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Original article

Clinical phenotypes and constipation severity in Parkinson’s disease: Relation to Prevotella species

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ABSTRACT

Background: The gut microbiome is speculated to play a crucial role in its pathogenesis of Parkinson’s disease (PD) as a triggering factor. Recent hypotheses suggested that Prevotella species regulate gut permeability, exert a neuroprotective effect, and interestingly, has been suspected to be deficient in PD patients, and so may play a role in this disease. Aim: This study was designed to compare between PD patients and their healthy controls as regards relative Prevotella abundance, prevalence of Prevotella-dominant Enterotype, and constipation severity. Also, to correlate Prevotella changes with the clinical phenotypes and severity of motor and non-motor symptoms (NMS) of PD. Methods: Twenty-five PD cases were enrolled in this study and cross-matched to 25 healthy subjects representing the control group. Overall NMS severity was assessed using the Non-Motor Symptoms Scale (NMSS). Quantitative SYBR green Real Time PCR was performed for the identification and quantitation of Prevotella in stool. Results: Prevotella relative abundance was 4-fold decreased in cases when compared to controls with postural instability and gait difficulty (PIGD) phenotype showing the lowest abundance, however the difference was not statistically significance. Prevotella-dominant Enterotype was less presented in cases compared to controls, the result was statistically significant. Severe and very severe constipation grades presented 64% of cases group vs 12% of control group. There was statistically significant positive correlation between total constipation score and Unified Parkinson’s Disease Rating Scale total score and motor symptoms phenotypes. Conclusion: Relative low Prevotella abundance in PD patients appears to be related to severe phenotypes of the disease; PIGD and mixed phenotypes. Severe constipation was more presented in PD cases which may be considered as a preclinical biomarker for PD.

Keywords: Parkinson’s disease
Gut microbiome
Dysbiosis
Prevotella
Non-motor symptoms

Introduction

Parkinson’s disease (PD) is a neurodegenerative disorder characterized by motor (rigidity, bradykinesia, and tremor) and non-motor symptoms (constipation, sleep disturbances, pain, and depression) [1], with idiopathic constipation is considered one of the strongest risk factors for PD [2].

Recent neuroscience research hypothesizes that the pathology of PD may start in the gut; where α-Syn aggregates in the enteric nervous system (ENS), then spreads in retrograde fashion to the...
central nervous system (CNS) along the vagus nerve [3]. Lewy bodies and α-synuclein, the neuropathological hallmarks of PD, were found to appear in the gut before they appear in the brain [4].

With regards to PD, gut dysbiosis might have a role in disease pathogenesis through interactions of the gut-brain axis, where bacterial metabolites increase gut permeability allowing for lipopolysaccharides and short chain fatty acids (SCFAs) to leak, stimulating the production of proinflammatory cytokines which can cause neuroinflammation upon crossing the blood-brain barrier [5]. This might promote α-Syn misfolding and subsequently neuronal degeneration which manifests as exacerbations of PD symptoms [6].

Of particular interest in the gut microbiome is the family Prevotellaceae, the genus Prevotella. A decrease in the Prevotella populations of PD patients compared to controls has been suggested [7]. Prevotella participates in mucin synthesis which maintains gut permeability, alongside production of neuroprotective SCFAs which may exert a protective effect on dopaminergic neurons against degeneration [5].

Prevotella are also found to be involved in protection of dopaminergic neurons through secretion of a gaseous gut neurotransmitter hydrogen sulfide. Decreased levels of Prevotella are found to be associated with lower levels of uric acid which is known to have a protective effect against PD. Lower levels of Prevotella in PD patients were also associated with decreased levels of ghrelin, which is a gut hormone known to regulate nigrostriatal dopamine function and is speculated to limit PD associated neurodegeneration. Given the dimension of the problem and its pathophysiologic and prognostic impact for PD, gut dysbiosis remains an issue that has to be clarified and better characterized [5-7].

This study aimed to correlate between prevalence of Prevotella and Prevotella-dominant Enterotype between PD patients and healthy controls. Also, to correlate between Prevotella abundance with PD clinical phenotypes and severity of its motor and non-motor symptoms.

Patients and Methods

This cross-sectional study included twenty-five PD cases, who presented to the movement disorder Clinic of Neurology Department of Alexandria University Hospital, were enrolled as well as a cross matching control group of 25 healthy subjects of similar age and sex. Patients were enrolled over a period of six months starting March till August 2020. An approval was obtained from Alexandria Faculty of Medicine Ethics Committee. After receiving informed consent from the cases, a clinical history & examination for each case and stool sample were obtained. Patients’ inclusion criteria were; age older than 50 years, no previous intake of any type of probiotics, antibiotic or antifungal medications within the last month, clinical evaluation of PD cases was performed by neurologist to confirm PD diagnosis. As for the control group, included healthy participants without any signs of PD or potential premotor symptoms. As for the exclusion criteria; patients with severe renal or liver impairment, inflammatory bowel disease or irritable bowel syndrome, patients with other neurological disorders, or history of recent antibiotic use.

