Gut Microbiota in Human Immunodeficiency Virus Infection

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ABSTRACT

Human microbiota, majority presents in human gastrointestinal tract, plays crucial role in body physiological functions, such as immune system. Human immunodeficiency virus (HIV) infection impairs gut barrier and alter the microbiota ecosystem, called dysbiosis. Several studies showed different composition of gut microbiota between healthy individual and HIV patient. Moreover, different phase of HIV infections had their own characteristic of gut microbiota. Antiretroviral treatment might improve the CD4 level, however, it inconsistently restore the gut ecosystem. Some studies in prebiotic and probiotic revealed the potential beneficial effect in gut microbiota. Probiotic might inhibit mucosal invasion, improve intestinal mechanical barrier integrity, reduce microbial translocation, restore mucosal immune function, counteract local inflammation and lower systemic inflammation.

Keywords: *gut microbiota, human immunodeficiency virus, dysbiosis, probiotic*

ABSTRAK

Microbiota pada manusia yang mayoritas terdapat pada saluran cerna memainkan peranan penting pada fungsi fisiologis tubuh seperti sistem imun. Human immunodeficiency virus (HIV) merusak sawar saluran cerna dan merubah ekosistem mikrobiota, yang disebut disbiosis. Beberapa studi menunjukkan perbedaan susunan mikrobiota usus antara orang sehat dengan pasien HIV. Bahkan tiap fase infeksi HIV memiliki karakteristik mikrobiota usus tersendiri. Terapi antiretrovirus dapat memperbaiki kadar CD4, tetapi tidak memperbaiki ekosistem saluran cerna secara konsisten. Beberapa penelitian pada prebiotik dan probiotik memperlihatkan potensi yang menguntungkan terhadap mikrobiota saluran cerna. Probiotik menghambat invasi mukosa, memperbaikan integritas sawar mekanik usus, mengurangi translokasi mikroba, memperbaikI sistem imun mukosa, menurunkan peradangan local dan sistemik.

Kata kunci: *mikrobiota saluran cerna, human immunodeficiency virus, disbiosis, probiotik*

INTRODUCTION

Studies of human microbiome are subject of current great interest nowadays. More than 90% of the cells within and on the human body are of microbial origin. It is estimated over 10^{14} microorganisms resides in human body. They are collectively called microbiota, the genomes of which act together as a living system known as microbiome. About 99% of the microbiota is present in the human gastrointestinal tract. The gut microbiota consists of large, diverse collection of trillions of bacteria, archaea, eukarya, and viruses. It contributes to the development and maintenance of physiological responses within the host. It also plays crucial role in maintaining intestinal homeostasis, mucosal barrier system, regulation of innate and adaptive immune system.[1-7]

Chronic inflammation and systemic illness such as inflammatory bowel disease, obesity, cardiovascular diseases affect the composition of the intestinal microbiota, resulting dysbiosis.[8-11] Human immunodeficiency virus (HIV) infection impairs gut barrier and alter the composition of microbiota. Gori et al firstly observed high prevalence of opportunistic pathogen microorganism (*Pseudomonas aeruginosa*, *Candida albicans*) and lower level of Bifidobacteria and Lactobacilli in HIV patient.[12] This finding has attracted attention and research dedicated to investigate the relationship is currently growing. Moreover, it has been proposed as a prospective novel therapeutic target in HIV patients.

This paper aimed to review the impact of HIV infection in intestinal microbiota during various HIV infection course ie., early infection, chronic infection and after anti retroviral treatment. We also discuss potentials clinical application of microbiota restorations in daily practice.

