Original Article

Effect of moxibustion on diversity of intestinal flora in 46 patients with immune reconstitution insufficiency after antiretroviral therapy

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ABSTRACT

Background: To analyze the effects of moxibustion + antiretroviral therapy (ART) on the intestinal bacterial community structure of patients with human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS), and to provide new ideas and methods for improving immune reconstitution in patients through intervention of intestinal disorders and inhibition of immunity activation.

Methods: 16S rRNA high-throughput sequencing was used to compare and analyze the differences in intestinal bacterial composition between patients in an ART group and a moxibustion + ART group.

Results: The results of principal component analysis show that the intestinal flora distribution of gut microbiota in patients in the ART group and the moxibustion group was similar at baseline. After 48 weeks of treatment, the distribution locations and the phase limits of the samples in the ART group and the moxibustion group were significantly different, and compared with the ART group, the points represented by the moxibustion group after treatment and before treatment were far apart, indicating that the composition and structure of microorganisms in the moxibustion group after treatment and before treatment were significantly different. In the moxibustion group, the number of dominant bacteria elevates, which is very different from that after ART treatment only.

Conclusion: Moxibustion can improve the intestinal microbiological composition of HIV/AIDS patients and the composition of intestinal microbes in HIV/AIDS patients after moxibustion differs greatly from that after ART treatment only. Moxibustion can improve the intestinal microbiological composition and the dominant bacteria of HIV/acquired immunodeficiency syndrome (AIDS) patients.

Key words: human immunodeficiency virus/acquired immunodeficiency syndrome, moxibustion, immune reconstitution insufficiency, intestinal flora

INTRODUCTION

In recent years, intestinal flora has become a major topic in the field of clinical research. Increasing evidence has shown that changes in intestinal flora are closely related to the development of many diseases, including those affecting the immune system and metabolism of the host, which can, in turn, influence the host's health, physical fitness, personality, and even thinking. The inflammatory response markers of patients with human immunodeficiency virus (HIV) are elevated, and special gut microbiota regulate mucosal immune responses. The disturbance of...
the microbiota can lead to defective immune regulation, resulting in the production of more pathogenic microorganisms, which promote inflammatory responses. Research has shown that traditional Chinese medicine can increase the proportion of beneficial bacteria in the gut by improving the diversity of the intestinal flora, which plays an important role in alleviating clinical symptoms and treating diseases. Moxibustion, as a noninvasive external treatment based on traditional Chinese medicine, has a great effect on enhancing the body's immunity and alleviating clinical symptoms. However, the efficacy mechanism of moxibustion that takes part in regulating the structure of the intestinal flora in patients with immune reconstitution insufficiency after antiretroviral therapy (ART) is unclear. With the aim of providing a new method and pathway for improving patients' immune reconstitution through the intervention of intestinal disorders and the inhibition of immunity activation, 16S rRNA high-throughput sequencing was used in this study to compare and analyze the effects of ART alone versus ART + moxibustion on the composition of intestinal flora in patients with HIV/AIDS.

METHODS

Participants and methods
The participants were patients with HIV/AIDS who were treated from June 2019 to June 2020 at Beijing Ditan Hospital, which is affiliated with Capital Medical University and the First Affiliated Hospital of Henan University of Chinese Medicine, and who voluntarily participated and signed informed consent forms. The study was approved by the Ethics Committee of the Institute of Clinical Basic Medicine of Traditional Chinese Medicine at the China Academy of Traditional Chinese Medicine (No. 2018NO. 07).

Diagnostic criteria

Inclusion criteria
The criteria for inclusion in this study were patients who were aged 18–65, male or female, positive for HIV antibodies, diagnosed by a Western Blot confirmatory test, had undergone ART treatment for two years or more, and had a plasma HIV load < 50 copies/mL for more than a year and a half, with CD4+ T lymphocyte levels < 300 cells/μL or < 20% increased from treatment baseline. The patients also had to be willing to sign an informed consent form.