Parkinsonian symptoms were measured using the Unified Parkinson’s Disease Rating Scale (UPDRS) [8]. The PD were clinically classified into 3 phenotypes; tremor dominant (TD), postural instability and gait difficulty (PIGD), and mixed phenotypes (MX) as described by Chaudhuri et al. [9].

The degree of constipation was quantified in more detail using the Wexner constipation score (Cleveland Clinic Constipation Scoring System) [7]. The questionnaire includes different variables: frequency of bowel movement, difficulty, completeness, abdominal pain, time, type of assistance for defecation, failure, and history (duration of constipation). A scoring ranges from 0 (normal) to 4 (severe condition) is derived. Finally, constipation is graduated as mild (score 1–5), moderate (6–10), severe (11–15), and very severe (15–20) [7].

Detection of Prevotella:

1-Stool specimens were collected and within the same day delivered to Alexandria University Main Microbiology laboratory frozen, where aliquots of each specimen were frozen at −80 °C until DNA extraction in the same week.

2-DNA was extracted from 180-220 mg stool samples using QIAamp® Fast DNA Stool Mini Kit. DNA extracts were stored at −80°C until PCR testing. Two µl of DNA extract was used in the PCR reaction.
Correlation between PD clinical parameters total p. According to Wexner Score 6-ery severe.

Comparison of symptoms phenotypes total constipation score and PD clinical motor unified PD rating scale (UPDRS). There was statistically significant positive correlation between total constipation score and PD clinical motor symptoms phenotypes where it was significant with both tremor dominant and postural instability and gait difficulty clinical phenotypes (Table 2).

Comparison between the studied groups and Enterotypes

Enterotype 1 is the most common enterotype detected in PD cases. Eighteen (72%) of the 25 PD patients had predominant Enterotype 1, 7 (28%) were assigned to Enterotype 2 and none to Enterotype 3. On the other hand, only 7 (28%) of the 25 control cases were assigned to Enterotype 1, 13 (52%) were Enterotype 2 and 5 (20%) were Enterotype 3. Statistically significant difference was detected between the 2 groups regarding the enterotype distribution (p value=0.001) (Table 3).

Comparison of Prevotella in the studied groups and in PD clinical phenotypes

Table 4 and Figure 1 show that patients with PD show a decrease in Prevotella relative abundance (2.26E-02) in comparison to control group (8.09E-02) showing that patients with PD have decreased level of Prevotella in their stool samples and gut microbiome relative to samples from healthy controls. However, this difference was not statistically significant (p=0.138).

When subgrouping patients with tremor, PIGD, and mixed predominant phenotypes and comparing them with the control subjects, all the phenotypes showed lower Prevotella relative abundance than the control group with PIGD phenotype showing the lowest. However, there was no statistically significant difference between the three phenotypes and the control (p-value=0.295). Moreover, there was no statistically significant difference between the three phenotypes (Table 5, Figure 2).

There was no significant correlation of Wexner Constipation Score with Prevotella relative abundance. Prevotella relative abundance was 4-fold decreased in cases when compared to controls, however it failed to reach statistical significance, Prevotella-dominant enterotype was less presented in cases compared to controls, the result was statistically significant (p=0.001). There was highly statistically significant difference between two groups as regards total constipation score, median (Min-Max) in cases group was 13 (2-18) Vs 4 (0-16) with (p value = 0.0001). Also, severe and very severe constipation grades presented 64% of cases group versus 12% of control group, the result was statistically significant (p value = 0.0001).

When studying the correlation between Prevotella relative abundance and the disease duration and
severity, no statistically significant correlation was detected except for; significant positive correlation between the age of PD cases and *Prevotella* relative abundance \((r=0.402, p=0.047)\) (Figure 3).