Microbiota in Human Intestine

Characterization of the microbiome profile in healthy intestine is an important initial step in understanding the role of microbiome. In recent years, new "omic" technologies have facilitated the analysis of human gut microbiome which was overlooked previously. Current studies in gut microbiota use 16S rRNA based sequencing of bacterial gene and bioinformatic analysis. Almost all studies have exclusively focused on the bacterial component of the gut microbiome. It is estimated that over 35,000 bacterial species reside in human gastrointestinal tract. There is a significant difference in genus and number of bacteria residing in human gastrointestinal tract, starting from esophagus to colon. Large intestine harbors over 70% of all microbes in human body, predominantly by Firmicutes and bacteroides phyla. Moreover, there is also an axial difference of genera in human intestine, existing from luminal, mainly composed of *Bifidobacterium*, *Streptococcus*, *Enterobacteriaceae*, *Enterococcus*, *Ruminococcus*, to mucosal surface, mainly composed of *Clostridium*, *Lactobacillus*, *Akkermansia* of human intestine. Gut microbiota plays important roles such as nutrient metabolism by fermenting carbohydrates and indigestible oligosaccharide which result in the synthesis of short chain fatty acid (butyrate, propionate, acetate) as a source of energy for gut mucosa. It also involve in production of vitamin K, several components of vitamin B, conjugated linoleic acid, citric acid, pyruvic acid, and demonstrates benefit in lipid metabolism by influencing lipoprotein lipase activity in adipocytes and lipid hydrolysis. Gut microbiota contribute to host innate and adaptive immune system through gut-associated lymphoid tissue (GALT), effector and regulatory T cells, IgA producing plasma cells, dendritic and macrophage cells. Furthermore,

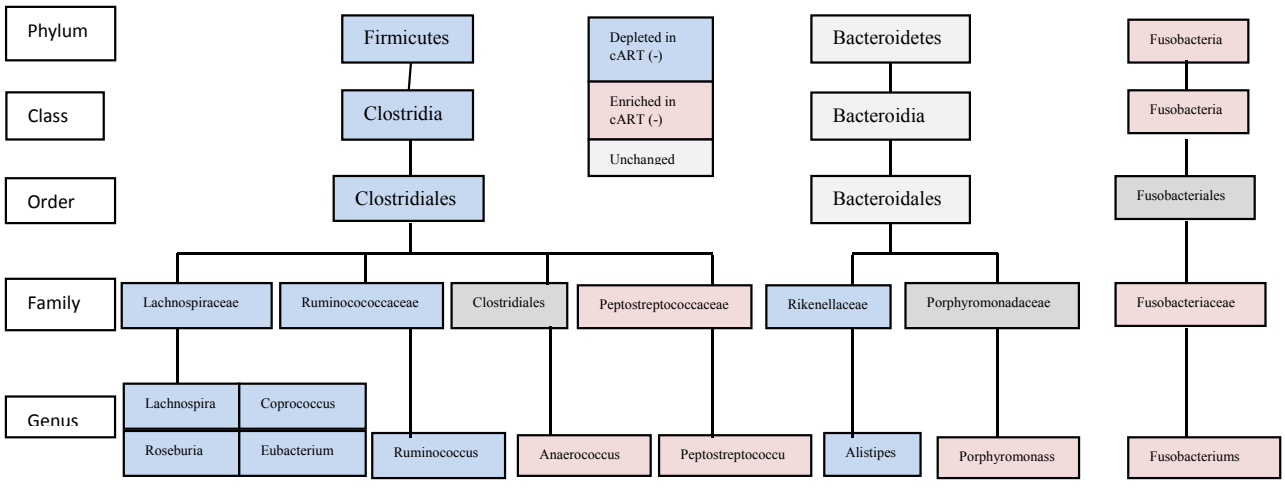

Figure 1. Differences in rectal mucosal microbiota between healthy subjects and HIV-positive subjects (Modified from McHardy et al)[22]

gut microbiota helps maintaining the integrity of gastrointestinal structure and function.[2,6,11,13-19]