Exclusion criteria
Patients were excluded for the following reasons: had severe opportunistic infection; participated in clinical trials for other drugs within a month before the current study; had immunomodulation treatment within a month before the current study; had a white blood cell count < 2.0 × 10^9/L, N < 1.0 × 10^9/L, hemoglobin < 90.0 g/L, or platelet count < 75.0 × 10^9/L; had abnormal liver or kidney function (abnormal liver function indicates that aspartate transaminase (AST), alanine transaminase (ALT), or total bilirubin (T-BIL) ≥ 2 times the upper limit of the reference value, and abnormal kidney function indicates that the creatinine clearance rate is lower than normal); were women who were pregnant, breastfeeding, or preparing for pregnancy; had other severe diseases (e.g., cancer, cirrhosis, cardiovascular diseases, etc.); had an intellectual disability; or spoke a different language and could not understand the content of the trial very well and thus could not provide good cooperation. Those who satisfied the inclusion criteria but had one of the above conditions were excluded.

Methodology

Groups
Twenty cases were included in the ART group treated with ART, while 26 cases were included in the moxibustion group treated with ART combined with moxibustion.

Feces collection
Fecal samples were collected using the natural defecation method. Before defecation, to prevent urine from contaminating the feces, the patients were required to urinate, and the toilet bowl was cleaned. Then, the patients defecated in a waterless place, and about 2 g of feces were dug out, ensuring no exposure to air by using a sampling spoon and tightening the cap on the sample tube. Three tubes of samples were taken and preserved in a self-sealing bag. The self-sealing bag was placed in an icebox filled with ice or ice packs to create a low-temperature environment, which can prevent the development of bacteria. The samples were stored at -80 °C for freezing within 2 h.

Genomic DNA extraction and polymerase chain reaction (PCR)
The following tests were used to extract biological information: an adopt kit for genomic DNA extraction, PCR amplification of the V3-V4 variable region of the bacterial 16S rRNA gene based on the Illumina Hiseq platform for high-throughput sequencing, split joint sequencing in the form of reads, operational taxonomic units (OTUs) clustering, species annotation, alpha diversity and beta diversity analyses, and other tests.

Statistical analyses
Statistical Package for the Social Sciences (SPSS) version
22.0 statistical analysis software was used to analyze the data, and measures were expressed as x ± s. A t-test was used for those that conformed to a normal distribution. To test the comparison of the categorical counting indicators, χ² or Fisher's exact probability method was used. Hypotheses were tested using two-sided tests to obtain the test statistics and their corresponding P-values, with P < 0.05 as the criterion for significant difference.

Analysis of microorganism diversity
The raw data for sequencing were dehybridized and spliced. All high-quality sequences were classified into OTUs using Vsearch (version 2.4.2) based on 97% similarity, and the sequence with the largest abundance was selected to represent the OTU sequencing. The Ribosomal Database Project's classifier algorithm for Naive Bayesian classification was used to compare representative sequences and obtain the OTU annotation information. Based on the OTU data, beta diversity was analyzed by principal component analysis (PCA) to compare the differences in the community structure of the different subgroups. Species composition and community structure differences of the grouped samples were statistically tested by community structure composition analysis, distribution ratio analysis, and chord analysis. All statistical analyses of microbial diversity were performed using R language. Statistical tests for differences in species composition and community structure of grouped samples were performed using one-dimensional statistical analysis methods, such as a t-test. All statistical analyses for microbial diversity were performed using R language and SIMCAP software.

RESULTS
Stool samples were collected from 46 patients after meeting the inclusion criteria and screening for exclusion criteria. Twenty cases were included in the ART group, with a mean age of 48.58 ± 10.32 years, and 26 cases were included in the moxibustion group, with a mean age of 47.36 ± 12.15 years. No statistically significant difference was found in the ages of the patients in the two groups (P > 0.05).

