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Gut microbiota in Parkinson's disease patients: hospital-based study

Eman M. Khedr^{1,2*} , Anwar M. Ali¹, Enas Deaf³, Hebatallah M. Hassan³, Ahmed Alaa¹ and Ayman Gamea⁴

Abstract

Background: Parkinson's disease (PD) is one of the most common neurodegenerative diseases. There is accumulating evidence that link gut microbiota to symptomatology and pathophysiology of PD. The aim of this study was to describe the pattern of gut microbiota and its association with PD and identify the effect of environmental factors on gut microbiota. This case-control study included 46 patients diagnosed as Parkinson's disease (PD) and 31 healthy volunteers age and sex matched. Detailed history including age of onset, duration of disease, environmental risk factors, diet data, treatment, Unified Parkinson's Disease Rating Scale (UPDRS), and gastrointestinal tract (GIT) domain of Non-Motor Symptoms Scale (NMSS) were assessed. After extraction of bacterial DNA from the fecal samples, bacterial abundance was quantified by qPCR using 16S rRNA group-specific primers.

Results: Significant high abundance of Clostridium cluster IV, Akkermansia, Bifidobacterium, and lactic acid bacteria were found in the PD group compared with the control group ($P < 0.001$, 0.04, 0.02 and < 0.001 , respectively), while Firmicutes were significantly less abundant in the PD group ($P < 0.001$) compared with the control group. The naive PD patients had significant abundance of Bifidobacterium, and lactic acid compared with control group. Interestingly, Akkermansia was more abundant in treated than untreated patients. There were significant associations between pesticide exposure and Bifidobacterium ($P = 0.002$), while no significant correlations between different gut microbiota and demographic, environment data, different rating scores or dominant type of PD. There was a significant negative correlation between the Bifidobacterium with the duration of illness ($P = 0.012$).

Conclusion: The present study highlighted a significant connection between PD and levels of certain types of gut microbiota, in support of a possible link between gut microbiota and a neurodegenerative cascade of PD.

Keywords: Gut microbiota, Parkinson's disease, GIT domain non-motor symptoms

Background

Parkinson's disease (PD) is a multisystem and progressive neurodegenerative disease that is mainly due to loss of dopaminergic neurons in the substantia nigra pars compacta, and characterized by the cardinal motor symptoms of tremor, bradykinesia and rigidity [1]. There are also a number of non-motor symptoms that affect the quality of life in PD patients over time [2]. A common complaint in this category are gastrointestinal (GIT) dysfunctions

including weight loss, gastroparesis, constipation and dysfunctional defecation [3] that often occur prior to motor symptoms. Indeed, early involvement of GIT dysfunction is considered a possible pre-symptomatic stage of PD [4].

Gut microbiota influences intestinal motility, permeability, secretion and immunity, and passes signals to central nervous system (CNS) through microbiota-gut-brain axis [5]. Each individual has their own specific gut microbiota and a variety of exogenous factors can affect their composition including diet, infection, lifestyle, use of antibiotics and hygiene preferences [6]. Very recently, studies revealed that diet changes, such as a high energy-dense diet and high-sugar diet, induce gut microbial

*Correspondence: emankhedr99@yahoo.com

¹ Department of Neuropsychiatry, Assiut University Hospital, Faculty of Medicine, Assiut, Egypt

Full list of author information is available at the end of the article

dysbiosis, increase gut inflammation and microglia activation, and disrupt vagal gut–brain communication [7]. A positive correlation between gut microbiota and clinical phenotype has been found between the abundance of Enterobacteriaceae and severity of postural instability and gait difficulty, suggesting that one or more changes in specific bacteria may be related to the symptoms or pathology of PD [8].

Few studies have evaluated the association between gut microbiota and Parkinson's disease [9–11] as they evaluated microbiota composition in fecal samples of PD patients and age-matched controls. There were discrepancies between different studies which may be attributed to many influencing factors, e.g., diet, geographic origin, and ethnicity. As there has been no previous study in Egypt, we aimed to describe the pattern of gut microbiota in sample of PD patients and its relationship with different environmental and risk factors for PD.