Gut Microbiota in Human Immunodeficiency Virus (HIV) Infection

Gut microbiota is a critical factor in the development and maintenance of physiological immune response within the host. Human gastrointestinal tract, especially GALT, is the major site of HIV replication. Moreover, around 60% of human CD4+ T cells reside in GALT. The HIV infection results in unfavorable pathologic manifestations: it breakdowns gut barrier by enhancing epithelial permeability and decrease repair function, increase production of inflammatory cytokines, disrupts the balance between gut bacteria and immunity, increases translocation of microbes, and shifts the composition of gut microbiota to pro-inflammatory or pathogenic dominant population.[5,20] This, results in disturbance of gut microbiota ecosystem, called dysbiosis.[14,19,21] Study by Dubourg et al showed decrease in local environment diversity, i.e. alpha diversity due to HIV infection ($p = 0.00078$).[3] McHardy demonstrated numerous different of rectal mucosal microbial taxa between healthy subjects and HIV-positive subjects (Figure 1).[22]

Gut Microbiota in Early Phases of HIV Infection

There is a rapid viral replication, high immune response, and massive destruction of immune system in early phases of HIV infection. Gori et al firstly demonstrated increase in opportunistic pathogen in fecal sampels: *Candida albicans* (10,000-fold higher in the HIV-positive subjects compared to healthy subjects) and *Pseudomonas aeruginosa* (10-fold increase in HIV-positive subjects compared to healthy subjects). In comparison with healthy subjects, fecal bifidobacteria and lactobacilli were decreased in HIV-positive subjects.[12] Study by Vujkovic-Cvijin et al showed decreased in Enterobacteriaceae family and Bacteroidia class in untreated HIV patient.[23]

Gut Microbiota in Chronic Human Immunodeficiency Virus (HIV) Infection

Observation study by Dillon et al showed different mucosal bacterial composition between chronic HIV-infected subjects and healthy subjects. Although both groups were dominated by Firmicutes, Bacteroidetes, Proteobacteria phyla, there was a significant lower median amount of Firmicutes ($p = 0.03$) and higher median abundance of Proteobacteria ($p = 0.03$) in HIV-infected subjects (Figure 2). Relative abundance of Proteobacteria phylum in HIV infection relates with production of hydrogen sulfide compounds and epithelial inflammation by Desulfovirbrio, increase production of IL-8 and lipopolysaccharide by Acinetobacter. Comparisons of bacteria at the family level showed differences in overall relative abundances ($p = 0.02$). Prevotellaceae was significantly higher ($p = 0.03$) and Lachnospiraceae, Christensenellaceae, Ruminococcaceae, Bacteroidaceae were significantly lower in HIV-infected subjects ($p = 0.09$, $p = 0.04$, $p = 0.04$, $p = 0.04$, respectively) (Figure 3). There was also significant difference at genera level ($p = 0.009$), with a decrease in Bacteroides: Prevotella ratio (Figure 4).[24] Observational study by Nowak et al showed reduced in gut microbiota alpha diversity (lower Shannon index) in viremic HIV subjects which correlated with CD4+ T-cell count ($p = 0.01$).[25] Patients with CD4 < 200 had significantly decreased in bacterial richness, bacterial phylogenetic diversity compared to patients with CD4 > 200.[26] A case control study by Dubourg et al also showed reduced in alpha diversity in HIV-infected subjects. There was an increase in aerobic bacteria (Enterobacteriaceae and Enterococcaceae family) and decrease in anaerobic bacteria (Ruminococcaceae family). This, associated with difference in chemokines, T-cell activation marker finding between HIV-infected subjects and healthy subjects.[3]

