Gut dysbiosis of bacteria and fungi associated with human immunodeficiency virus infection

Xueqi Wu[1], Yuanhong Xu[2], Qingfeng Li[2], Fengjiao Yang[1], Shenghua He[2], Yingjie Li[2], Shujuan Yang[1], Peibin Zeng[1]

[1]West China School of Public Health and West China Fourth Hospital, Sichuan University, Chengdu, Sichuan 610041, China;
[2]Chengdu Public Health Clinical Center, Chengdu, Sichuan 610095, China.

To the Editor: There have been growing concerns about the gut bacterial dysbiosis associated with HIV infection, addressing a critical role of gut bacteria in persistent chronic inflammation and severe intestinal dysfunction among the human immunodeficiency virus (HIV) infected population. However, little is known about the gut fungal dysbiosis caused by HIV-infection. Therefore, we launched a case-control study aiming to explore the gut dysbiosis of bacteria and fungi in HIV infection, which may reveal a more comprehensive feature of HIV infection-related gut dysbiosis of bacteria and fungi that possibly play a role in the chronic systemic immune activation observed in HIV infection.

The ethics committee of West China Fourth Hospital approved the study protocol (No. KS2020310). A total of 130 participants were enrolled in Chengdu Public Health Clinic Center, including 75 HIV-infected patients (HIV+) and 55 HIV-uninfected participants [HIV–, matched with HIV+ for sex, age, and body mass index (BMI)]. Fecal and peripheral blood samples were collected, and all participants reported without any antibiotic usage in the past one month at the time of fecal sample collection. The peripheral blood CD4+ and CD8+ cell counts and plasma HIV viral load were determined by standard methods for HIV+ participants ($n = 75$).[1] Ten immune biomarkers were quantitatively measured by quantitative enzyme-linked immunosorbent assays (ELISA) (Jiangsu MEIMIAN Industry Co., Ltd., Yancheng, Jiangsu, China), including 1) four biomarkers indicating the microbial translocation: lipopolysaccharide (LPS), lipopolysaccharide-binding protein (LBP), soluble form of the LPS co-receptor CD14 (sCD14) and mannose-binding protein/lectin (MBP/MBL); 2) six inflammatory-related cytokines: interleukin (IL)-6, tumor necrosis factor (TNF)-α, IL-1β, IL-17, IL-10, and IL-22. DNA was extracted from fecal samples using an E.Z.N.A.® Soil DNA Kit (Omega Biotek, Inc., Norcross, GA, USA) according to manufacturer's protocols. The V3-V4 region of bacterial 16S ribosomal RNA (rRNA) gene and internal transcribed spacer (ITS) region were amplified using universal primers. Amplicons were sequenced on Ion Torrent S5 system (Life Technologies, Carlsbad, USA) and Illumina Hiseq 2500 platform (Illumina Inc., San Diego, CA, USA) for bacteria and fungi, respectively. Sequence data was analyzed by bioinformatics analysis using QIIME (v.1.17, http://qiime.org/), UCHIME (v4.2.40, https://www.drive5.com/usearch/manual/uchime_algo.html), VSEARCH (v2.4.4, https://github.com/torognes/vsearch/releases), and Mothur (v1.39.5, https://www.mothur.org/). Subsequent statistical analysis was performed in R software (v4.1.2, https://www.r-project.org/). Sequence data have been deposited in the Sequence Read Archive under BioProject accession PRJNA762595 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA762595/).

HIV+ patients and HIV− participants shared similar demographic characteristics in sex, age and BMI. The levels of five immune biomarkers (IL-10, IL-1β, MBP/MBL, TNF-α, and IL-22) are significantly different between HIV+ and HIV–, among which, IL-10 ($Z = -2.320$, $P = 0.020$), IL-1β ($Z = -1.773$, $P = 0.048$), and MBP/MBL ($Z = -2.022$, $P = 0.043$) in HIV+ were lower than HIV–. By contrast, TNF-α ($Z = -3.224$, $P = 0.001$) and IL-22 ($Z = -2.855$, $P = 0.004$) were higher in HIV+ [Supplementary Table 1, http://links.lww.com/CM9/B86].

