

Salivary microbiome in patients undergoing hemodialysis and its associations with the duration of the dialysis

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Supplementary Table S1. Dissimilarity Tests between the Healthy Controls and the HD Patients.

Adonis	P-value	ANOSIM	<i>P</i> value
0.0936	0.001	0.2371	0.001

Supplementary Table S2. Relative Abundance of the Top 30 Bacterial Genera.

Gerena	Groups		P value
	Healthy controls (N=100)	HD Patients (N=108)	
<i>Streptococcus</i>	19.49±14.60	21.90±14.56	0.178
<i>Neisseria</i>	9.58±10.63	9.90±8.66	0.294
<i>Lautropia</i>	12.05±19.16	4.21±6.82	0.031
<i>Oribacterium</i>	6.89±11.35	5.64±9.55	0.569
<i>Porphyromonas</i>	3.16±3.41	4.62±4.79	0.004
<i>Ruminococcaceae</i>	1.63±3.48	5.74±6.91	0.000
<i>Capnocytophaga</i>	1.86±3.27	4.77±6.12	0.000
<i>Prevotella [G-7]</i>	4.12±5.00	2.05±3.12	0.000
<i>Actinomyces</i>	4.52±4.80	1.36±1.78	0.000
<i>Haemophilus</i>	2.77±3.96	2.39±2.24	0.710
<i>Veillonellaceae</i>	1.95±3.29	2.85±3.53	0.000
<i>Selenomonas [G-3]</i>	2.05±3.91	2.05±3.91	0.935
<i>Veillonella</i>	2.99±2.39	1.26±1.49	0.000
<i>Granulicatella</i>	1.42±1.46	2.37±2.26	0.000
<i>Fusobacterium</i>	2.03±2.17	1.64±1.33	0.641
<i>Prevotella</i>	1.91±2.22	1.71±1.43	0.499
<i>Selenomonas</i>	1.74±3.59	1.59±2.49	0.153
<i>Gemella</i>	1.09±1.11	1.72±2.21	0.034
<i>Rothia</i>	1.58±1.91	0.84±1.27	0.000
<i>Catonella</i>	0.87±0.99	1.36±1.45	0.003
<i>Leptotrichia</i>	1.33±1.63	0.89±1.10	0.027

Gerena	Groups		<i>P</i> value
	Healthy controls (N=100)	HD Patients (N=108)	
<i>Campylobacter</i>	0.52±0.87	1.40±2.04	0.000
<i>Selenomonas [G-4]</i>	0.78±1.78	1.12±1.59	0.006
<i>Norank f XIII</i>	0.98±2.14	0.83±1.70	0.994
<i>Fretibacterium</i>	0.56±1.17	0.90±1.29	0.002
<i>Eubacterium yurii</i>	0.63±1.36	0.69±1.24	0.016
<i>Alloprevotella</i>	0.69±1.22	0.58±1.17	0.181
<i>Treponema [G-2]</i>	0.56±1.27	0.68±0.85	0.002
<i>Aggregatibacter</i>	0.25±0.45	0.88±1.15	0.000
<i>Bergeyella</i>	0.12±0.19	0.84±1.50	0.000

The data are presented as “Mean ± SD.”

Supplementary Table S3. Relative abundance of the top 30 bacterial genera with significant difference between the groups.

Gerena	Groups		P value
	Healthy controls	HD Patients	
	(N=100)	(N=108)	
Healthy group			
Lautropia	12.05±19.16	4.21±6.82	0.031
Prevotella [G-7]	4.12±5.00	2.05±3.12	0.000
Actinomyces	4.52±4.80	1.36±1.78	0.000
Veillonella	2.99±2.39	1.26±1.49	0.000
Rothia	1.58±1.91	0.84±1.27	0.000
Leptotrichia	1.33±1.63	0.89±1.10	0.027
HD group			
Ruminococcaceae	1.63±3.48	5.74±6.91	0.000
Capnocytophaga	1.86±3.27	4.77±6.12	0.000
Porphyromonas	3.16±3.41	4.62±4.79	0.004
Veillonellaceae	1.95±3.29	2.85±3.53	0.000
Granulicatella	1.42±1.46	2.37±2.26	0.000
Gemella	1.09±1.11	1.72±2.21	0.034
Campylobacter	0.52±0.87	1.40±2.04	0.000
Catonella	0.87±0.99	1.36±1.45	0.003
Selenomonas [G-4]	0.78±1.78	1.12±1.59	0.006
Fretibacterium	0.56±1.17	0.90±1.29	0.002
Aggregatibacter	0.25±0.45	0.88±1.15	0.000
Bergeyella	0.12±0.19	0.84±1.50	0.000
Eubacterium yurii	0.63±1.36	0.69±1.24	0.016
Treponema [G-2]	0.56±1.27	0.68±0.85	0.002

