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The Gut Microbiota Correlates with HIV Control and Immune Status

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INTRODUCTION

The human microbiota refers to the ensemble of microorganisms inhabiting the human body and it is clustered in particular parts of the body including skin, mouth, vagina and gastrointestinal tract. The gut microbiota harbours tens of trillions of microorganisms of at least 1000 known bacterial species and presents a wide bacterial metabolic spectrum supported by more than 3 million genes. It is known to have an essential role in human physiology including digestion functioning, production of vitamins and nutrients, and development and maintenance of a fully active and competent host immune system. Compositional shifts in the gut microbiota have been described for many health disorders.

As recent studies suggest a role of the gut microbiome on HIV/AIDS pathogenesis, here we aim to explore if bacterial communties inhabiting the gastrointestinal tract of HIV-1 infected subjects differ by HIV-1 control and immune status.

STUDY DESIGN

This study included 80 subjects: 64 HIV-1 infected individuals and 16 HIV-1 negative individuals that served as our negative control group.

		VIH-1 POSITIVE (n=64)	NEGATIVE (n=16)	P Value	
Gender, n (%)	Male	45 (70.3%)	13 (81.25%)		0.5356
	Female	19 (29.7 %)	2 (12.5%)	0.1106	0.2141
	Transgender	0	1 (6.25%)		0.2
Age, years, median (IQR)		45 (37, 52)	41 (37, 45.25)		0.2839
Body Mass Index (BMI), median (IQR) *		23.8 (22.3, 26.2)	24.3 (21.9, 28.15)		0.892
Group of risk, n (%)	MSM	31 (48.4%)	13 (81.25%)		**0.02434**
	MSW	24 (27.5%)	2 (12.5%)		0.0749
	PWID	7 (10.9%)	0	**0.02985**	0.3344
	TGW	0	1 (6.25%)		0.2
	Unknown	2 (3.12%)	0		1
Ethnic group, n (%)	Caucasian	49 (76.6%)	12 (75%)		1
	Hispano-Latino	11 (17.2%)	4 (25%)	0.8892	0.4853
	Asiatic	1 (1.6%)	0		1
	Others	3 (4.6%)	0		1
Antibiotic usage within previous 3 months		2 (3.16%)	0		1
Antibiotic usage within previous 6 months		16 (25%)	0		**0.0322**
Serology HBV *		0	1 (8.3%)		0.2105
Serology HCV *		7 (13.2%)	0		0.334
Serology Syphilis *		10 (17.5%)	0		0.5576

Table 1. Demographic data for the HIV-1 infected and HIV-1 non infected groups. P-values were obtained with Fisher tests for categorical data, and the Mann-Whitney rank sum test for continuous data. The total number of individuals was 64 and 16 for the HIV-1 positive and HIV-1 negative groups, except for the following variables (*): BMI (n=63 for HIV-1 positive and n=11 for HIV-1 negative groups); Serology HBV (n=45 for HIV-1 positive and n=12 for HIV-1 negative groups), Serology HCB (n=53 for HIV-1 positive and n=15 for HIV-1 negative groups) and Serology Syphilis (n=57 for HIV-1 positive and n=6 for HIV-1 negative groups).

Within the HIV-1 positive group, individuals were classified into 7 different phenotypes according to their AIDS progression status and response to antiretroviral treatment recorded by their CD4$^+$ T cell counts and viral loads (VL).

	Number of patients (n)	Antiretroviral treatment (ART)	CD4$^+$ T cell counts (cells/mm^3)	Viral load (VL) (copies/ml)
CONCORDANT	27	YES (> 2 years)	>500	<50
DISCORDANT	11	YES (> 2 years)	<300	<50
EARLY TREATED	5	YES (<6 months after infection)	-	<50
LATE PRESENTERS	5	NO	<200	-
NAIVE	7	NO	>500	>2000
ELITE CONTROLLERS	3	NO	-	<50 for 1 year
VIREMIC CONTROLLERS	6	NO	-	50-2000 for 1 year

Table 2. Classification criteria for the 7 HIV-1 infected phenotypes.

MATERIALS AND METHODS

DNA was isolated from fecal samples and variable regions V3V5 from bacterial molecular marker 16S rRNA gene were amplified by PCR and sequenced using Illumina MiSeq (Figure1). The generated amplicons were about 450 base pair long and were expected to allow genus or even up to species-level taxonomic characterization of bacteria present within our mixed microbial communities.

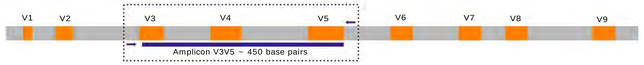


Figure 1. Schematic representation of the 16S rRNA gene used as molecular marker for bacterial characterization.

16S rRNA gene amplicons were filtered and clustered into OTUs (Operational Taxonomic Units) at 97% sequence similarity using Mothur. Taxonomic assignment of sequences was performed with SILVA database.