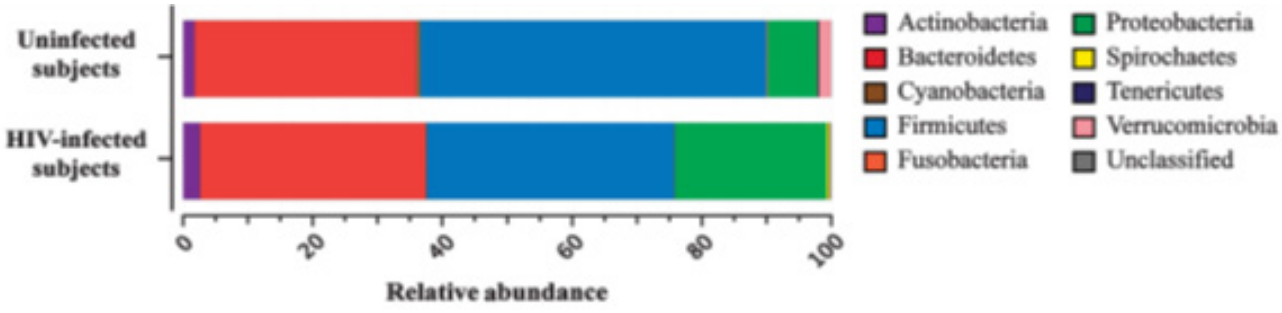

Figure 2. Comparison of the relative abundance of the Bacteroidetes, Firmicutes, Proteobacteria and other phyla between healthy subjects and HIV-infected subjects[24]

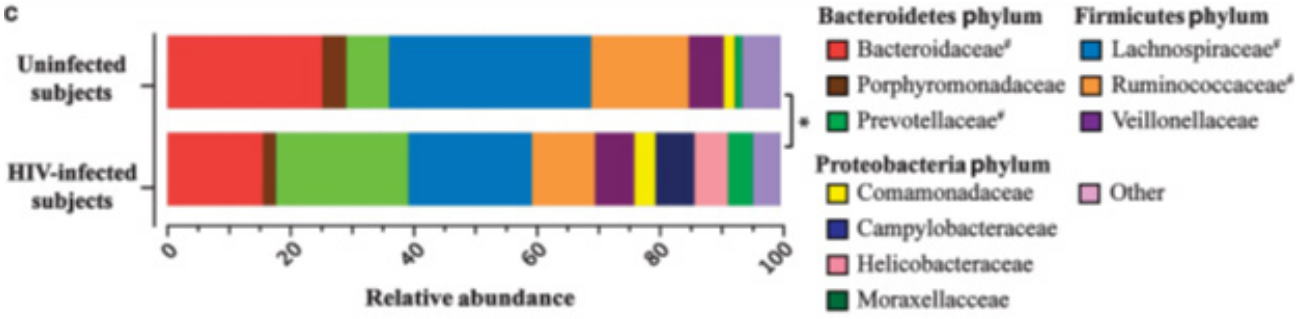

Figure 3. Comparison of the average relative abundance of the top 10 most abundant bacterial families[24]

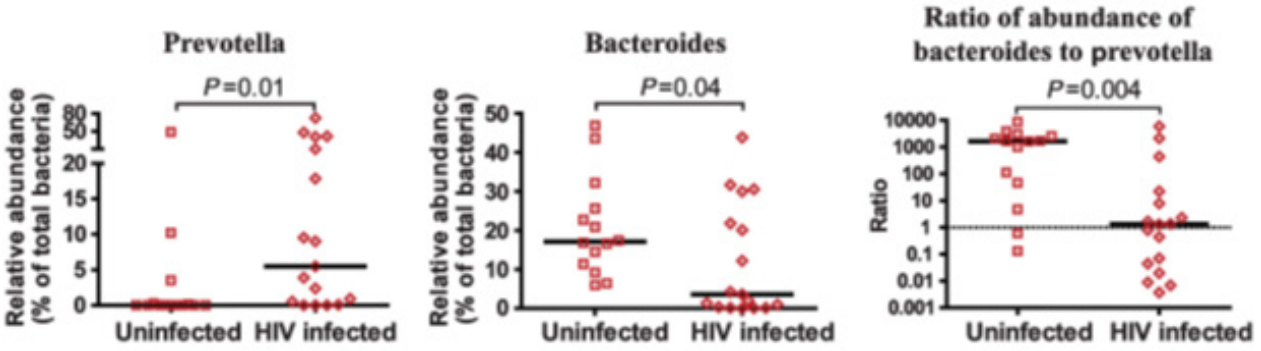

Figure 4. Significant decrease in Bacteroides: Prevotella ratio in HIV subjects[24]