Table 1. Primer sequences and different bacteria types.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Primer Name</th>
<th>Primer Sequence (5'-3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bacteria</td>
<td>UnivF</td>
<td>TCCTACGGGAGGCACGAGT</td>
</tr>
<tr>
<td></td>
<td>UnivR</td>
<td>GGACTACCAGGGTATCTATCCGTT</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>B3F</td>
<td>CGATGGATAGGGGTTCTGAGAGGA</td>
</tr>
<tr>
<td></td>
<td>B3R</td>
<td>GCTGGCACCGAGTTAGCCGA</td>
</tr>
<tr>
<td>Prevotella</td>
<td>PrevF</td>
<td>CACCAAGGCAGACGATCA</td>
</tr>
<tr>
<td></td>
<td>PrevR</td>
<td>GGATAACGCCYGACCT</td>
</tr>
<tr>
<td>Ruminococcus</td>
<td>Rflbr730F</td>
<td>GGCATGCTTGTGGCTT</td>
</tr>
<tr>
<td></td>
<td>Clep866mR</td>
<td>CCAGGTTGATWACCTTATGGTTAA</td>
</tr>
</tbody>
</table>

Table 2. PD phenotypes and Wexner Constipation Score.

<table>
<thead>
<tr>
<th>Total Constipation score (n = 25)</th>
<th>Phenotype</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tremors Dominant (TD) (n = 17)</td>
<td>Postural Instability Gait Difficulty (PIGD) (n = 5)</td>
</tr>
<tr>
<td>Min. – Max.</td>
<td>0 – 16</td>
<td>2 – 18</td>
</tr>
<tr>
<td>Median</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>(p_{control})</td>
<td>0.008*</td>
<td>0.048*</td>
</tr>
</tbody>
</table>

\(p_1: p\) value for association between Tremors and PIGD
\(p_2: p\) value for association between Tremors and Mixed
\(p_3: p\) value for association between PIGD and Mixed
\(p_4: p\) between cases phenotypes

Table 3. Comparison between the two study groups according to the Enterotypes.

<table>
<thead>
<tr>
<th>Enterotypes</th>
<th>PD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterotype 1</td>
<td>18 (72%)</td>
<td>7 (28%)</td>
</tr>
<tr>
<td>Enterotype 2</td>
<td>7 (28%)</td>
<td>13 (52%)</td>
</tr>
<tr>
<td>Enterotype 3</td>
<td>0 (0%)</td>
<td>5 (20%)</td>
</tr>
<tr>
<td>Total</td>
<td>25 (100%)</td>
<td>25 (100%)</td>
</tr>
<tr>
<td>(MC) (p) value</td>
<td>0.001*</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. *Prevotella* in PD and control groups.

<table>
<thead>
<tr>
<th><em>Prevotella</em></th>
<th>Control</th>
<th>PD Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>1.75E-01</td>
<td>1.79E-01</td>
</tr>
<tr>
<td>Min</td>
<td>1.11E-03</td>
<td>4.33E-06</td>
</tr>
<tr>
<td>Max</td>
<td>6.91E-01</td>
<td>7.61E-01</td>
</tr>
<tr>
<td>Median</td>
<td>8.09E-02</td>
<td>2.26E-02</td>
</tr>
<tr>
<td>(p) value</td>
<td>0.138</td>
<td></td>
</tr>
</tbody>
</table>
Table 5. *Prevotella* in PD phenotypes and control group.

<table>
<thead>
<tr>
<th><em>Prevotella</em></th>
<th>Control</th>
<th>Tremors Dominant (TD) (n =17)</th>
<th>Postural Instability Gait Difficulty (PIGD) (n = 5)</th>
<th>Mixed (n = 3)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min. – Max.</td>
<td>1.11E-03–6.91E-01</td>
<td>2.98E-05–6.26E-01</td>
<td>4.33E-06–7.61E-01</td>
<td>3.77E-03–2.26E-02</td>
<td>0.295</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>1.75E-01±2.03E-01</td>
<td>2.14E-01±2.52E-01</td>
<td>1.60E-01±3.36E-01</td>
<td>1.30E-02±9.42E-03</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>8.09E-02</td>
<td>3.01E-02</td>
<td>1.42E-03</td>
<td>1.27E-02</td>
<td></td>
</tr>
<tr>
<td>p&lt;sub&gt;control&lt;/sub&gt;</td>
<td>0.5</td>
<td>0.11</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. *Prevotella* in PD and control groups.

Figure 2. *Prevotella* in PD phenotypes and control group.

Figure 3. Correlation between *Prevotella* and age.
Discussion

Many studies illustrated significant difference between PD patients and their healthy controls regarding gut microbiome composition. Decreased abundance of Prevotella was one of the most prominent overlaps between the results of these studies [14,15].