The different fecal microbiota compositions between the ART and moxibustion groups
The PCA score plot reflected the differences in flora in each group at the genus level. These results showed that patients in the ART group and the moxibustion group had similar distributions in the score plots at enrollment in the study, indicating a similar baseline for fecal gut microbiological composition. After 48 weeks of treatment, the samples from patients in the ART and moxibustion groups had significantly different distribution locations and phase limit intervals in the PCA score plots, indicating that the compositions of the two groups' fecal microbiota had changed after treatment. Compared with the ART group after treatment, the spots in the moxibustion group after treatment had long distances. In other words, the compositions of the microbiota in the moxibustion group differed greatly before and after treatment (Figure 1).

Differences in fecal microbiota in the ART group before and after treatment
Changes in fecal microbiota in the ART group at the phylum level before and after treatment: The results of the community composition analysis indicated that, after 48 weeks of ART treatment, Latescibacteria (Staphylocoanc), Tenericutes (Soft-walled), Firmicutes (Thick-walled), and Nitrospirae (Nitrospirae) were significantly elevated, while Enothoneillaeota (Enterobacteriaceae), Planctomycetes (Planctomycetes), Cyanobacteria (Cyanobacteria), Bacteroides (Bacteriophages), and Preoteobacteria (Ascomycetes) were obviously decreased. The differences in the above flora were statistically significant at the phylum level (P < 0.05, Figure 2).

Changes in fecal microbiota in the ART group at the class level before and after treatment: The results of the community composition analysis indicated that, after 48 weeks of ART treatment, Mollicutes (Fleabacteria), Erysipelotrichia (Danubacteria), Clostridia (Clostridia), and Delta proteobacteri (Deltaproteobacteria) were obviously elevated, whereas Ktedonobacteria (Ciliobacteria), alphaproteobacteria (α-Ascomycetes), Bacterioda (Bacteriophages), gammaproteobacteria (γ-Ascomycetes), and Actinobacteria were obviously decreased. The differences in the above flora were statistically significant at the class level (P < 0.05, Figure 3).

Changes in fecal microbiota in the ART group at the family level before and after treatment: The results of the community composition analysis indicated that, after 48 weeks of ART treatment, Sphingomonadaceae (Sphingomonadaceae), Clostridiales_vadi (Clostridiales), Prevotellaceae (Prevotellaceae), Succinivibrionaceae (Succinivibrionaceae), Clostridiales_1 (Clostridiales) were obviously decreased, whereas Erysipelotrichaceae (Erysipelotrichaceae), Bacteroidales (Bacteroidales), Tannerellaceae (Tannerellaceae), Lactobacillales (Lactobacillales) Bifidobacteriaceae and Enterobacteriaceae(Enterobacteriaceae) were obviously elevated. The differences in the above flora were statistically significant at the family level (P < 0.05, Figure 4).

Changes in fecal microbiota in the ART group at the genus level before and after treatment: The results of the community composition analysis indicated that, after 48 weeks of ART treatment, Fusobacterium, Prevotella_9, and...
Figure 1. Analysis of intestinal microbial beta diversity before and after treatment in two groups of patients based on a principal component analysis 3D score map. ART, antiretroviral therapy.

Figure 2. Changes in fecal microbiota in the ART group at the phylum level before and after 48 weeks of treatment. ART, antiretroviral therapy.

Prevotella_2 were obviously elevated, while Streptococcus, Lachnospira, Dialister, Succinivibrio, and Bifidobacterium were obviously decreased. The differences in the above flora were statistically significant at the genus level ($P < 0.05$, Figure 5).

Differences in fecal microbiota in the moxibustion group before and after treatment

Changes in fecal microbiota in the moxibustion group at the phylum level before and after treatment: The results of the community composition analysis indicated that, after 48 weeks of the moxibustion treatment, *Firmicutes* (Phylum Thick-walled), *Entothelionellaota* (Enterobacteriaceae), *Proteobacteria* (Acyomycetes), and *Actinobacteria* (Actinobacteria) were significantly higher, while *Sporobactes* (Spirochaetes), *Cyanobacteria* (Cyanobacteria), *Nitrospirae* (Nitrospirae Phylum), *Gemmatimonadetes* (Bacillus phylum), and *Bacteroidetes* (Bacteroidetes Phylum) were significantly lower. The differences in the above flora were statistically significant at the phylum level ($P < 0.05$, Figure 6).