Impact of Antiretroviral Treatment in Gut Microbiota

Studies on the short-term and long-term impact of antiretroviral treatment on the gut microbiota have convincingly demonstrated that antiretroviral treatment is unable to consistently restore human gut health. A study of rectal mucosal microbiota by McHardy et al demonstrated incompletely improvement in rectal mucosal microbiota diversity after antiretroviral treatment.[22] In contrast, the reduced in diversity was not restored by antiretroviral treatment according to the study by Dubourg.[3] In a stool gene sequencing study by Lozupone et al relatively short term antiretroviral treatment (6.7-8.5 months) insufficiently restored the microbiota composition; however, longer duration of treatment might not predict complete restoration.[27] Further study with greater subjects by Lozupone et al demonstrated different response to antiretroviral treatment between bacterial taxas. The increase abundance of Prevotella, Prevotellaceae in untreated HIV-positive subjects did not decrease significantly toward level in healthy subjects after antiretroviral treatment, but Peptococcus was significantly decrease with antiretroviral treatment. Bacteroides and Odoribacter were remain at low prevalence in spite of antiretroviral treatment.[28]

Probiotics as Potential Therapeutic Approach

Although the invention of antiretroviral drugs have successfully suppressed viral load and improved CD4 counts, most studies still inconsistently showed restoration of gut microbiota in HIV-treated subjects. This, commonly associated with persistence of chronic inflammation, activation of immune system, bacterial translocation. Probiotics are non-pathogen microorganisms, which in adequate amounts, confers health benefit on the host. Prebiotics are natural or processed non-digestible food, generally oligosaccharides, which also have benefit on the host. Many efforts are currently done in this field to assess their potential role in modifying, restoring gut microbiota and reducing inflammatory process.[29,30] Several studies reported beneficial effect of probiotics supplementation. Gori et al in a pilot randomized, placebo-controlled double blind study demonstrated dietary prebiotic supplementation significantly increased bifidobacterium and decreased pathogenic clostridium.[31] A pilot study by Scagnolari et al reported decreased expression of indolamine-2,3-dioxygenase (IDO), an enzyme associated with neuronal toxicity and neurocognitive disorders, in GALT after probiotic supplementation in antiretroviral-treated HIV infected patients.[32] d'Ettorre et al showed increased in Th1 cell in GALT ($p = 0.005$), lower in intraepithelial lymphocytes at cecum, ileum, transverse, descending

colon (p = 0.049, p = 0.027, p = 0.004, p = 0.002, respectively), lower frequencies of CD4+ and CD8+ T-cells expressing alternately CD38 or HLA-DR (CD4+ cells: p = 0.005 and p = 0.005, respectively; CD8+ cells: p = 0.037 and p = 0.005, respectively) after probiotic supplementation.[33] A 3-month double-blind placebo-controlled trial of *Bacillus coagulans* by Yang et al reported significant increase in the percentage of blood CD4+ (+ 2.8% vs. -1.8%; p = 0.0018) in subjects with HIV- infection on optimal antiretroviral treatment. However, the pro inflammatory biomarkers did not change significantly.[34] Moreover, a retrospective observational study by Irvine et al in Tanzania revealed an increased in CD4+ 0.17 cells/mL/day (95% CI: 0.01-0.34; p = 0.04) in *Lactobacillus rhamnosus* yogurt consumer after adjusting for length of time using antiretroviral medication.[35] Several mechanisms are proposed to explain the benefit effect of probiotic. Probiotic can inhibit mucosal invasion, improve intestinal mechanical barrier integrity, reduce microbial translocation, restore mucosal immune function, counteract local inflammation and lower systemic iinflammation, stimulate production antimicrobial substances, increase intestinal immunoglobulin.[5,20,36,37]

CONCLUSION

HIV infection results in unfavorable change of human gut microbiota composition. The invention of antiretroviral contributes significantly in HIV treatment; however, it inconsistently restores gut microbiota composition. Probiotic, non-pathogen microorganisms, might play a promising role in improving microbiota composition.

