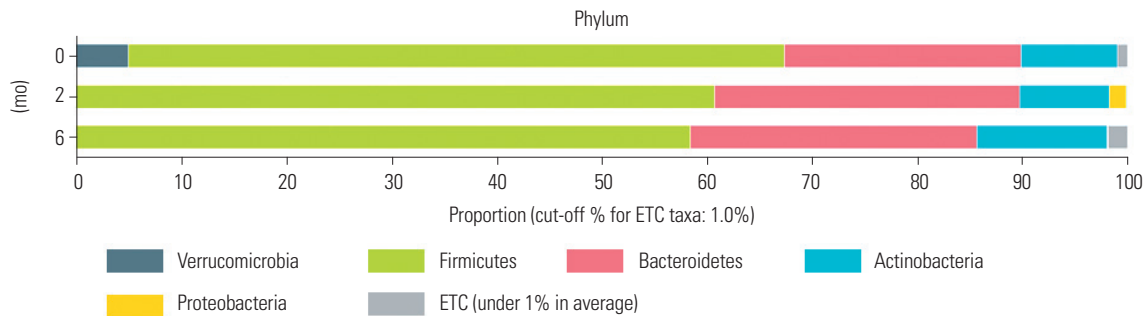


Fig. 5. Taxonomic composition of stool microbiome of patients with intestinal tuberculosis at enrollment, 2 months, and 6 months after anti-tuberculosis medication.

study, patients with Crohn’s disease are known exhibit fewer butyrate-producing bacteria.²⁰⁻²² Although we could not directly compare gut microbiomes between patients with intestinal tuberculosis and those with Crohn’s disease, we expect that analysis of butyrate-producing bacteria in stool samples could help differentiate intestinal tuberculosis from Crohn’s disease in difficult cases, before trying empirical anti-tuberculosis treatments. In contrast, the genus *Megasphaera*, which decreased in patients with intestinal tuberculosis, is a type of lactic acid-producing bacteria. This bacterium is usually reported as being related to bacterial vaginosis,^{23,24} and we could not find any reports on its association with tuberculosis. Therefore, further studies are required to elucidate the link between *Megasphaera* and the pathogenesis of intestinal tuberculosis.

In this study, the unique bacterial composition of patients with intestinal tuberculosis was not significantly affected by anti-tuberculosis treatment. Although mucosal healing was

achieved after treatment, initial dysbiosis was neither aggravated nor recovered, and this was represented as lower alpha-diversity than that in controls. The use of anti-tuberculosis drugs has caused concern about gut dysbiosis since it includes rifampin, which is active against a broad spectrum of both gram-positive and gram-negative bacteria.²⁵ However, there are contradictory reports regarding changes in the gut microbiome after anti-tuberculosis treatment in patients with pulmonary tuberculosis. Hu, et al.⁵ reported that anti-tuberculosis treatment causes dysbiosis, while Wipperman, et al.²⁶ reported that this treatment appeared to have little overall effect on bacterial diversity in the gut. Since the intestine itself is the infection site in intestinal tuberculosis, translating the results of gut microbiome analysis after anti-tuberculosis treatment in this form of tuberculosis is more complicated than that in pulmonary tuberculosis. Dysbiosis induced by antibiotics could be offset by decreasing inflammation after the resolution of