Changes in fecal microbiota in the moxibustion group at the class level before and after treatment: The results of the community composition analysis indicated that, after 48 weeks of the moxibustion treatment, *Clostridia*
Figure 3. Changes in fecal microbiota in the ART group at the class level before and after 48 weeks of treatment. ART, antiretroviral therapy.

Figure 4. Changes in fecal microbiota in the ART group at the family level before and after 48 weeks of treatment. ART, antiretroviral therapy.
Figure 5. Changes in fecal microbiota in the ART group at the genus level before and after 48 weeks of treatment. ART, antiretroviral therapy.

Figure 6. Changes in fecal microbiota in the moxibustion group at the phylum level before and after 48 weeks of treatment.
(Clostridia), Gamma proteobacteria (γ-proteobacteria), and Actinobacteria (Actinobacteria) were obviously elevated, while Bacteroidia (Bacteroidetes), Deltaproteobacteria (δ-proteobacteria), Bacilli (Bacillariophyta), Alphaproteobacteria (α-proteobacteria), and Erysipelotrichia (Erysipello- trichia) were obviously decreased. The differences in the above flora were statistically significant at the class level (P < 0.05, Figure 7).

Changes in fecal microbiota in the moxibustion group at the family level before and after treatment: The results of the community composition analysis indicated that, after 48 weeks of the moxibustion treatment, Succinivibrionaceae (Vibrio succinicum), Streptococaceae (Streptococcus pepsinis), Prevotellaceae (Prevotella spp.), Fusobacteriaceae (Clostridia), and Burkholderiaceae (Burkholderiaceae) were obviously decreased, whereas Erysipelotrichaceae (Dentamaea spp.), Enterobacteriaceae (Enterobacteriaceae), Clostridiales_1 (Clostridiales), Bifidobacteriaceae (Bifidobacteriaceae), and Lactobacillaceae (Lactobacillaceae) were obviously elevated. The differences in the above flora were statistically significant at the family level (P < 0.05, Figure 8).

Changes in fecal microbiota in the moxibustion group at the genus level before and after treatment: The results of the community composition analysis indicated that, after 48 weeks of the moxibustion treatment, Lachnospira (Trichoderma spp.), Fusobacterium (Clostridium spp.), Alloprevotella (Prevotella anmolanae), and Succinivibrio (Vibrio succinicolica spp.) were obviously elevated, whereas Dorea (Dorea spp.), Dialister (genus of small analogue bacilli), Subdoligranulum (genus of rare micrococci), Bifidobacterium (bifidobacteria), and Escherichia_Shigella (Escherichia coli Shigella) were obviously decreased. The differences in the above flora were statistically significant at the genus level (P < 0.05, Figure 9).

**Differences in fecal microbiota between the two groups after treatment**

Differences in fecal microbiota at the phylum level between the two groups after treatment: The results of the community composition analysis indicated that, compared at the phylum level after treatment, Enterobacteriaceae (Enterobacteriaceae), Proteobacteria (Proteobacteria), and Fusobacteria (Clostridia) were dominant in the ART group (P < 0.05), while Gemmatimonadetes (Bassilis phylum), alphaproteobacteria (α-proteobacteria), deltaproteobacteria (δ-proteobacteria), Erysipelotrichia (Dendanum filament) Actinobacteria (Actinobacteria), and Clostridia (Clostridia) were dominant in the moxibustion group (P < 0.05, Figure 11).

