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Gut Microbiome Changes with Osteopathic Treatment of Constipation in Parkinson's Disease: A Pilot Study

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Abstract

Introduction: The gut microbiome appears to be predictive of Parkinson's disease (PD) with constipation. Chronic constipation frequently manifests prior to motor symptoms and impairs quality of life. An osteopathic manipulative medicine (OMM) sequence used physical exam assessment and manual treatment of neuromusculoskeletal dysfunctions pertinent to constipation in PD for this prospective ABA-design study, IRB-NYITBHS1065. The effects of 4 weekly treatments on the gut microbiome among men and women over 40 years old with chronic constipation and PD were investigated. Severity of PD was rated with the Movement Disorders Society-Unified PD rating scale (UPDRS) in six subjects with constipation. Also, the Bristol stool scale and questionnaires validated for constipation were administered for diagnosis, symptom severity, and quality of life during a 4-week control-period (A), 4-weekly OMM-treatments (B), and 2-weeks no-intervention (A). Biweekly stool samples were assessed for normalized microbiota abundance.

Results: The mean Bristol rating improved from type 2 (± 1) Pre-OMM to 3 (± 1; p = .167; d = 0.677) Post-OMM. Mean constipation severity significantly decreased (p = .010; d = 1.508) Post-OMM. Mean quality of life significantly improved (p = .041; d = 1.072) Post-OMM. The Pre-OMM mean number of families within the phylum Firmicutes decreased by 3 (p = .043; d = 1.177) Post-OMM. There were significant changes in the normalized abundance of phyla Actinobacteria (p = .040; d = 0.845) and Verrucomicrobia (p = .024; d = 0.675) as well as in genus Roseburia (p = .033; d = 1.109), Intestinimonas (p = .035; d = 0.627) and Anaerotruncus (p = .004) Post-OMM.

Conclusion: The gut microbiome shifted among individuals with constipation and PD after four weekly treatments with the OMM-sequence. Changes in the gut microbiome Post-OMM were associated with UPDRS results and constipation measures. Clinical trials and studies to develop the gut microbiome into a validated biomarker for PD are necessary to understand the impact of OMM in patients with PD and constipation.

Keywords: *Parkinson's Disease; Constipation; Manual Therapy; Microbiota; Gastrointestinal System; Autonomic Nervous System*

Abbreviations

PD: Parkinson's Disease; CC: Chronic Constipation; PD-CC: PD with CC; OMM: Osteopathic Manipulative Medicine; WCCSS: Wexner Cleveland Constipation Scoring System; MDS: Movement Disorder Society; UPDRS: Unified PD Rating Scale; ANS: Autonomic Nervous System; ENS: Enteric Nervous System; SNS: Somatic Nervous System; H&Y: Hoehn & Yahr; PAC-SYM: Patient Assessment of Constipation- Symptom; PAC-QOL: Quality of Life

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder with disabling motor and non-motor symptoms [1,2]. Chronic constipation (CC) is present in up to 80% of patients with PD [2-4]. Direct costs of CC management range from $1,912 to $7,522 per-patient per-year in the United States [5]. It can precede the initial diagnosis of PD, which involves the onset of motor symptoms [2-4]. There is growing evidence that the gut microbiome is indirectly involved in the clinical development of PD and imbalances in gut microbial abundance with CC was found to be predictive of PD with CC (PD-CC) [6-8]. There is no direct evidence that treating the gut microbiome in prodromal CC prevents further constipation, PD-related microbiome imbalance, or PD. Osteopathic manipulative medicine (OMM) involves clinical diagnoses and manual treatments previously found to significantly improve CC in otherwise-healthy and cerebral palsy subjects [9-11]. However, the use of OMM for constipation in PD or gut microbiome imbalance is not well defined. The microbiome may become an important, non-invasive biomarker for early diagnosis, treatment, and objective monitoring in PD-CC.

Previous reports suggest that small bowel bacterial overgrowth may contribute to slow transit constipation in PD, which is thought to delay the absorption of nutrients and medications [12]. When the fecal microbiomes of PD patients were compared to healthy, age-matched controls, the mean normalized abundance of Prevotellaceae (Bacteroidetes phylum) was significantly lower [6,7]. A normalized abundance of Prevotellaceae ≤ 6.5% of the total gut microbiota had 86.1% sensitivity and 38.9% specificity for PD [6]. Fecal samples from PD patients were found to have significantly decreased Bacteroidetes [7]. The abundance of other bacterial families (Lactobacillacae, cae, Bradyrhizobiacae, Clostridiales Incertae Sedis IV and Ruminococcicae) combined with the constipation severity score on the Wexner Cleveland Constipation Scoring System (WCCSS) also identified PD patients with 66.7% sensitivity and 90.3% specificity [6]. Additionally, Enterobacteriaceae abundance was significantly greater in PD [6,7] and has been positively associated with the severity of postural instability and gait difficulty as assessed by the Movement Disorder Society (MDS) -Unified PD Rating Scale (UPDRS) [6]. Certain genetic or metabolic elements from the microbiome may functionally complement human genetics and neurophysiology [7,13].