REFERENCES

1. Brenchley JM, Douek DC. Microbial translocation across the GI tract. Ann Rev Immunol 2012;30:149-73.
2. Dinh DM, Volpe GE, Duffalo C, Bhalchandra S, Tai AK, Kane AV, et al. Intestinal microbiota, microbial translocation, and systemic inflammation in chronic HIV infection. J Infect Dis 2014;211:19-27.
3. Dubourg G, Lagier J-C, Hüe S, Surenaud M, Bachar D, Robert C, et al. Gut microbiota associated with HIV infection is significantly enriched in bacteria tolerant to oxygen. BMJ Open Gastroenterol 2016;3:e000080.
4. Saxena D, Li Y, Yang L, Pei Z, Poles M, Abrams WR, et al. Human microbiome and HIV/AIDS. Curr HIV/AIDS Rep 2012;9:44-51.
5. D'Angelo C, Reale M, Costantini E. Microbiota and probiotics in health and HIV infection. Nutrients 2017;9:615.
6. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature 2010;464:59.
7. Gootenberg DB, Paer JM, Luevano J-M, Kwon DS. HIV-associated changes in the enteric microbial community: Potential role in loss of homeostasis and development of systemic inflammation. Curr Opin Infect Dis 2017;30:31.
8. Brown K, DeCoffe D, Molcan E, Gibson DL. Diet-induced dysbiosis of the intestinal microbiota and the effects on immunity and disease. Nutrients 2012;4:1095-119.
9. Frank DN, Zhu W, Sartor RB, Li E. Investigating the biological and clinical significance of human dysbioses. Trends Microbiol 2011;19:427-34.
10. Tang WW, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. NEJM 2013;368:1575-84.
11. Shreiner AB, Kao JY, Young VB. The gut microbiome in health and in disease. Curr Opin Gastroenterol 2015;31:69.
12. Gori A, Tincati C, Rizzardini G, Torti C, Quirino T, Haarman M, et al. Early impairment of gut function and gut flora supporting a role for alteration of gastrointestinal mucosa in human immunodeficiency virus pathogenesis. J Clin Microbiol 2008;46:757-8.
13. Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Reddy DN. Role of the normal gut microbiota. WJG 2015;21:8787.
14. Marchesi JR, Adams DH, Fava F, Hermes GD, Hirschfield GM, Hold G, et al. The gut microbiota and host health: A new clinical frontier. Gut 2015:309990.
15. Bäckhed F, Fraser CM, Ringel Y, Sanders ME, Sartor RB, Sherman PM, et al. Defining a healthy human gut microbiome: Current concepts, future directions, and clinical applications. Cell Host Microbe 2012;12:611-22.
16. Devillard E, McIntosh FM, Paillard D, Thomas NA, Shingfield KJ, Wallace RJ. Differences between human subjects in the composition of the faecal bacterial community and faecal metabolism of linoleic acid. Microbiology 2009;155:513-20.
17. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-y M, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science 2013;341:569-73.
18. Geuking MB, Cahenzli J, Lawson MA, Ng DC, Slack E, Hapfelmeier S, et al. Intestinal bacterial colonization induces mutualistic regulatory T cell responses. Immunity 2011;34:794-806.
19. Lu W, Feng Y, Jing F, Han Y, Lyu N, Liu F, et al. Association between gut microbiota and CD4 recovery in HIV-1 infected patients. Front Microbiol 2018;9:1451.
20. Zilberman-Schapira G, Zmora N, Itav S, Bashiardes S, Elinav H, Elinav E. The gut microbiome in human immunodeficiency virus infection. BMC Med 2016;14:83.
21. Mosca A, Leclerc M, Hugot JP. Gut microbiota diversity and human diseases: should we reintroduce key predators in our ecosystem? Frontiers in microbiology 2016;7:455.
22. McHardy IH, Li X, Tong M, Ruegger P, Jacobs J, Borneman J, et al. HIV Infection is associated with compositional and functional shifts in the rectal mucosal microbiota. Microbiome 2013;1:26.
23. Vujkovic-Cvijin I, Dunham RM, Iwai S, Maher MC, Albright RG, Broadhurst MJ, et al. Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. Science translational medicine 2013;5:193ra91-ra91.
24. Dillon S, Lee E, Kotter C, Austin G, Dong Z, Hecht D, et al. An altered intestinal mucosal microbiome in HIV-1 infection is associated with mucosal and systemic immune activation and endotoxemia. Mucosal immunology 2014;7:983.