Differences in fecal microbiota at the family level between the two groups after treatment: The results of the community composition analysis indicated that, compared at the family level after treatment, Fusobacteriaceae (Clostridiales), Prevotellaceae (Prevotellaceae), and Burkholderiaceae (Burkholderiaceae) were dominant in the ART group (P < 0.05), Enterobacteriaceae (Enterobacteriaceae), Bacteroidaceae (Bacteroides), Lachnospiraceae (Trichoderma), Bifidobacteriaceae (Bifidobacteriaceae), and Succinivibrionaceae (Succinivibrionaceae) were dominant in the moxibustion group (P < 0.05, Figure 12).

Differences in fecal microbiota at the genus level between the two groups after treatment: The results of the community composition analysis indicated that, compared at the genus level after treatment, Alloprevotella (Propionibacterium spp.), Prevotella_9 (Prevotella spp.-9), Prevotella_2 (Prevotella spp.-2), and Parabacteroides (Parabacteroides spp.) were dominant in the ART group (P < 0.05), while Bifidobacterium (Bifidobacterium), Succinivibrio (Vibrio succinogenes), Dialister (small class of bacilli), and Escherichia_Shigella (Escherichia coli Shigella) were dominant in the moxibustion group (P < 0.05, Figure 13).

**DISCUSSION**

To some degree, the human body is a multicomponent complex that combines human biology with commensal flora (including bacteria, viruses, fungi, etc.). The normal gastrointestinal tract of the human body includes a complex and balanced microbial community. These microbes are beneficial to the host in many ways, including to the host's immune system and metabolism, and can influence the host's health, constitution, personality, and to some degree, even thinking.

**Gut microbiological characteristics of AIDS patients**

Mulu et al. found that patients infected with HIV had low microbiota diversity in the mucosa of the lower intestine compared to patients without HIV and had more potential for pathogenic consortia. Usually, the diversity of mucosal microbiota is lower than that of fecal microbiota. This reduction in the mucosal microbiota of patients infected by HIV can make the subtle changes easier to determine or more obvious in the mucosal samples. This may be an important reason for the greater difference between mucosal and fecal...
Figure 7. Changes in fecal microbiota in the moxibustion group at the class level before and after 48 weeks of treatment.

Figure 8. Changes in fecal microbiota in the moxibustion group at the family level before and after 48 weeks of treatment.
samples. The progress of the HIV-1 virus becoming HIV in infected patients is very long.\textsuperscript{7} Although it is difficult to be sure whether the changes in fecal microflora are causes or effects of HIV-1 infection, the diversity of fecal microflora may be connected to the progress and duration of the disease. Nowak \textit{et al.} demonstrated that a reduction in the alpha diversity of the number of bacterial taxa was observed in patients within a highly immunodeficient group (CD4\textsuperscript{+} T lymphocyte count between 120 and 150 cell/µL) compared to chronic untreated individuals, suggesting that opportunistic infection occurs in advanced diseases\textsuperscript{8} and that a decrease in bacterial resistance may be the potential mechanism.

\textbf{The influence of ART treatment on HIV patients' intestinal flora}

Gori \textit{et al.} first witnessed a higher prevalence of \textit{Pseudomonas aeruginosa} and \textit{Candida albicans} and a lower abundance of \textit{Bifidobacterium} and \textit{Lactobacillus} in the fecal microbiota of HIV patients compared to healthy controls, indicating the potential for intestinal ecosystem
disorder in the early stages of HIV-1 infection. The results of the current study showed that, compared with before treatment, intestinal microorganisms \textit{Firmicutes} (Thick-walled Bacteria), \textit{Bacteroidetes} (Anaerobes), and \textit{Bifidobacteriaceae} (Bifidobacteria) were obviously elevated in patients after ART treatment. This indicates that ART can play a role in regulating the disorder of intestinal microecology.