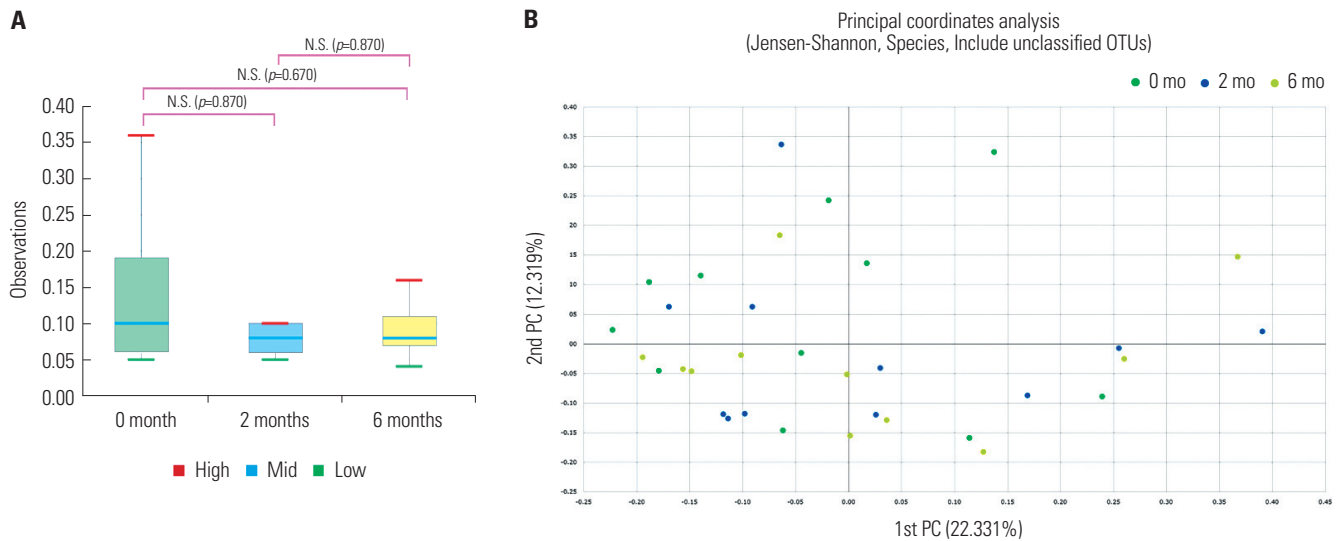


Fig. 6. Alpha- and beta-diversity of stool microbiome of patients with intestinal tuberculosis at enrollment, 2 months, and 6 months after anti-tuberculosis medication. (A) Simpson index. (B) Jensen-Shannon-based principal coordinates analysis at the species level. OTU, operational taxonomic unit; PC, principal component.

tuberculosis. Our study suggests that despite 6 months of anti-antibiotic treatment, gut dysbiosis is not severe in patients with intestinal tuberculosis. This could partly explain why the incidence of *Clostridioides difficile* infection is exceptionally low (0.3%) among patients who take anti-tuberculosis drugs.²⁷ Long-term follow-up studies are required to evaluate the final effects of anti-tuberculosis treatment on the gut microbiome in patients with intestinal tuberculosis.

The proportion of *Verrucomicrobia*, which was higher in patients with intestinal tuberculosis than in controls, decreased after anti-tuberculosis treatment. However, *Akkermansia muciniphila* (*A. muciniphila*), which is the only genus from the phylum *Verrucomicrobia* in the intestinal microbiota, was not a significant determinant in LEfSe analysis. Therefore, we were unable to determine the significance of the changes in *Verrucomicrobia*. Studies regarding the relationship between *A. muciniphila* and *M. tuberculosis* are very scarce. Only one study has reported that unfavorable lipid profiles after long-term treatment of multidrug-resistant tuberculosis was negatively associated with *Verrucomicrobia* and *A. muciniphila*.²⁸

Our study has several strengths. First, to the best of our knowledge, this is the first study to have evaluated the gut microbiome in patients with intestinal tuberculosis. Although a few mechanisms have been suggested,^{29,30} the detailed pathogenesis of intestinal tuberculosis is still unclear. Microbial analysis using next-generation sequencing technology will shed some light on this. Second, we prospectively enrolled the study patients and serially collected their stool for up to 6 months.

However, our study also has some limitations. First, the patients included in the final analysis comprised those diagnosed with probable intestinal tuberculosis. Among patients with suspected intestinal tuberculosis, the proportion that is definitively diagnosed is low.³⁰ Thus, empirical anti-tuberculo-

sis treatment is performed in most of these patients; this was the impetus for our concerns. Therefore, we set out to evaluate stool microbiome in these patients and designed our study to exclude patients with a definite diagnosis from the start. To compensate for this, every component of the criteria for probable intestinal tuberculosis was carefully reviewed in each patient. The interferon gamma release assay was seropositive in more than 90% of the patients, and endoscopic findings were also indicative for intestinal tuberculosis. Mucosal healing after anti-tuberculosis treatment was confirmed in all patients. Second, the small sample size is another limitation of our study. Intestinal tuberculosis is not a common disease, and it was not easy to persuade patients to try empirical antibiotics first and to collect stool samples serially. Therefore, it was difficult to enroll patients despite our institution being a tertiary hospital. Due to the small sample size, we could not perform microbiome analysis according to the endoscopic findings of the patients. In the future, multicenter research would be optimal to increase the sample size. Third, we could not collect long-term follow-up stool samples after completing anti-tuberculosis treatment for the same reasons as mentioned above. Lastly, we could not evaluate immunologic alterations related to tuberculosis infection and resolution. Our results suggest butyrate might play a role in the immune response and regulation of the pathogenesis of intestinal tuberculosis. However, because we did not measure metabolites or cytokines, such as interleukin-10, we could not prove this hypothesis. Further studies are needed to confirm our findings.