25. Nowak P, Troseid M, Avershina E, Barqasho B, Neogi U, Holm K, et al. Gut microbiota diversity predicts immune status in HIV-1 infection. Aids 2015;29:2409-18.
26. Monaco CL, Gootenberg DB, Zhao G, Handley SA, Ghebremichael MS, Lim ES, et al. Altered virome and bacterial microbiome in human immunodeficiency virus-associated acquired immunodeficiency syndrome. Cell host & microbe 2016;19:311-22.
27. 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;14:329-39.
28. 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;5:562-70.
29. Hardy H, Harris J, Lyon E, Beal J, Foey AD. Probiotics, prebiotics and immunomodulation of gut mucosal defences: homeostasis and immunopathology. Nutrients 2013;5:1869-912.
30. Tanabe S. The effect of probiotics and gut microbiota on Th17 cells. Int Rev Immunol 2013;32:511-25.
31. Gori A, Rizzardini G, Van't Land B, Amor KB, Van Schaik J, Torti C, et al. Specific prebiotics modulate gut microbiota and immune activation in HAART-naive HIV-infected adults: results of the "COPA" pilot randomized trial. Mucosal immunology 2011;4:554.
32. Scagnolari C, Corano Scheri G, Selvaggi C, Schietroma I, Najafi Fard S, Mastrangelo A, et al. Probiotics differently affect gut-associated lymphoid tissue indolamine-2, 3-dioxygenase mRNA and cerebrospinal fluid neopterin levels in antiretroviral-treated HIV-1 infected patients: a pilot study. Int J Mol Sci 2016;17:1639.
33. d'Ettorre G, Rossi G, Scagnolari C, Andreotti M, Giustini N, Serafino S, et al. Probiotic supplementation promotes a reduction in T-cell activation, an increase in Th17 frequencies, and a recovery of intestinal epithelium integrity and mitochondrial morphology in ART-treated HIV-1-positive patients. Immun Inflamm Dis 2017;5:244-60.
34. Yang OO, Kelesidis T, Cordova R, Khanlou H. Immunomodulation of antiretroviral drug-suppressed chronic HIV-1 infection in an oral probiotic double-blind placebo-controlled trial. AIDS research and human retroviruses 2014;30:988-95.
35. Irvine SL, Hummelen R, Hekmat S, Looman CW, Habbema JDF, Reid G. Probiotic yogurt consumption is associated with an increase of CD4 count among people living with HIV/AIDS. J Clin Gastroenterol 2010;44:e201-e5.
36. Nair MS, Amalaradjou M, Venkitanarayanan K. Antivirulence properties of probiotics in combating microbial pathogenesis. Advances in applied microbiology. Elsevier; 2017.p.1-29.
37. Kim CJ, Walmsley SL, Raboud JM, Kovacs C, Coburn B, Rousseau R, et al. Can probiotics reduce inflammation and enhance gut immune health in people living with HIV: study designs for the Probiotic Visbiome for Inflammation and Translocation (PROOV IT) pilot trials. HIV clinical trials 2016;17:147-57.