Methods

Forty-six consecutive patients (33 males, 13 females) who fulfilled the UK Parkinson's Disease Brain Bank criteria for idiopathic PD [12] were recruited via a non-probability sampling with ages between 50 and 70 years from those who attended the Department of Neurology of our University Hospital from March 2019 to September 2020. Patients with features supporting other forms of Parkinsonism (such as repeated head injury, cerebrovascular strokes or encephalitis), oculogyric crises, supranuclear gaze palsy, a history of prior abdominal surgeries and/or history of metabolic disorders or autoimmune diseases were excluded. In addition, we excluded patients who had taken antibiotics or probiotic supplements for the 3 months prior to taking samples. Patients with a history of using medications that have been shown to affect gut microbiota, including COMT inhibitors, and metformin were also excluded. Thirty-one age- and sex-matched healthy relatives were included as control group. Recruiting the relatives of PD patients as controls could help offsetting the potential impact on gut microbiota by the dietary factor, physical and socioeconomic stress that is present in both PD patients and their relatives. They have no symptoms or signs of parkinsonism or potential pre-motor symptoms with the same exclusion criteria of the patients above.

All participants gave written informed consent before participation in the study following a full explanation of the protocol. The IRB committee approved the study protocol.

The procedures involving human subjects are done in accord with the ethical standards of the Committee on Human Experimentation of the institution in which the

experiments were done or in accord with the Helsinki Declaration of 1975 [13].

Demographic and clinical data were collected including age, gender, residency (rural/urban), occupation, age of onset, duration of illness, severity of disease, clinical symptoms and signs environmental risk factors (including smoking and exposure to pesticides), diet data (vegetarian/non-vegetarian), type of vegetable consumption (raw/cooked), fruits intake, source and amount of drinking water), past history of trauma, family history of PD and or dementia, treatment as well as rating scores of the Unified Parkinson's Disease Rating Scale (UPDRS) [14], modified Hoehn and Yahr scale (H&Y) [15], Self-Assessment Scale for PD, it is 30 items self-completed questionnaire with response yes or no covers the various non-motor symptoms of PD experienced by the patients in last month [16], gastro-intestinal tract domain of the Non-Motor Symptom Assessment Scale (NMSS) [17]. As the constipation has impact on gut microbiota, we classified the patients according to the presence or absence of constipation to compare between them in different microbiota. Patients were divided into two subgroups, tremor-dominant and akinetic-rigid, on the basis of individual items of the UPDRS III scale as described by Schiess and colleagues [18]. Patients were classified as tremor-dominant if the tremor score was at least twice the non-tremor score. Conversely, the patient was classified as akinetic-rigid type if the non-tremor score was at least twice the tremor score. The remaining patients, in whom the tremor and non-tremor score differed by less than a factor 2, were classified as mixed type.

Stool samples were collected and stored at $-18\text{ }^{\circ}\text{C}$ in subject's home freezer and at $-70\text{ }^{\circ}\text{C}$ upon arrival the laboratory. The total bacterial DNA was extracted from fecal samples using the QIAamp[®] DNA Stool mini kit (Qiagen GmbH, Germany, Cat No 12830-50) according to the manufacturer's protocol. Following this, the DNA concentration and RNA purity were determined using a spectrophotometer (GeneQuart 1300, Germany). The Bacterial abundance was quantified by qPCR using SYBR Green qPCR in a Rotor gene 3000 (Corbett Life Science, Australia) using 16S rRNA group specific primers as this is the available in our lab.

Statistics

The statistical software SPSS (IBM SPSS Statistics for Windows, Version 24.0 Armonk, NY: IBM Corp.) was used for data processing and analysis. The central tendency and variability of the numerical data were presented in the form of mean \pm standard deviations (SD). Categorical variables were summarized by frequency counts and percentages. Because the data had a non-normal distribution as detected by Shapiro–Wilk test,

we used the Mann–Whitney test to compare the two groups with each other and the Kruskal–Wallis H test to compare three or four groups with each other. Non-parametric Spearman correlations between the copy number of each microbiome and different clinical scales were performed. A P -value < 0.05 was regarded as statistically significant.