Constipation in PD manifests from one or more human pathophysiology features of PD, including the autonomic nervous system (ANS) dysregulation, enteric nervous system (ENS) dysregulation [2-4,6,12], outlet dysfunction due to pelvic floor muscle dyssynergy [12,14,15], bradykinesia and rigidity [4,16,17], and restrictive pulmonary syndrome [18-20]. The α-synuclein pathology, Lewy body accumulation, and neuron loss occur in the somatic nervous system (SNS), ANS, and ENS [2-4,6,12]. The ENS mechanoreceptors of interstitial cells of Cajal and Ruffini are involved in communication with the ANS. The decreased rate and coordination of colonic peristalsis causes slow transit constipation [2,4,12,17,22,23]. Constipation from dyssynergic outlet dysfunction occurs in two-thirds of individuals with PD. The outlet dysfunction leads to obstruction due to dystonia (spasmodic muscles) and/or paradoxical contraction and tone imbalance of the puborectalis muscle and/or anal sphincter [12,14-16], which directly impairs segmental transit through the large bowels [15,16]. Additionally, decreased activity from bradykinesia and rigidity or other features in PD makes acquiring and tolerating fluids more difficult [22,24,25]. Walking, running, and ventilation increase circulation and decrease stasis of metabolic waste. However, bradykinesia, rigidity, and restrictive pulmonary syndrome limit activity. Constipation and colonic transit time have been shown to be related to physical inactivity [18-20,22,24-28]. High quality ventilation continually moves the abdominal organs and its fluids [28]. However, the decreased pumping mechanics in restrictive pulmonary syndrome (hypoventilation, decreased thoracic excursion, and diaphragmatic motion) likely further impairs gut health.

OMM is the examination and treatment of the function of neuromusculoskeletal system anatomy to improve functional body mechanics such as joint range of motion, muscle tone, circulation, body fluid pressures and exchanges, and nervous impulses utilizing non-invasive, generally safe manual techniques [9,25]. An OMM sequence combining techniques to specifically address normalization of the ANS dysregulation, balance tone of the pelvic floor muscles that may be dystonic, improve gastrointestinal inertia to reduce the bradykinesia effects on the gastrointestinal system and release myofascial restrictions to allow for better thoracic excursion and diaphragm motion to

enhance their pumping mechanics has not been tested for gut microbiome imbalance in PD-CC. Further research is needed to assess how gut microbiome imbalances relate to previously validated measures of PD and CC. By improving CC severity without molecularly perturbing the microbiome, the natural ecology or response in ecology can be studied.

Severity of PD motor and non-motor signs and symptoms may be quantified by the MDS UPDRS [1-3,12]. Part-1 rates nonmotor aspects of PD, including constipation. This validated rating scale also includes the Hoehn & Yahr (H&Y) scale (1 - 5 points) [34], dyskinesia, and dystonia in PD, making it useful for PD subtypes. The tools validated in non-PD studies, WCCSS and Patient Assessment of Constipation-Symptom (PAC-SYM) and Quality of Life (PAC-QOL) surveys characterize and quantify the patient experience and include questions consistent with obstructive dysfunction [29-31]. The Bristol Stool Scale is a non-invasive, validated tool depicting 7-types of stool that may be used in an out-patient office to evaluate gastrointestinal motility [29,30,32,33].

Aim of the Study

The aim of this study was to determine if four weekly treatments with the OMM-sequence for CC in PD alters the balance of normalized microbial abundance in the gut. The hypothesis is that there will be a significant difference in the normalized abundances of bacteria in fecal samples after four weekly treatments with the pre-defined OMM-sequence for PD-CC.