In conclusion, the composition of the stool microbiome in patients with probable intestinal tuberculosis differs from that of healthy controls; however, anti-tuberculosis treatment over 6 months did not appear to significantly change the stool microbiome.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author. The data are not publicly available since the original study from which the data of the control group were extracted is still ongoing.

ACKNOWLEDGEMENTS

This research was funded by the Seoul National University Bundang Hospital Research Fund, grant number 02-2016-043, and by the Bio & Medical Technology Development program of the National Research Foundation of Korea (NRF), funded by the Ministry of Science and ICT (MIST) of the Republic of Korea (Project No. 2016M3A9F3947027).

AUTHOR CONTRIBUTIONS

Conceptualization: Hyuk Yoon and Young Soo Park. **Data curation:** Hyuk Yoon and Young Soo Park. **Formal analysis:** Hyuk Yoon. **Funding acquisition:** Hyuk Yoon and Dong Ho Lee. **Investigation:** Hyuk Yoon. **Methodology:** Hyuk Yoon. **Project administration:** Young Soo Park. **Resources:** Hyuk Yoon and Young Soo Park. **Software:** Hyuk Yoon. **Supervision:** Young Soo Park. **Validation:** Hyuk Yoon. **Visualization:** Hyuk Yoon. **Writing—original draft:** Hyuk Yoon. **Writing—review & editing:** Young Soo Park, Cheol Min Shin, Nayoung Kim, and Dong Ho Lee. **Approval of final manuscript:** all authors.

ORCID iDs

Hyuk Yoon <https://orcid.org/0000-0002-2657-0349>
 Young Soo Park <https://orcid.org/0000-0003-1893-7726>
 Cheol Min Shin <https://orcid.org/0000-0003-2265-9845>
 Nayoung Kim <https://orcid.org/0000-0002-9397-0406>
 Dong Ho Lee <https://orcid.org/0000-0002-6376-410X>