The data are presented as “Mean ± SD”

Supplementary Table S4. Relative Abundance of the Species Associated with Diseases in the Healthy Controls and the HD Patients.

Specie	Groups		<i>P</i> value
	Healthy controls (N=100)	HD Patients (N=108)	
The species belonging to the “Red Complex”			
<i>Porphyromonas gingivalis</i>	0.68±1.68	1.61±3.73	0.000
<i>Tannerella forsythia</i>	0.16±0.23	0.28±0.32	0.000
<i>Treponema denticola</i>	0.07±0.11	0.10±0.13	0.011
The species belonging to the “Orange Complex”			
<i>Campylobacter showae</i>	0.09±0.14	0.80±1.73	0.000
<i>Fusobacterium nucleatum</i>	0.69±0.82	0.90±0.81	0.001
<i>Fusobacterium periodonticum</i>	1.34±1.73	0.74±0.94	0.001
<i>Prevotella intermedia</i>	0.28±0.64	0.39±0.63	0.001
<i>Prevotella melaninogenica</i>	0.04±0.07	0.01±0.01	0.000
<i>Prevotella nigrescens</i>	0.05±0.12	0.10±0.22	0.147

The data are presented as “Mean ± SD”

Supplementary Table S5. The detailed information of the Cytoscape analyzation.

OTU		Phylum	Family	P value	correlation r
Positive correlations					
OTU1433	<i>Lachnospiraceae</i> [G-2] sp. HMT_096	<i>Firmicutes</i>	<i>Lachnospiraceae</i> [XIV]	0.0128	0.31867
OTU1433	<i>Lachnospiraceae</i> [G-2] sp. HMT_096	<i>Firmicutes</i>	<i>Lachnospiraceae</i> [XIV]	0.0001	0.33249
OTU27	<i>Erysipelothrix tonsillarum</i>	<i>Firmicutes</i>	<i>Erysipelotrichaceae</i>	0.0015	0.30225
OTU902	<i>Lachnoanaerobaculum orale</i>	<i>Firmicutes</i>	<i>Lachnospiraceae</i> [XIV]	0.0003	0.33813
OTU505	<i>Oribacterium</i> sp. HMT_102	<i>Firmicutes</i>	<i>Lachnospiraceae</i> [XIV]	0.0010	0.31225
OTU17	<i>Olsenella</i> sp. HMT_809	<i>Actinobacteria</i>	<i>Atopobiaceae</i>	0.0020	0.30447
OTU788	<i>Peptostreptococcaceae</i> [XI][G-7] sp. HMT_081	<i>Firmicutes</i>	<i>Peptostreptococcaceae</i> [XI]	0.0021	0.30173
OTU914	<i>Pseudoramibacter alactolyticus</i>	<i>Firmicutes</i>	<i>Eubacteriaceae</i>	0.0020	0.30372
Negative correlations					
OTU1301	<i>Vibrio gigantis</i>	<i>Proteobacteria</i>	<i>Vibrionaceae</i>	<0.0001	-0.40778
OTU1017	<i>Streptococcus anginosus</i>	<i>Firmicutes</i>	<i>Streptococcaceae</i>	<0.0001	-0.40319
OTU1009	<i>Pseudomonas marincola</i>	<i>Proteobacteria</i>	<i>Pseudomonadaceae</i>	<0.0001	-0.40864
OTU1160	<i>Agrobacterium tumefaciens</i>	<i>Proteobacteria</i>	<i>Rhizobiaceae</i>	<0.0001	-0.41443
OTU1367	<i>Pseudomonas orientalis</i>	<i>Proteobacteria</i>	<i>Pseudomonadaceae</i>	<0.0001	-0.40259
OTU1183	<i>Arthrospira platensis</i>	<i>Cyanobacteria</i>	<i>Oscillatoriales</i>	<0.0001	-0.40193

Supplementary Table S6. Demographics and Clinical Parameters of Patients in the Three Groups.