Other studies have shown that the disorder of gut flora affects the efficacy of ART treatment and prevents ART treatment from working properly and can make HIV
patients more sensitive to opportunistic infections and other complications, thus indirectly increasing mortality in HIV patients.\textsuperscript{10} In addition, in a previous study, after successful long-term ART treatment, the gut microbiota of HIV-positive patients were found to be less similar than that of HIV-negative patients, and the extent to which ART restored health-related prevalence varied by bacterial classification.\textsuperscript{11} It has been determined that short-term effective ART inhibits the virus load below the low limit and that the index of the alpha diversity is not reduced; however, the index of the beta diversity indicated that the fecal microbiota of patients infected with HIV-1 had different performance and could even distinguish the patients with HIV from the healthy control group.\textsuperscript{9} Thereby, opportunistic pathogen infections and the migration of microbes were shown to have a possible connection with the unchanged diversity of the total bacteria. This diversity is closely associated with an increase in the serotypes of fecal microbiota of patients infected with HIV-1.\textsuperscript{12} Meanwhile, short-term ART can effectively inhibit the duplication of the HIV-1 virus but cannot influence the total bacteria diversity of the gut microbiota.

\textbf{The influence of moxibustion treatment on HIV patients' intestinal flora}

Previous studies have shown that HIV patients with immune reconstruction insufficiency have obviously high inflammatory response markers compared to patients with immune reconstruction sufficiency.\textsuperscript{13} The aviation of CD4\textsuperscript{+} T lymphocytes has a negative connection with the aviation of \textit{Erysipelotrichaceae}, and the ratio of CD4\textsuperscript{+}/CD8\textsuperscript{+} lymphocytes has a positive connection with the aviation of \textit{Succinivibrionaceae}. In addition, the levels of proinflammatory factor were found to be relatively high in patients with immune reconstruction insufficiency.\textsuperscript{14} In a comparison after moxibustion treatment and before treatment, the current study showed that patients' intestinal \textit{Firmicutes} (Thick-walled Bacteria), \textit{Entotheonellaeota} (Enterobacteriaeae), \textit{Succinivibrionaceae} (\textit{Vibrio succinii}), \textit{Bifidobacteriaeae} (\textit{Bifidobacteriaceae}), \textit{Lactobacillaceae} (\textit{Lactobacillaceae}), and \textit{Enterobacteriaeae} (\textit{Enterobacteriaceae}) were obviously elevated, while \textit{Nitrospiraceae} (\textit{Nitrospira}), \textit{Bacteroidetes} (\textit{Bacteroides}) \textit{Erysipelotrichaceae} (\textit{Denitrifying filamentous bacteria spp.}), \textit{Escherichia Shigel} (\textit{Escherichia coli Shigel}), and other bacteria were obviously decreased. Meanwhile, the composition of intestinal microorganisms in patients in the moxibustion group also differed significantly from that in the ART group. For example, \textit{Entotheonellaeota} (\textit{Enterobacteriaeae}), \textit{Fusobacteria} (\textit{Clostridium}), \textit{Bacteroidia} (\textit{Bacteroidetes}), and \textit{Alloprevotella} (\textit{Prevotella spp.}) predominated in the patients with ART only, but \textit{Firmicutes} (\textit{Thick-walled Bacteria}), \textit{Bacteroidetes} (\textit{Mycobacteria}), \textit{Actinobacteria} (\textit{Actinobacteria}), \textit{Erysipelotrichia} (\textit{Denitrofilamentous Bacteria}), \textit{Clostridia} (\textit{Clostridium}), \textit{Enterobacteriaeae} (\textit{Enterobacteriaceae}), \textit{Bacteroidaceae} (\textit{Bacteroidetes}), \textit{Bifidobacteriaceae} (\textit{Bifidobacteriaeae}), and \textit{Dialister} (small bacillus-like genera) prevailed in the patients with moxibustion.