Methods

This study involving the collection of repeated human stool samples to determine changes in gut microbiota before, during, and after 4 weekly treatments with an OMM-sequence for the management of constipation in PD was approved by the New York Institute of Technology College of Osteopathic Medicine (NYITCOM) institutional review board (IRB #: BHS1065; Clinicaltrials.gov# NCT02344485). Potential subjects were recruited from the NYITCOM PD Center and screened using an IRB-approved screening questionnaire that included past medical history to assess for eligibility prior to consent. Collection and use of the de-identified samples and their analysis by an external lab were included in the IRB-approved consent process. All participants elected to have their caregiver present for consent and all visits.

Inclusion criteria

Medically diagnosed with PD, ability to provide photos of stool for assessment, age 40 years or older and diagnosis of constipation according to the Rome III criteria.

Exclusion criteria

No diagnosis of PD; presence of other neurological diseases or disorders causing constipation (excluding diagnosed headache or migraine); cancer of the colon, pelvis, gastrointestinal tract or abdomen; unexplained or new onset constitutional signs of fever, night sweats, weight loss; rectal bleeding or black stool; anemia that has not been medically evaluated; active hepatitis; acute infectious mononucleosis; splenomegaly; irritable bowel syndrome; abdominal aortic aneurysm; other known or suspected secondary cause for chronic constipation (e.g. organic pathology, congenital gastrointestinal malformation); age of less than 40 years; pregnancy; inability to tolerate the OMM treatment protocol; unable to provide stool photos; acute infectious gastrointestinal illness within the past one month; surgery within the past 6 weeks in or around the abdomen or pelvis; use of oral antibiotics within the last one month. All subjects who have contraindications to the techniques were excluded from participation. Power analysis for sample size was calculated based on previous PAC-QOL estimated effect sizes. Participants were requested to not change usage and document all usage and changes in medications, supplements, or food that might cause or relieve constipation were not exclusion criterion due to the high likelihood that participants are taking multiple approaches to manage their conditions.

In visit one, eligible, consented subjects were assigned a de-identified number. Severity of PD was rated by an international MDS-certified investigator with permission purchased from MDS. The subjects' medical history, tobacco use, past month medication and supplement use and potentially confounding variables were documented. Subjects were asked to avoid changing their diet during the study, unless under direction of their health care provider. Taking medications, supplements, or food that might cause or relieve constipation were not exclusion criteria in an effort to avoid worse subject discomfort or illness. However, participants were requested to not alter usage if possible and to document all usage. Changes in medical history, new illnesses, and subject-perceived effects of treatment were documented weekly.

To prospectively test the OMM-sequence, subjects were monitored for 10 weeks with the repeated measures WCCSS, PAC-SYM, PAC-QOL and feces sample for gut microbiota on visits 3, 5, 7, 9 and 10. Subjects were given a $20.00 gift card at each visit. Subjects and their caregivers maintained a diary of Bristol rating and any constipation management used. They used their phone or were provided with a digital camera to take photos of every stool to bring to each visit 2-10. Bristol Stool Scale rating was performed by one of three physician-investigators weekly from subject-provided photos. Severity was measured by the WCCSS. The subjects' assessment of constipation symptoms and quality of life were measured by PAC-SYM and PAC-QOL surveys. Stool swab samples were collected by subjects using Ubiome kit as directed, and mailed to Ubiome (San Francisco, CA 94105) CLIA-certified lab for analysis using amplification of the 16S ribosomal RNA genes. Outcome measurements and interventions were conducted with one subject per patient room in an out-patient setting. This study was conducted separately from any care as a patient. Subjects and their caregivers were not debriefed on any outcomes of the study.

OMM-sequence intervention

In weekly visits 5 - 8, the 30-minute OMM-sequence intervention was performed as described in figure 1 [35] by a licensed osteopathic physician (board certified in family medicine and osteopathic manual therapy and/or neuromusculoskeletal medicine and OMM). Each technique was applied for 2 minutes with the subject supine, except for the sacral rock and sometimes the mesenteric release, which was in the lateral recumbent position. The ANS was treated by steps 1. Suboccipital Release, 3. Celiac, Superior Mesenteric, and Inferior Mesenteric Ganglion Inhibition, 4. Bilateral T10-L2 Paraspinal Inhibition, 5. Bilateral Sacroiliac Joint Decompression, and 6. Sacral Rock [35]. The ENS-related dysmotility was treated by steps 7 - 8. Mesenteric Release of ascending colon and descending colon, and step 9. Colonic Stimulation [35]. The thoracic excursion and diaphragm restrictions of restrictive pulmonary syndrome were treated by step 2. Respiratory Diaphragm Release [35]. The pelvic floor muscle imbalance and dystonia was treated by the steps 5. Bilateral Sacroiliac Joint Decompression and 6. Sacral Rock to improve muscle tone and balance [35]. The OMM techniques performed are commonly used, non-invasive, and considered minimal risk [35,36]. The most common anticipated side effect was soreness and/or tenderness lasting for 1 - 2 days. In visit-9 of their participation in the study, the subjects did not have treatment in order to assess any longer-term effects on their final visit-10.