Characteristics	Groups			<i>P</i> value
	HD1 (N=23)	HD2 (N=61)	HD3 (N=24)	
Age [years] (Mean ± SD)	44.61±13.23	46.24±13.45	47.58±13.53	0.698
Male/Female	11/12	35/26	16/8	0.426
BMI [kg/m ²] (Mean ± SD)	21.96±4.10 (17.21-31.45)	22.83±3.65 (16.22-36.33)	21.93±3.23	0.304
Length of HD therapy (Mean ± SD)	0.78±0.29-	3.17±0.94	8.33±2.04	-
CPI ^c				0.299
0	0 (0.0%)	0 (0.0%)	0 (0.0%)	-
1	1 (4.3%)	0 (0.0%)	0 (0.0%)	0.430
2	4 (17.4%)	4 (6.5%)	3 (12.5%)	0.311
3	10 (43.5%)	27 (44.3%)	8 (33.3%)	0.694
4	8 (34.8%)	30 (49.2%)	13 (54.2%)	0.382
X	0 (0.0%)	0 (0.0%)	0 (0.0%)	-
Number of Decayed teeth (Mean, range)	0.70, 0-3	0.97, 0-5	0.71, 0-3	0.707
Number of Missing teeth (Mean, range)	0.96, 0-5	1.05, 0-15	1.35, 0-15	0.632
Number of Filled teeth (Mean, range)	0.39, 0-4	0.43, 0-3	0.33, 0-3	0.925

“-” = vacancy.

CPI: The data are presented as n (%). The categories of CPI were defined as: 0 = healthy gingiva; 1 = bleeding observed directly or by using a mouth mirror after probing; 2 = calculus detected during probing but with all of the black bands on the probe visible; 3 = pocket of 4 to 5 mm (gingival margin within the black band on the probe); 4 = pocket of 6 mm (black band on the probe was not visible); X = excluded sextant (<2 teeth present)

Supplementary Table S7. Comparison of Bacterial Diversity, Richness, Observed Operational Taxonomic Units (OTUs) and Uncultured Species among the Three Groups.

	Groups			<i>P</i> value
	HD1 (N=23)	HD2 (N=61)	HD3 (N=24)	
Chao	263.44±46.04	257.10±37.52	258.00±46.67	0.826
Shannon	3.51±0.33	3.40±0.54	3.40±0.44	0.829
Observed OTUs	221.30±39.45	217.87±33.39	212.67±38.68	0.639
Uncultured species	21.93±10.00-	23.63±14.04	23.96±15.15	0.896

The data are presented as “Mean ± SD”

Supplementary Table S8. Relative Abundance of the Top 10 Bacterial Phyla.