Studies have shown that the microbial populations of healthy individuals compared to those treated with highly active antiretroviral therapy are very similar, with the phyla \textit{Firmicutes}, \textit{Bacteroidetes}, \textit{Proteobacteria}, \textit{Actinobacteria}, and \textit{Fusobacteria} accounting for the richest gut microbiome.\textsuperscript{15} In this experiment, the gut microbiota of the moxibustion group was dominated by \textit{Firmicutes} (\textit{Thick-walled Bacteria}), \textit{Bacteroidetes} (\textit{Bacteroidetes}), \textit{Actinobacteria} (\textit{Actinobacteria}), \textit{Erysipelotrichia} (\textit{Danubacteria}), \textit{Clostridia} (\textit{Clostridium}), \textit{Enterobacteriaeae} (\textit{Enterobacteriaceae}) \textit{Bacteroidaceae}, \textit{Bifidobacteriaeae}, and \textit{Dialister}, suggesting that the gut microbial populations of HIV patients treated with ART were closer to those of the normal
population than to those treated with just ART. In general, beneficial anti-inflammatory short-chain fatty acid-producing bacteria, such as *Fusobacteria*, *Enterobacteriaceae*, *Prevotella*, and *Proteobacteria*, are the dominant species in healthy individuals but are reduced in HIV patients.\(^\text{[6]}\) Moxibustion can increase the numbers of some flora and exert anti-inflammatory effects.

Gut flora influences the immune response in HIV patients, mainly through two pathways. The first is through the action of the gut microbiota itself, which is closely linked to the gut epithelial and mucosal immune responses. The microbiota plays an important role in ensuring the integrity of the gut barrier against pathogen microinfections through a variety of mechanisms, including the release of antimicrobial molecules and anti-inflammatory short-chain fatty acids and the activation of cellular receptors essential to the immune response.\(^\text{[16]}\) Second, gut bacteria can also exert anti-inflammatory effects through the production of microbial metabolites, which support the integrity of the intestinal epithelial barrier by regulating the expression of tight junction proteins. This aids in intestinal motility, as tight junction proteins have anti-inflammatory properties and decrease the nuclear factor KPAPAB signaling pathway, which is involved in the transcription of genes for inflammatory cytokines, thus playing an important role in the immune response.\(^\text{[17]}\)

**CONCLUSION**

The results of the current study showed that moxibustion can improve the composition of gut microbiota, and a significant difference was found in the improvement of the dominant bacteria with ART therapy. As suggested by the results of previous studies, moxibustion can promote the repair of the immune reconstruction function and alleviate clinical symptoms in AIDS patients by regulating the intestinal flora–immune response.

This study has some limitations. First, the progress of our study was relatively short, and we only observed the influences of short-term ART and moxibustion treatments on gut microbiota in order to make a significant connection between gut microbiota and immune parameters. Future studies need to investigate the changes in gut microbiota and the repairing of the immune function after a long and effective course of treatment. Second, in this study, the fecal microbiota was an alternative to using gut microbiota, which is the only true sample in a large noninvasive epidemiological study. However, the faecal microbiota may only represent the composition of the luminal gut microbe and not the composition of the mucosal surface, because the microbiota related to the mucosal surface may interact directly with the gut-associated lymphoid tissue of HIV-infected patients rather than with the gut microbiota, which is an important difference between the faecal microbiota and the mucos-associated microbiota. Third, this study did not include a control of healthy people, which should be included in future studies to observe the influences of moxibustion on the intestinal flora of HIV-infected patients.

**DECLARATION**

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**Author contributions**

Gao GJ, Zhou Q, Li SY: Conceptualization, Writing—Original draft preparation, Writing—Reviewing and Editing. Li X, Guo HJ: Conceptualization, Supervision. Liu Y, Wang J: Supervision, Project administration. All authors have read and approved the final version of the manuscript.

**Ethics approval**

The study was approved by the Ethics Committee of the Institute of Clinical Basic Medicine of Traditional Chinese Medicine at the China Academy of Traditional Chinese Medicine (No. 2018NO. 07).

**Informed consent**

The authors declare that they have obtained the patient’s informed consent to publish in this article.

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**Conflict of interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Data availability statement**

No additional data.

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