REFERENCES

- Naidoo CC, Nyawo GR, Wu BG, Walzl G, Warren RM, Segal LN, et al. The microbiome and tuberculosis: state of the art, potential applications, and defining the clinical research agenda. *Lancet Respir Med* 2019;7:892-906.
- Rampal R, Kedia S, Wari MN, Madhu D, Singh AK, Tiwari V, et al. Prospective validation of CD4+CD25+FOXP3+ T-regulatory cells as an immunological marker to differentiate intestinal tuberculosis from Crohn's disease. *Intest Res* 2021;19:232-8.
- Namasivayam S, Sher A, Glickman MS, Wiperman ME. The microbiome and tuberculosis: early evidence for cross talk. *mBio* 2018; 9:e01420-18.
- Eribo OA, du Plessis N, Ozturk M, Guler R, Walzl G, Chegou NN. The gut microbiome in tuberculosis susceptibility and treatment response: guilty or not guilty? *Cell Mol Life Sci* 2019;77:1497-509.
- Hu Y, Yang Q, Liu B, Dong J, Sun L, Zhu Y, et al. Gut microbiota associated with pulmonary tuberculosis and dysbiosis caused by anti-tuberculosis drugs. *J Infect* 2019;78:317-22.
- Maji A, Misra R, Dhakan DB, Gupta V, Mahato NK, Saxena R, et al. Gut microbiome contributes to impairment of immunity in pulmonary tuberculosis patients by alteration of butyrate and propionate producers. *Environ Microbiol* 2018;20:402-19.
- Marshall JB. Tuberculosis of the gastrointestinal tract and peritoneum. *Am J Gastroenterol* 1993;88:989-99.
- Park SH, Yang SK, Yang DH, Kim KJ, Yoon SM, Choe JW, et al. Prospective randomized trial of six-month versus nine-month therapy for intestinal tuberculosis. *Antimicrob Agents Chemother* 2009;53: 4167-71.
- Chun J, Kim KY, Lee JH, Choi Y. The analysis of oral microbial communities of wild-type and toll-like receptor 2-deficient mice using a 454 GS FLX Titanium pyrosequencer. *BMC Microbiol* 2010;10:101.
- Hur M, Kim Y, Song HR, Kim JM, Choi YI, Yi H. Effect of genetically modified poplars on soil microbial communities during the phytoremediation of waste mine tailings. *Appl Environ Microbiol* 2011;77:7611-9.
- Kim BS, Kim JN, Yoon SH, Chun J, Cerniglia CE. Impact of enrofloxacin on the human intestinal microbiota revealed by comparative molecular analysis. *Anaerobe* 2012;18:310-20.
- Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. *Genome Biol* 2011;12:R60.
- Chang HS, Lee D, Kim JC, Song HK, Lee HJ, Chung EJ, et al. Isolated terminal ileal ulcerations in asymptomatic individuals: natural course and clinical significance. *Gastrointest Endosc* 2010;72:1226-32.
- Winglee K, Eloe-Fadrosh E, Gupta S, Guo H, Fraser C, Bishai W. Aerosol Mycobacterium tuberculosis infection causes rapid loss of diversity in gut microbiota. *PLoS One* 2014;9:e97048.
- Hu Y, Feng Y, Wu J, Liu F, Zhang Z, Hao Y, et al. The gut microbiome signatures discriminate healthy from pulmonary tuberculosis patients. *Front Cell Infect Microbiol* 2019;9:90.
- Luo M, Liu Y, Wu P, Luo DX, Sun Q, Zheng H, et al. Alternation of gut microbiota in patients with pulmonary tuberculosis. *Front Physiol* 2017;8:822.
- Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 2013;504:446-50.
- Silva JPB, Navegantes-Lima KC, Oliveira ALB, Rodrigues DVS, Gaspar SLE, Monteiro VVS, et al. Protective mechanisms of butyrate on inflammatory bowel disease. *Curr Pharm Des* 2018;24:4154-66.
- Lachmandas E, van den Heuvel CN, Damen MS, Cleophas MC, Netea MG, van Crevel R. Diabetes mellitus and increased tuberculosis susceptibility: the role of short-chain fatty acids. *J Diabetes Res* 2016;2016:6014631.
- Takahashi K, Nishida A, Fujimoto T, Fujii M, Shioya M, Imaeda H, et al. Reduced abundance of butyrate-producing bacteria species in the fecal microbial community in Crohn's disease. *Digestion* 2016;93:59-65.
- Laserna-Mendieta EJ, Clooney AG, Carretero-Gomez JF, Moran C, Sheehan D, Nolan JA, et al. Determinants of reduced genetic capacity for butyrate synthesis by the gut microbiome in Crohn's disease and ulcerative colitis. *J Crohns Colitis* 2018;12:204-16.
- Wang Y, Gao X, Ghozlane A, Hu H, Li X, Xiao Y, et al. Characteristics of faecal microbiota in paediatric Crohn's disease and their dynamic changes during infliximab therapy. *J Crohns Colitis* 2018;12: 337-46.
- Fredricks DN, Plantinga A, Srinivasan S, Oot A, Wisner A, Fiedler TL, et al. Vaginal and extra-vaginal bacterial colonization and risk for incident bacterial vaginosis in a population of women who have sex with men. *J Infect Dis* 2020 May 7. [Epub]. Available at: <https://doi.org/10.1093/infdis/jiaa233>.
- Singh R, Ramsuran V, Mitchev N, Niehaus AJ, Han KSS, Osman F, et al. Assessing a diagnosis tool for bacterial vaginosis. *Eur J Clin*

- Microbiol Infect Dis 2020;39:1481-5.
25. Hong BY, Maulén NP, Adami AJ, Granados H, Balcells ME, Cervantes J. Microbiome changes during tuberculosis and antituberculous therapy. *Clin Microbiol Rev* 2016;29:915-26.
 26. Wipperman MF, Fitzgerald DW, Juste MAJ, Taur Y, Namasivayam S, Sher A, et al. Antibiotic treatment for tuberculosis induces a profound dysbiosis of the microbiome that persists long after therapy is completed. *Sci Rep* 2017;7:10767.
 27. Lee YM, Huh KC, Yoon SM, Jang BI, Shin JE, Koo HS, et al. Incidence and clinical outcomes of *Clostridium difficile* infection after treatment with tuberculosis medication. *Gut Liver* 2016;10:250-4.
 28. Wang J, Xiong K, Zhao S, Zhang C, Zhang J, Xu L, et al. Long-term effects of multi-drug-resistant tuberculosis treatment on gut microbiota and its health consequences. *Front Microbiol* 2020;11:53.
 29. Sharma MP, Bhatia V. Abdominal tuberculosis. *Indian J Med Res* 2004;120:305-15.
 30. Choi EH, Coyle WJ. Gastrointestinal tuberculosis. *Microbiol Spectr* 2016;4:4.6.21.