Results

Forty-six cases of idiopathic PD and 31 healthy controls were included. PD patients had mean age of onset 60 ± 3.7 years with illness duration of 35.5 ± 23.9 months. The UPDRS motor section score was 47.3 ± 32 , and the NMSS of GI was 13.6 ± 9.5 . There were no significant differences between PD patients and controls in age, sex, duration of smoking, or number of cigarettes per day. Sixteen patients each were classified as tremor-dominant and akinetic-rigid. There were no significant differences in environmental risk factors between PD patients and controls. Details are illustrated in Table 1.

Thirty-nine patients were on PD medications including carbidopa/levodopa and dopamine agonists and 7 patients were naive without any medication when the samples were collected.

As a primary outcome there was a significantly higher copy number of Clostridium cluster IV, Akkermansia, Bifidobacterium, and lactic acid bacteria and lower abundance of Firmicutes in PD than in the control group ($P < 0.001$, 0.04, 0.02 and < 0.001 , < 0.001 , respectively), particularly in treated patients.

On the other hand, the naive PD patients had significant abundance of Bifidobacterium, and lactic acid compared with control group. Interestingly, no significant difference between treated and untreated except in Akkermansia as it is lower in untreated than treated patients and nearly similar to control group (Table 2).

There were significant associations between pesticide exposure and Bifidobacterium ($P = 0.002$) as PD patients who had been exposed to pesticide had a lower level of Bifidobacterium than non-pesticide exposed patients (Table 3).

There was a significant negative correlation between the level of Bifidobacterium and duration of illness ($\rho = -0.414$, $P = 0.012$). There were no significant correlations between any gut microbiomes and different PD assessment scales (UPRS, Self-assessment Scale, and NMSS). On the other hand, there was a borderline significant correlation between Hoehn and Yahr staging and Akkermansia ($P = 0.056$). There were no other significant correlations between gut microbiota and stages or predominant types of PD (Table 4).

Likewise, there were no significant differences of gut microbiota between tremor-dominant, akinetic-rigid, and mixed types of PD (Table 5).

On comparison between PD patients with constipation and patients without constipation, there was a significantly higher copy number of Akkermansia in constipating patients than patients without constipation (mean \pm SD; 8.6 ± 1.6 and 7.2 ± 1.9 , respectively, with $P = 0.02$) while no significant differences in other gut microbiota between constipating versus non-constipating patients (Table 6).

Discussion

Gut microbiota and PD

This study provides further insight into the association between gut microbiota and PD. The main finding was a significantly higher copy number of gut microbiota from Clostridium cluster IV, Akkermansia, Bifidobacterium, and lactic acid bacteria in the PD group compared with the control group, while Firmicutes were significantly lower in PD ($P < 0.001$).

The higher Clostridium cluster IV in PD patients was consistent with data reported by Qian and colleagues [19]. Clostridium produces elevated levels of noxious brain metabolites that can lead to neurological symptoms [20].

The high abundance of Akkermansia microbiome was consistent with Heintz-Buschart and colleagues, and Keshavarzian and coworkers [10, 11]. They evaluated microbiota composition in fecal samples of PD patients and age-matched controls, and all found that Akkermansia was more abundant in fecal samples from PD patients. Previous studies have shown that Akkermansia causes alterations in the function of the mucosal barrier that substantially increases the intestinal permeability. Akkermansia uses mucus as a source of energy, which leads to increased exposure of microbial antigens to the immune cells [21]. Endotoxins derived from the gut can also activate α -synuclein aggregation or neuronal damage [21, 22].

The result of the present study was also on line with Bedarf and colleagues [9] as they found an increase in Akkermansia in PD patients. Kang and colleague on their experimentally induced colitis found that the protective effects of extracellular vesicles derived from *Akkermansia muciniphila* support a beneficial influence on intestinal immunity [23]. Inflammatory and regulatory properties have been reported for Akkermansia, probably mediated due to an increased exposure of immune cells to microbial antigens upon breaking down the mucosal mucin layer [24]. As previous studies on colonic biopsies and feces samples from treated and drug-naïve PD participants the authors suggested that an altered mucosal