In the present study, the alpha-diversity of gut bacteria in HIV+ was significantly lower than HIV– according to the observed species ($P < 0.001$) and Shannon index ($P = 0.033$). The result of the beta-diversity analysis presented significantly different clusters in bacterial communities between HIV+ and HIV– ($R = 0.041$, $P = 0.023$). In HIV+, there was a difference in the composition of gut bacteria compared to HIV–. *Firmicutes*, *Bacteroides*, *Proteobacteria,* and *Fusobacteria* were the four predominant genera in phylum level for

Access this article online	
Quick Response Code:	Website: www.cmj.org
	DOI: 10.1097/CM9.0000000000002194

Correspondence to: Dr. Peibin Zeng, West China School of Public Health and West China Fourth Hospital, Sichuan University, Chengdu 610041, Sichuan, China
E-mail: zengpeibin@live.cn

Copyright © 2022 The Chinese Medical Association, produced by Wolters Kluwer, Inc. under the CC-BY-NC-ND license. This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

Chinese Medical Journal 2022;135(00)
Received: 06-12-2021 **Edited by:** Yuanyuan Ji

both HIV+ and HIV–. For example, expansion of *Fusobacteria* ($P = 0.001$) and decreased proportion of *Firmicutes* ($P = 0.001$) were observed in HIV+. Top 15 genera in relative abundance were observed, with increased proportions of *Fusobacterium* ($P = 0.001$), *Prevotella, 9* ($P = 0.018$), and decreased *Faecalibacterium* ($P = 0.001$), *Alistipes* ($P = 0.004$), *Akkermansia* ($P = 0.042$), and *Ruminococcaceae. UCG-014* ($P = 0.002$) in HIV+ [Supplementary Figure 1, http://links.lww.com/CM9/B82].

Our study demonstrated the gut fungal dysbiosis due to HIV infection with increased gut fungal alpha-diversity and altered fungal composition. According to the observed species ($P < 0.0001$), the alpha-diversity of fungi in HIV+ was marginally higher than that in HIV–. Furthermore, our findings on beta-diversity revealed distinct clusters of fungal community between HIV+ and HIV- participants ($R = 0.195$, $P = 0.001$), which could be attributed to the expansion of opportunistic fungal infection, which has also been observed in HIV+ with oral fungal dysbiosis.[2] In HIV+, the ratio of *Basidiomycota-Ascomycota* was 0.09, which was lower than in HIV– (0.15). The top fungal genus was *Candida*, followed by *Cladosporium*, *Gibberella*, *Rhodotorula,* and *Saccharomyces*. For example, higher proportions of *Microsporum* ($P = 0.028$), and lower *Cystobasidium* ($P = 0.013$), *Rhodotorula* ($P = 0.048$) were found in HIV+ [Supplementary Figure 2, http://links.lww.com/CM9/B83].

When the specific gut bacterial and fungal taxa of HIV+ and HIV– were compared, 5 bacterial taxa from HIV+ and 17 taxa from HIV– had different abundance [Supplementary Figure 3, http://links.lww.com/CM9/B84]. In HIV+, all the 5 bacterial taxa were from the phylum of *Fusobacteria* with similar linear discriminant analysis (LDA) scores ~4.0. While in HIV–, 15 taxa from *Firmicutes* and 2 taxa (*Alistipes* and *Rikenellaceae*) from *Bacteroidetes* were presented, among which, *Clostridia* and *Clostridiales* were the top 2 taxa, both having scores greater than 4.0. The depletion of potentially beneficial bacteria like *Bacteroidaceae* and *Ruminococcaceae* in HIV + people could be linked to the loss of CD4+ T cells, as both effector and regulatory CD4+ T cells were specifically targeted and attacked by HIV, resulting in beneficial bacteria being killed by innate immune cells.[3] For fungal LDA analysis, 4 taxa from *Ascomycota* and 16 taxa from *Basidiomycota* were found to be differentiated between HIV+ and HIV–. *Nectriaceae*, *Hypocreales,* and *Sordariomycetes* were the top 3 fungal taxa with scores greater than 4.0 in HIV+. While, *Basidiomycota* (LDA = 3.94), *Phallaceae* (LDA = 3.88), and *Phallales* (LDA = 3.82) were verified to be particularly enriched in HIV–. The gut fungal dysbiosis in HIV infection was characterized by altered fungal biodiversity and compositions, which might also play an important role in HIV pathogenesis.