1. Suboccipital Release: The physician placed his/her finger pads over the suboccipital region noting areas with chronic tissue changes. A slow and gentle anterior pressure into the musculature was applied until tissue softening. This was repeated as needed for 2 min.

2. Respiratory Diaphragm Release: The physician placed one hand across the thoracolumbar junction posteriorly and the other hand and forearm across the lower ribcage anteriorly. Rotational restriction of the diaphragm was tested for by moving the anterior and posterior torso in opposite directions (left and right). Sufficient direct force was applied to match the diaphragmatic tension until it released in 1-2 min.

3. Celiac, Superior Mesenteric, and Inferior Mesenteric Ganglion Inhibition: The physician placed two fingers over each of the ganglia: celiac - inferior to the xiphoid process, inferior mesenteric - superior to the umbilicus, and superior mesenteric - midway between the xyphoid and the umbilicus. Fingers Slowly sank into the abdomen with respirations (resisting on inhalation and advancing further on exhalation) until a sense of decreased tension or maximal comfortable subject tolerance was reached (whichever occurs sooner) for ≤ 2 min and then slowly released.

4. Bilateral Para-Spinal Inhibition to T10-L2: The physician placed his/her fingertips over the belly of the medial paraspinal muscles of levels T10 to L2 noting areas with chronic tissue changes. An anterior force sufficient to engage these muscles was exerted until softening of the tissues for one min. on each side.

5. Bilateral Sacroiliac Joint Decompression: The physician placed fingertips of the cephalad hand on the medial aspect of the PSIS (Posterior Superior Iliac Spine) and the caudad hand on the medial aspect of the ischial tuberosity. Lateral traction was applied to match the tension for one min. on each side.

6. Sacral Rock: The physician placed the cephalad hand with the heel of the hand at the sacral base and fingers pointing towards the coccyx. The caudad hand overlied the cephalad hand but with fingers pointing in the opposite direction. Gentle pressure was exerted to increase the rocking motion of the sacrum synchronous with the subject's sacral extension during inhalation and sacral flexion during exhalation for 1-2 min.

7. Mesenteric Release of Ascending Colon: The physician's hands were placed on the right lateral abdominal wall with fingers curled slightly into the border of the ascending colon. Fingers were gently pushed towards the back as tolerated. Then, the fingers were drawn towards the subject's left side until meeting the restrictive barrier where it was held for 20 - 30 sec. as the tissue softened and became more fluctuant with breathing. Technique was repeated as tolerated ≤ 2 min.

8. Mesenteric Release of Descending Colon: The physician's hands were placed on the left lateral abdominal wall with the fingers curled slightly into border of the descending colon. Fingers gently pushed towards the back as tolerated, and then drawn towards the subject's right side until meeting the restrictive barrier where it was for 20-30 sec. as the tissue softened and became more compliant with breathing. Technique was repeated as tolerated ≤ 2 min.

9. Colonic Stimulation: The physician placed finger pads on the abdominal wall overlying the splenic flexure of the colon. With gentle pressure, the fingers rolled along the bowel in the direction of colonic flow, releasing and repositioning the hands farther along the colon to repeat this sequence until covering the length of the descending colon up to the suprapubic level. After several excursions down the descending colon, the hands were repositioned to begin at the hepatic flexure, and worked along the transverse and descending colon. After several of these excursions, the hands were repositioned to begin over the cecum.

Figure 1: *Description of the techniques in the OMM-sequence for adjunctive management of constipation in PD [35].*

Data and statistical analyses

The non-parametric, 2-tailed correlations, Kendall's tau (t) and Spearman's rho (r_s), of normalized abundance of microbiota during the Pre-OMM control-period were utilized to test for linear relationships to the validated outcome measures, $p \leq .05$ was significant; Strength of r_s: +1 -1 Perfect; +0.9 -0.7 Strong; +0.6-0.4 Moderate; +0.3-0.1 Weak; 0-0 None) [37]. The sum of the UPDRS subscores related to Mobility, Gait, and Posture included items Part 2: 2.11 Getting out of bed, a car, or a deep chair (0-4pts); 2.12 Walking and balance (0-4pts); 2.13 Freezing (0-4pts); Part 3: 3.3 Rigidity (0-4pts); 3.9 Arising from chair (0-4pts); 3.10 Gait (0-4pts); 3.11 Freezing of gait (0-4pts); 3.12 Postural stability (0-4pts); 3.13 Posture (0-4pts); and Part 4: 4.6 Painful off-state dystonia.