Table 1 Demographic and clinical characteristics of the studied groups

	Parkinson's patients (n = 46)	Control (n = 31)	P-value
Age (mean ± SD years)	62.8 ± 4.7	61.1 ± 3.1	0.07
Range	50–70	55–65	
Sex: (number)			
Male/female	33/13	23/8	0.57
Age of onset (mean ± SD years)	60 ± 3.7		
Range	50–70		
Duration of illness (mean ± SD months)	35.5 ± 23.9		
UPDRS (mean ± SD)	47.3 ± 32		
Self-assessment (mean ± SD)	11.3 ± 6.3		
Non-motor scale of GIT (mean ± SD)	13.6 ± 9.5		
Number of cigarettes per day (mean ± SD)	4.1 ± 8.2	5.2 ± 9.7	0.65
Duration of smoking in years (mean ± SD)	3 ± 6.3	2.1 ± 3.3	0.43
Predominant type: Number (%)			
Tremor dominant type	16 (34.8%)		
Akinetic-rigid type	16 (34.8%)		
Mixed type	14 (30.4%)		
Hoehn and Yahr staging number (%)			
Stage 1	20 (43.5%)		
Stage 2	16 (34.8%)		
Stage 3	6 (13%)		
Stage 4	4 (8.7%)		
Residency (rural)	26 (56.5%)	16 (51%)	0.32
Residency (urban)	20 (43.5%)	15 (49%)	
Source of water (private water system)	44 (95.7%)	31 (100%)	0.16
Source of water (private and mineral water)	2 (4.3%)	0	
Drinking water (1–2 L/days)	21 (45.7%)	12 (38.7%)	0.16
Drinking water (> 2–3 L/days)	20 (43.5%)	10 (32.3%)	
Drinking water (> 3–4 L/days)	5 (10.9%)	9 (29%)	
Smoking (cigarette, tobacco, shisha)	25 (54.3%)	17 (54.8%)	0.89
Pesticide (no) number (%)	35 (76.1%)	18 (51%)	0.57
Pesticide (yes) number (%)	11 (23.8%)	15 (49%)	
Pesticide (rare)	3 (6.5%)	5 (16.2%)	
Pesticide (occasional)	6 (13%)	6 (19.8%)	
Pesticide (often)	2 (4.3%)	4 (13%)	
Family history of PD or dementia (no)	33 (71.7%)	23 (74%)	0.53
Family history of PD or dementia (yes)	13 (28.3%)	8 (26%)	

PD Parkinson's disease

barrier function and PD patients exhibited significantly greater intestinal permeability than controls might be associated with unexplored disease-related impact on mucosal barrier function [21].

In addition, Bifidobacterium produces lactic acid, vitamins B1, B2, and K and decreases oxidative stress markers. Lactic acid prevents the growth of pathogenic and harmful bacteria, maintains intestinal microbial homeostasis, and modulates the local and systemic immune response [22, 25]. Taken together, the observed

bacterial pattern in our PD samples may be related to yet unexplored mechanisms of a disturbed intestinal and immune function in PD pathogenesis.

Patients with PD had higher levels of lactic acid bacteria than controls. Studies have shown that Lactobacillaceae is associated with decreased levels of ghrelin intestinal hormone [26], which regulates nigrostriatal dopamine activity, prevents neurodegeneration, modulates afferents of the enteric nervous system (ENS) and accelerates gastric emptying. The latter may contribute

Table 2 Copy number of gut microbiota among Parkinson’s disease patients and control group

	Total PD patients (n = 46)	PD patients under treatments (n = 39)	Newly diagnosed (non-treated patients) (n = 7)	Control (n = 31)	P-value			
					Total PD versus controls	Treated PD versus controls	Newly PD versus controls	Newly versus treated patients
Clostridium cluster IV	11.5 ± 3.7	11.9 ± 3.7	9.8 ± 4.3	8.04 ± 1.38	< 0.001	< 0.001	0.39	0.378
Firmicutes	7.6 ± 2.3	7.3 ± 2.2	9.0 ± 2.7	9.64 ± 1.89	< 0.001	< 0.001	0.90	0.061
Akkermansia	8.0 ± 1.9	8.3 ± 1.9	6.5 ± 0.97	6.75 ± 1.92	0.012	0.005	1.0	0.043
Bifidobacterium	10.8 ± 1.4	10.7 ± 1.4	11.4 ± 1.2	9.36 ± 1.69	< 0.001	0.006	0.004	0.238
Lactic acid bacteria	12.4 ± 2.1	12.3 ± 2.3	13.3 ± 2.2	9.98 ± 3.84	0.003	0.04	0.022	0.289