Ecological analyses over the OTU table were performed using R Vegan and Phyloseq packages. Statistical analyses for richness estimators and diversity indices among groups were done with t-student test and ANOVA plus post-hoc Tukey-Kramer test as corresponded. LEfSe algorithm was used to identify which bacterial taxa contributed to differences between HIV-1 infected and HIV-1 negative individuals and Non-metric Multidimensional Scaling (NMDS) ordination analysis was performed over whole genus abundance dataset using the Bray-Curtis ecological distance with R package Phyloseq.

RESULTS

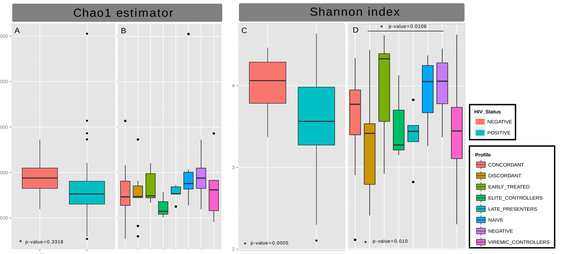


Figure 2. Richness estimator (Chao1) and diversity index (Shannon) values for HIV-1 positive and HIV-1 negative subjects (A, C) and by HIV-1 phenotype (B,D).

The gut microbiome of HIV-1 infected subjects is significantly less rich (Chao1 estimator) and diverse (Shannon index) than that of HIV-1 negative individuals (Figure2A, 2C). HIV-1 phenotypes corresponding to more advanced HIV-1 disease stages, ie: Late Presenters (LP), Immune Discordants (ID), but also Immune Concordant (IC) have lower richness and diversity than HIV-1 negative individuals. Antiretroviral Naive (AN) subjects have very similar richness and diversity values to HIV-1 negative individuals. Early Treated (ET) subjects show a considerable increase in diversity compared to HIV-1 negative individuals, but not in estimated richness (Figure 2B, 2D). Both, Elite Controllers (EC) and Viremic Controllers (VC) show reduced richness and diversity compared to HIV-1 negative patients.

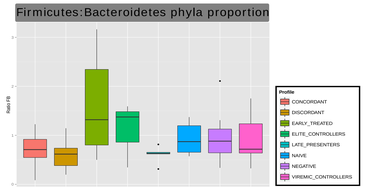


Figure 3. Firmicutes:Bacteroidetes ratio values for all 7 phenotypes of study and HIV-1 negative individuals.

HIV-1 infected subjects in the LP, ID, and IC groups also show the lowest Firmicutes:Bacteroidetes phyla ratio (ratio <1). While AN and VC show a similar ratio to HIV-1 negative individuals (ratio ~1), EC and ET groups show the highest Firmicutes:Bacteroidetes phyla ratio (ratio >1).

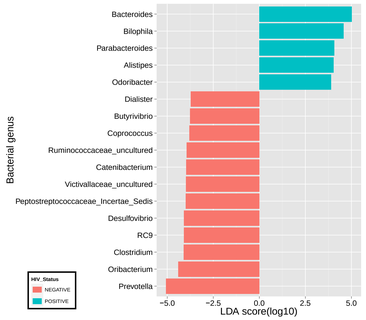


Figure 4. LEfSe results showing statistically significant biomarkers at bacterial genus level for both HIV-1 positive and negative subjects.

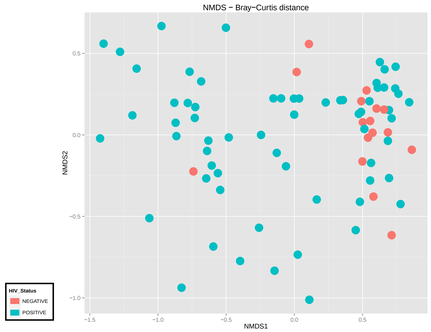


Figure 5. NMDS ordination plot showing potential clustering effect on HIV-1 positive and negative subjects based on Bray-Curtis distances calculated over genus abundance data.

Using LEFse, the only unbalanced phylum between HIV positive and negative subjects is Lentisphaerae, which is enriched in HIV-1 negative individuals. At the family level, HIV-1 positive subjects are enriched in Bacteroidaceae and Porphyromonadaceae, and depleted in Victivallaceae, Peptostreptococcaceae, Clostridiaceae and Prevotellaceae. At the genus level (Figure4), HIV-1 infected individuals are enriched for Bacteroides, Bilophila, Parabacteroides, Alistipes and Odoribacter, and depleted for Dialister, Butyrivibrio, Coprococcus, Catenibacterium, Desulfovibrio, RC9, Clostridium, Oribacterium, Prevotella and uncultured or unclassified genus (one Ruminococcaceae, one Victivallaceae and one Peptostreptococcaceae).

The NMDS plot shows how samples are clustered based on their similarity in bacterial genus composition. In contrast to HIV-1 positive subjects which are widely distributed, HIV-1 negative subjects do show a clustering tendency towards the right part of NMDS1 and the middle-top part of NMDS2 in where Prevotella and Veillonella genus are enriched.

CONCLUSIONS

HIV-1 infection is associated with significant decreases in gut microbiome richness and diversity as well as to a dysbiotic bacterial profile leaded by an increase in Bacteroidetes and a decrease in Prevotella within the gut microbial communities. Thus, our findings suggest an important interaction between the HIV-1 infection and the intestinal microbiota composition.