Between HIV+ and HIV–, there were distinct bacteria-fungi correlation patterns [Supplementary Figure 4A, http://links.lww.com/CM9/B85]. The top 15 taxa in relative abundance, as well as differential taxa at the genus level from each group, were used to analyze the relationship between bacteria ($n = 25$) and fungi ($n = 15$). Less bacteria-fungi associations with 15 pairs were identified in HIV+, compared to HIV– with 23 pairs. In HIV–, *Candida* was observed to be significantly related to 9 bacteria, such as positive associations with *Escherichia. Shigella* and Ruminococcus gnavus group, and negatively correlated to *Bacteroides* and *Alistipes*. By contrast in HIV+, *Candida* was just associated with two bacteria: *Bacteroides* (negative) and *Ruminococcaceae. UCG-005* (positive). *Gibberella* was observed positively correlated with 7 bacteria such as *Fournierella* and *Ruminococcaceae* in HIV–, whereas no correlation between *Gibberella* and bacteria was found in HIV+. *Rhodotorula* exhibited significant correlations with 4 bacteria, including positive associations with *Christensenellaceae* R7 *group*, *Ruminiclostridium. 6* and *Ruminococcaceae.UCG-014*, as well as negative association with *Bacteroides*. Furthermore, bacteria-fungi interactions were found to cause tissue damage by producing extracellular enzymes, which increased pro-inflammatory cytokine secretion, resulting in increased oxidative damage and apoptotic cell death.[4]

Associations of immune biomarkers with gut bacteria and fungi in HIV+ and HIV– were presented [Supplementary Figure 4B, http://links.lww.com/CM9/B85]. Neither group had a significant association of biomarkers with fungi. For bacteria in HIV+, 1) *Dialister* exhibited a positive correlation with two microbial translocation markers (sCD14 and MBP/MBL) and five inflammatory-related cytokines (IL-6, IL-17, IL-1β, IL-22, and IL-10), which may suggest *Dialister* play a dual role of pro-inflammation and anti-inflammation in HIV infection; 2) *Fournierella*, was positively associated with three microbial translocation markers (LPS, LBP and MBP/MBL) and four inflammatory-related cytokines (IL-17, TNF-α, IL-22, and IL-10); 3) negative correlation was found between *Fusobacterium* and LPS, sCD14, IL-6, IL-1β, and MBP/MBL. By contrast in HIV-, increased abundance of *Ruminococcaceae. UCG-014* was associated with elevated levels of LBP, sCD14, and MBP/MBL. It is widely recognized that the complexity of interactions between gut microbes and host and the proposed relationships between immune biomarkers and specific taxa remained to be verified by experimental evidence.[5]

Our study should address several limitations, such as the small sample size and lack of experimental proof. Future research could include larger samples and the use of metagenomics and metabolomics tools, as well as experimental evidence, to confirm the key microorganisms and investigate the functions of these strains linked to gut dysbiosis and immune status in HIV infection.

In conclusion, our findings contribute to a better understanding of the alterations in the gut bacterial and fungal microbiome and their associations with chronic systemic immune activation observed in HIV infection by providing a more comprehensive picture of HIV infection-related dysbiosis of the gut microbiome.

Declaration of patient consent

The authors certify that participants' records were anonymized. Written informed consents were obtained from all participants prior to the study procedure.

Funding

The study was supported by grants from the Key Research and Development Project, Department of Science and Technology, Sichuan Province (Nos. 2018SZ0212 and 2018JY0383) and the Fundamental Research Funds for the Central Universities, Project of Sichuan Health and Family Planning Commission (No. 18PJ015).

Conflicts of interest

None.

References

1. Lozupone CA, Li M, Campbell TB, Flores SC, Linderman D, Gebert MJ, *et al*. Alterations in the gut microbiota associated with HIV-1 infection. Cell Host Microbe 2013;14:329–339. doi: 10.1016/j.chom.2013.08.006.
2. Mukherjee PK, Chandra J, Retuerto M, Tatsuoka C, Ghannoum MA, McComsey GA. Dysbiosis in the oral bacterial and fungal microbiome of HIV-infected subjects is associated with clinical and immunologic variables of HIV infection. PLoS One 2018;13:e0200285. doi: 10.1371/journal.pone.0200285.
3. Lozupone CA, Rhodes ME, Neff CP, Fontenot AP, Campbell TB, Palmer BE. HIV-induced alteration in gut microbiota: driving factors, consequences, and effects of antiretroviral therapy. Gut Microbes 2014;5:562–570. doi: 10.4161/gmic.32132.
4. Ghannoum M. Cooperative Evolutionary Strategy between the Bacteriome and Mycobiome. mBio 2016;7:01951–01916. doi: 10.1128/mBio.01951-16.
5. Libertucci J, Young VB. The role of the microbiota in infectious diseases. Nat Microbiol 2019;4:35–45. doi: 10.1038/s41564-018-0278-4.

How to cite this article: Wu X, Xu Y, Li Q, Yang F, He S, Li Y, Yang S, Zeng P. Gut dysbiosis of bacteria and fungi associated with human immunodeficiency virus infection. Chin Med J 2022;00:00–00. doi: 10.1097/CM9.0000000000002194