PD Parkinson’s disease

Table 3 Correlation between copy number of different gut microbiota and Parkinson’s disease characteristics

Spearman correlation	Clostridium cluster IV	Firmicutes	Akkermansia	Bifidobacterium	Lactic acid bacteria
Duration of illness (years)					
Rho-value	0.064	− 0.042	0.204	− 0.414	− 0.054
P-value	0.704	0.793	0.214	0.012*	0.742
UPDRS score					
Rho-value	0.103	− 0.092	0.286	− 0.172	− 0.037
P-value	0.538	0.567	0.078	0.315	0.821
Self-assessment score					
Rho-value	0.088	0.033	0.074	− 0.031	− 0.032
P-value	0.601	0.836	0.655	0.860	0.848
NMS of GIT score					
Rho-value	− 0.055	0.038	0.100	− 0.143	− 0.101
P-value	0.742	0.814	0.543	0.405	0.542
Predominant type Tremors versus akinetic versus mixed)					
Rho-value	− 0.198	0.241	− 0.261	0.160	0.130
P-value	0.234	0.129	0.108	0.352	0.431
Hoehn and Yahr stages (stage I, II, III, IV)					
Rho-value	0.120	− 0.158	0.308	− 0.022	0.054
P-value	0.473	0.323	0.056	0.898	0.745

PD Parkinson’s disease, UPDRS score Unified Parkinson’s Disease Rating Score, NMS of GIT score; Non-motor symptoms of gastrointestinal tract score

*Correlation is significant P < 0.05 level (2-tailed)

Table 4 Correlation between gut microbiota and risk factors of Parkinson’s disease

Risk factors	Clostridium cluster IV	Firmicutes	Akkermansia	Bifidobacterium	Lactic acid bacteria
Pesticide (exposed versus non-exposed)	0.848	0.786	0.552	0.002	0.174
Smoking versus non-smoking	0.400	0.808	0.303	0.289	0.509
Constipating (n = 22) versus non-constipating patients (n = 24)	0.559	0.639	< 0.001	0.924	0.911
Water (1–2 L/D versus > 2–4 L/D)	0.602	0.672	0.545	0.424	0.520
Residency (urban versus rural)	0.976	0.528	0.367	0.728	0.887
Family history of dementia or PD (positive versus negative)	0.353	0.537	0.368	0.877	0.974

L/D liter per day, PD Parkinson’s disease

Table 5 Gut microbiota and predominant types of Parkinson's disease in comparison to each other

Bacteria	Number			Tremors versus akinetic rigid			Tremors versus mixed type			Akinetic-rigid versus mixed type		
	T	A	M	Mean ± SD		P value	Mean ± SD		P value	Mean ± SD		P value
Clostridium cluster IV	14	12	12	12.6 ± 3.7	10.7 ± 3.5	0.19	12.6 ± 3.8	11 ± 3.8	0.29	10.7 ± 3.5	11 ± 3.8	0.84
Firmicutes	15	14	12	7.0 ± 2.0	7.0 ± 2.5	0.98	7.3 ± 2.4	8.0 ± 2.0	0.42	7.0 ± 2.5	8.0 ± 2.0	0.27
Akkermansia	13	14	12	8.5 ± 1.7	8.0 ± 2.0	0.49	8.5 ± 1.7	7.0 ± 1.6	0.07	8.0 ± 2.0	7.0 ± 1.6	0.17
Bifidobacterium	13	13	10	10.7 ± 1.0	10.0 ± 1.0	0.09	10.8 ± 1.0	11.3 ± 1.4	0.24	10.0 ± 1.4	11.0 ± 1.4	0.13
Lactic acid bacteria	14	14	11	12.0 ± 2.0	12.0 ± 2.0	0.98	12.0 ± 2.0	13 ± 2.5	0.27	12.0 ± 2.0	13.0 ± 2.5	0.27

T tremor dominant, A akinetic-rigid, M mixed type

Table 6 Copy number of gut microbiota among PD with constipation versus patients without constipation

	Patients with constipation (n = 22)	Patients without constipation (n = 24)	P value
Clostridium cluster IV	12.03 ± 3.59	10.99 ± 3.87	0.559
Firmicutes	7.50 ± 2.36	7.70 ± 2.38	0.639
Akkermansia	9.06 ± 1.37	6.79 ± 1.66	<0.001
Bifidobacterium	10.85 ± 1.27	10.78 ± 1.50	0.924
Lactic acid bacteria	12.46 ± 2.14	12.42 ± 2.21	0.911

to delayed gastric emptying in some patients with PD [27].