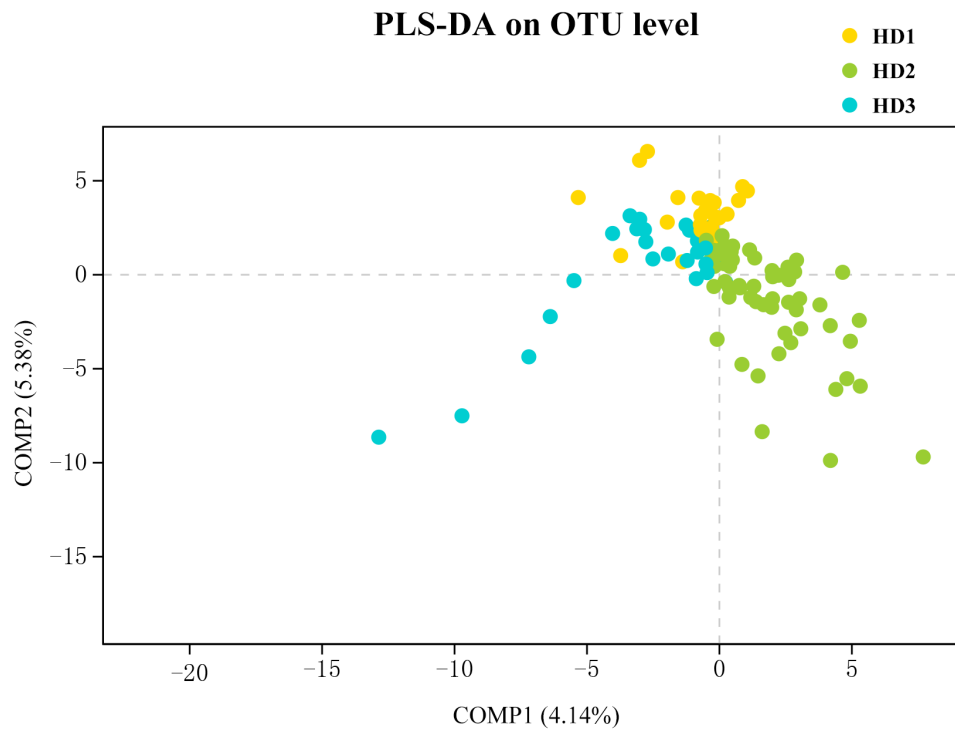
	Groups			<i>P</i> value
	HD1 (N=23)	HD2 (N=61)	HD3 (N=24)	
<i>Firmicutes</i>	51.04±9.24	53.36±15.04	53.85±14.93	0.749
<i>Proteobacteria</i>	19.70±8.55	19.66±11.64	20.74±11.84	0.917
<i>Bacteroidetes</i>	18.58±8.03	16.26±10.74	15.66±9.02	0.545
<i>Actinobacteria</i>	3.47±2.60	4.26±3.71	3.04±2.63	0.510
<i>Fusobacteria</i>	2.88±2.17	2.46±1.88	2.61±1.91	0.770
<i>Synergistetes</i>	1.08±1.33	0.83±1.06	1.09±1.89	0.594
<i>Tenericutes</i>	0.93±1.08	0.84±1.27	0.72±1.09	0.158
<i>Spirochaetae</i>	0.73±1.00	0.78±0.94	0.86±0.99	0.819
<i>Gracilibacteria</i>	0.93±2.38	0.69±0.95	0.88±2.34	0.758
<i>Saccharibacteria</i>	0.35±0.39	0.51±1.37	0.26±0.29	0.431
Others	0.30±0.31	0.35±0.30	0.28±0.26	0.439

The data are presented as “Mean ± SD”

Supplementary Table S9. Relative Abundance of the Species Associated with Diseases in the Three Groups.

Specie	Groups			<i>P</i> value
	HD1	HD2	HD3	
	(N=23)	(N=61)	(N=24)	
The species belonging to the “Red Complex”				
<i>Porphyromonas gingivalis</i>	1.12±2.02	1.86±4.03	1.45±1.85	0.269
<i>Tannerella forsythia</i>	0.28±0.25	0.28±0.33	0.27±0.35	0.724
<i>Treponema denticola</i>	0.12±0.20	0.09±0.11	0.10±0.12	0.902
The species belonging to the “Orange Complex”				
<i>Campylobacter showae</i>	0.59±0.74	0.97±2.22	0.57±0.61	0.953
<i>Fusobacterium nucleatum</i>	0.97±0.79	0.95±0.94	0.71±0.37	0.727
<i>Fusobacterium periodonticum</i>	1.06±1.49	0.70±0.77	0.54±0.59	0.517
<i>Prevotella intermedia</i>	0.29±0.59	0.42±0.61	0.42±0.73	0.705
<i>Prevotella melaninogenica</i>	0.01±0.03	0.01±0.01	0.01±0.01	0.633
<i>Prevotella nigrescens</i>	0.07±0.12	0.08±0.15	0.16±0.39	0.634

The data are presented as “Mean ± SD”



Supplementary Figure S1. PLS-DA discriminant analysis plot. PLS-DA: Partial least squares-discriminant analysis; HD1 group (N=23): $3m < \text{duration of HD} \leq 1y$; HD2 group (N=61): $1y < \text{duration of HD} \leq 5y$; HD3 group (N=24): $5y < \text{duration of HD}$.