According to the results of the current study, relative abundance of Prevotella was 4-fold reduced in PD patients compared to controls, but it was statistically nonsignificant. This is in accordance with some previous studies like those done by Scheperjans et al. 2015 [16], Keshavarzian et al. (2015) in United States of America [12], Hasegawa et al. (2015) in Japan [17], Unger et al. (2016) in Germany [14] and Li et al. (2017) in China [18], who have demonstrated a non-significant decrease of Prevotella in PD patients.

On the other hand, Hill-Burn et al. [19] as well as Barichella et al. [20] failed to find association between Prevotella and Parkinson’s disease in the 2 large studies performed in USA 2017 and Italy 2018 respectively. This difference may, in part, be attributed to dissimilarity in race, sample size, methods used in analysis, confounding factors and dietary habits among these study groups.

In their study Keshavarzian et al., tested both colonic mucosal biopsies and fecal samples for microbiota composition, mucosal biopsies demonstrated also reduced Prevotella abundance which failed to reach statistical significance [12].

Diet is one of the most prominent factors that can influence gut microbiome composition, one of the major limitations in Scheperjans et al study is missing information about the dietary habits of the study participants [16]. In the current study, dietary patterns of the study participants were assessed using a food frequency questionnaire which demonstrated no marked differences between the two study groups.

In the current study Wexner Constipation Scoring System was used to quantify constipation severity in both groups, there was highly statistically significant difference between 2 groups as regards total constipation score.

There was statistically significant positive correlation between total constipation score and both; motor symptoms and UPDRS total score. Frazzitta G et al. [21] demonstrated the same results. The current study confirms higher prevalence of constipation especially severe and very severe grades among PD patients, and association between constipation and severity of PD symptoms. These results support the concept of considering people with severe constipation may have a relatively high risk of developing PD and thus constipation can be used as a preclinical biomarker for PD.

In most PD gut microbiome studies, constipation is a potential confounder. In the current study there was no significant correlation between constipation and relative Prevotella abundance in study samples. Many studies demonstrated the same results [7,21] this suggests that in our subject cohort, constipation had little effect on Prevotella relative abundance.

Disease duration is also a potential confounder in PD gut microbiome studies, in the current study there was no relation between relative Prevotella abundance and disease duration, this was consistent with many recent studies [11,14]. Only Minato et al demonstrated significant reduction in Prevotella abundance between the start of the study and after 2 years of follow up, so disease duration can explain part of the discrepancies in results between PD microbiome studies [22].

In the current study, when contrasting PD motor phenotypes, PIGD subtype (which is associated with faster deterioration of motor and cognitive functions, worse α-Syn pathology in ENS, more resistant to treatment, and has worse prognosis) had lower Prevotella relative abundance than TD subtype, however it did not reach statistical significance. Our results were consistent with Scheperjans et al. [16] and Aho et al. [23] In their study illustrated significant association between Prevotella abundance and UPDRS-III total score, however these results did not match our study results. It was suggested that their results were biased by effect of medications as this association disappeared after eliminating the effect of this confounder. In the current study all patients were on the same group of dopaminergic medications.

Heintz-Buschart et al. [24] in their study provided an important piece of evidence, where Prevotella abundance was decreased in idiopathic rapid eye movement sleep disorder which predates PD in most cases, which supports the hypothesis that in the early stages of PD this family and its genus Prevotella change in PD prodromal stages. It also suggests that Prevotella reduction may contribute to triggering PD pathology and hence can be viewed as
a potential biomarker for PD diagnosis and a possible disease modifying treatment [24].

Finally, although considered sensitive, decreased *Prevotella* abundance alone is not specific for PD. Also, the case control study design cannot rule out the possibility that PD itself could be the reason behind gut microbiome composition changes instead of vice versa, however gut microbiome studies represent huge advancement in our understanding of PD pathology and a promising research area for new treatment modalities.

**Conclusion**

Parkinson’s disease is the second most common neurodegenerative disorder following Alzheimer’s disease. Preclinical PD is the stage preceding the onset of the typical motor symptoms and may last many years making it an excellent research target to seek biomarkers that can help in early disease diagnosis and investigate new disease modifying treatments. Many preclinical biomarkers have been suggested, gut microbiome composition changes have evolved as a recent research area to investigate possible pathologic mechanisms, biomarkers, and treatment modalities. Results of this study showed that *Prevotella* relative abundance was 4-fold decreased in cases when compared to controls, however it failed to reach statistical significance. *Prevotella*-dominant enterotype was less presented in cases compared to controls, the result was statistically significant denoting a correlation between low *Prevotella* level in gut microbiome of PD patients. There was highly statistically significant difference between PD clinical phenotypes as regards total constipation score, showing that it may be a promising tool for predicting and modulating the disease.

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**Conflict of interest**

The authors declare no conflict of interest.

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