In the present study, there was significantly less abundance of Firmicutes in the PD group than in the control group which was perfectly in line with the data of Keshavarzian and coworkers [11] who found a lower proportion of Firmicutes in PD. The lower abundance of this short chain fatty acid (SCFA) butyrate-producing bacteria could have harmful effects for the PD colon, including intestinal barrier integrity and immune function [28, 29]. The main site of action for SCFA appears to be the colon, but SCFAs are also taken up in the blood and exert effects on other organs, including the brain [30]. SCFA butyrate has anti-inflammatory properties thought to be due to an epigenetic mechanism [31] or to the effects of SCFA receptors that are anti-inflammatory, anti-microbial, and decrease intestinal barrier leakiness [32, 33]. Thus, the low abundance of butyrate-producing bacteria in feces from PD subjects may be one mechanism contributing to intestinal leakiness [34] and inflammation reported in PD [21].

Another reason for the heterogeneous finding across studies may be due to dietary habits. Short-term consumption of diets composed entirely of animal or plant products alters microbial community structure and

contributes to inter-individual differences in microbial gene expression. The animal-based diet increased the abundance of bile-tolerant microorganisms (Alistipes, Bilophila and Bacteroides) and decreased the levels of Firmicutes that metabolize dietary plant polysaccharides (Roseburia, Eubacterium rectale and Ruminococcus bromii) [35].

In the present study, a significant negative correlation between Bifidobacterium and duration of illness (P-value = 0.012) was recorded. Keshavarzian and coworkers [11] also reported a significant correlation between PD duration and numerous bacterial taxa regardless of medications, indicating that PD duration has a significant impact on microbial communities. However, it is unclear at present whether the relationship is causal.

Like Unger and colleagues [36], we found no correlations between gut microbiota and severity of symptoms (UPDRS, self-assessment scale, predominant type, disease stage, and non-motor scale of GIT). They found that the abundance of gut microbiota did not differ between PD subgroups, even after comparing hypokinetic-rigid and mixed types to a tremor-dominant group. However, Hasegawa and colleagues [37] found that non-tremulous PD patients had a higher level of Bacteroides than in tremor subtypes. Others found that Bacteroides abundance correlated with motor symptom severity as defined by UPDRS part III motor scores [38] and in another study, the abundance of Enterobacteriaceae was positively associated with the severity of postural instability and gait difficulty [39]. Tan and colleagues [40] detected a small intestinal bacterial overgrowth in 25% of the PD patients, regardless of disease duration, which could be used as a predictor of worse motor function but not GIT symptoms. Gabrielli and colleagues [41] showed that using antibiotics to eradicate the small intestinal bacterial overgrowth improved motor fluctuations without affecting levodopa's pharmacokinetics.

The influence of environmental risk factors on gut microbiota

An important issue in the present study was that the control group were recruited from relatives of our patients with nearly same age- and sex-distribution in order to limit any differences in diet, residency, job, water source, pesticide exposure, and daily intake of fluid and water, or fruits as confirmed by the absence of significant differences between PD patients and controls in any of these items. Our findings showed that there was a significant association between pesticide exposure and Bifidobacterium levels (P -value = 0.002) in patients with PD, which is higher in non-pesticide exposed patients (11 ± 0.18) than pesticide-exposed patients (10 ± 1.3). Our data with Hill-Burns and colleagues [42] who found that there is may be a correlation secondary to long-standing exposure to pesticide/herbicide contaminant in stream and ground water and the increased activity in PD of pathways that degrade xenobiotics.

In agreement with our findings, exposure to pesticides and herbicides in agricultural setting, including well water drinking, is known to increase the risk of developing PD [43, 44] and causes dopaminergic cell death and motor abnormalities in animal models [45]. The evidence for increased xenobiotics degradation in the gut, therefore, raises testable hypotheses on the role of xenobiotics in initiating the dysbiosis of the microbiome, and whether recent or continued exposure to PD-associated xenobiotics may contribute to the progression of neurodegeneration. Also, Yang and colleagues [46] showed that chronic rotenone administration, at a dose found commonly in pesticides caused fecal microbiota alterations, mitochondrial disruption and dopaminergic neuronal loss along with behavioral and neuropathological features of PD.

Effect of medication on gut microbiota

Most of the previous studies including PD patients with advanced and treated with L-DOPA [8, 11, 36] which affects colonic motility and may promote intestinal bacterial overgrowth [47]. To avoid alterations of gut microbiota related to late-stage PD or L-DOPA-induced intestinal effects, we included 7 untreated naive PD patients with early stage. In the present study, the naive PD patients had significant abundance of Bifidobacterium, and lactic acid bacillus compared with control group. Supporting our results Bedarf and colleagues [9] found that early, L-DOPA-naïve PD patients carried an altered gut microbiota composition. Weis and colleagues found that the relative abundances of the bacterial genera including Bifidobacterium, and Enterococcus, were significantly influenced by medication with L-dopa and

entacapone, respectively [48]. This study was performed on fecal samples of naïve 34 PD patients and followed up 3 months after medication.

On the other hand, Lactobacillus strains are low abundant members of the gut microbiota and their abundance varies greatly across human disease and chronic conditions [49]. Some strains of Lactobacillus are able to produce enzymes that can degrade levodopa into dopamine, suggesting that their abundances might be a consequence of the use of this medication in PD [50, 51]. Levodopa is absorbed in the small intestine, but it has been reported that 10–20% can reach the large intestine [52] and could thus help these bacteria to proliferate. However, the presence of abundance of lactic acid bacteria in naïve PD patients in our study suggested that the abundance of it is not related to medication.

Interestingly, there was no significant difference between treated and untreated except in Akkermansia as it is more abundant in treated than non-treated patients. Constipated individuals have been shown to have a gut microbiome enriched in Akkermansia [53, 54], and constipation is one of the major non-motor symptoms in PD. As a number of medications (anticholinergic) used to treat PD can cause constipation so the abundance of Akkermansia in treated patients may be related to both medication and chronicity of disease-related constipation.

The differences between the findings of our and other studies might also be based on the distinct geographic and cultural background of our study cohort, since the human gut microbiota is regionally different [55].

Limitations

The main limitation of our study was the relatively small sample size, and few numbers of gut microbiota analyzed. Further studies with larger sample sizes, and analysis of more gut microbiota and control of dietary factors are needed, as different geographical locations have different dietary habits.

Conclusions

Our study highlights a significant connection between PD and gut microbiota. The maintenance of a healthy microbiota is important for gut barrier integrity and immunity.

Abbreviations

CNS: Central nervous system; ENS: Enteric nervous system; GIT: Gastrointestinal tract; H&Y: Modified Hoehn and Yahr scale; NMSS: Non-Motor Symptoms Scale; PD: Parkinson's disease; SCFAs: Short chain fatty acids; UPDRS: Unified Parkinson's Disease Rating Scale.

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Authors' contributions

EMK, AM, ED, HH, AA, AG: contributed to study concept and design, acquisition of data, draft and revision of the report, statistical analyses, and interpretation of data. ED, HH, and AA contributed to case recruitments, acquisition of data and statistical analyses. HH, AA, AM, AG contributed to editing of this report. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author reasonable on request.

Declarations**Ethics approval and consent to participate**

An informed written consent was obtained from all the patients before participating in the study. The protocol was approved in October 2018 by IRB committee of Assiut University Hospital and all participants or relatives gave written informed consent before participation in the study. The ethical approval Reference number was 17100608. The confidentiality of the patients' information was maintained during all the steps of the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Neuropsychiatry, Assiut University Hospital, Faculty of Medicine, Assiut, Egypt. ²Department of Neuropsychiatry, Aswan University Hospital, Faculty of Medicine, Aswan, Egypt. ³Department of Medical Microbiology and Immunology, Faculty of Medicine, Assiut University, Assiut, Egypt. ⁴Department of Neuropsychiatry, South Valley University Hospital, Qena, Egypt.

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