The Pre-OMM control-period normalized abundance of gut microbiota was collected from the same time-points as the repeated outcome measures for constipation, WCCSS, PAC-SYM, PAC-QOL. Disease-specific measures of UPDRS (0-199) and constipation severity scores of the WCCSS, PAC-QOL, and PAC-SYM use greater scores for worse severity. The variables were independent of one another and not influenced by any other observation. Mean control-period measures were compared across the sessions to Post-OMM using one way, repeated measures ANOVA (mean 95% CI) and Bonferroni pairwise comparison using, $p \leq .05$ considered significant. Variables from the weekly medical and health history (medical and PD symptoms; gastrointestinal and constipation symptoms; medications and supplements; fluid and caffeine intake; physical activity; number of bowel movements; new therapies; intake of fermented foods, prebiotics, and probiotics) were quantified and evaluated for variability and effect on repeated measures. To investigate individual microbial changes, the normalized abundances of phyla from Pre-OMM control-period were compared to Post-OMM with two-tailed, repeated measures ANOVA. The families, genera, and species within phyla found to have significantly changed Post-OMM were selected for comparisons also using

two-tailed, repeated measures ANOVA. To assess overall changes in microbiome diversity, the mean Pre- and Post-OMM Shannon Diversity Index (SDI) (stdev) were compared by repeated measures [38]. The effect sizes were calculated using Cohen's d [d = (M_pre - M_post)/sqrt(((SD_pre^2)+(SD_post^2))/2)] or Hedges' g depending on the number of subjects having the bacteria present in their samples because Hedges' g allows for different sample sizes (d & g categories: ≤ .2 = small, ≤ .5 = moderate, ≥ .8 = large).

Results

Nine participants started the study, and six completed the study, including submitting stool samples for analysis. Withdrawals occurred during the control period secondary to a urinary tract infection, discomfort with sampling stools and inability to commit to time requirements for 10 weeks. The mean age of subjects (n = 6) was 72 (95%CI 63, 81) years. There were 3 females (50%) and 3 males (50%) (Table 1). One subject was African American and five were Caucasian. The PD means and ranges were MDS-UPDRS 87 (95%CI 52,122) and 55-135 pts, UPDRS-Part-I 16 (95%CI 8, 20) and 7-29 pts, and H&Y 3 (± 1) and 1-4 pts. None of the covariate measures UPDRS Total score, H&Y and UPDRS-Part-I non-motor aspects, age, years-with-PD, or years-with-constipation significantly correlated with mean control-period constipation instruments (WCCSS, PAC-SYM, or PAC-QOL). There was a significant negative, strong correlation of the number of years-with-constipation (τ_b = -.733, p = .039; r_s = -.829, p = .042) with the mean control-period Bristol. Subjects denied experiencing any side effects or reported adverse effects. None of the subjects had worsening constipation severity. None of the confounding variables significantly changed during the study. The mean Bristol rating improved from type 2 (± 1) Pre-OMM to 3 (± 1) (p = .167; d = 0.677) Post-OMM (Table 2). The mean WCCSS significantly decreased by 4 (p = .010; d = 1.508) Post-OMM. The mean PAC-QOL significantly decreased by 9 (p = .041; d = 1.072) (Table 2) Post-OMM. The PAC-SYM improved by 6 (p = .099; d = 0.795) Post-OMM.

	Mean (95% CI)
UPDRS Total	87 (52,122)
UPDRS Part 1	14 (8,20)
Hoehn and Yahr	3 (2,4)
Age (years)	72 (63,81)
Years with PD	16 (2,29)
Years with Constipation	18 (6,29)
Weight (lbs)	149 (108,189)

Table 1: *Movement disorders society-unified Parkinson's disease rating scale (UPDRS) total score (no symptoms = 0 to most severe = 199) and its subscores (Hoehn and Yahr (1-5), Part 1: Non-motor aspects of experiences of daily living), (n = 6). Abbreviations: 95% CI: 95% Confidence Interval.*

Outcome Measure	**Control Mean (SD)**	**Post-OMM Mean (SD; p-value)**	**Effect Size (d)**
WCCSS	15 (4)	9 (4; p = .010)*	1.508
PAC-SYM	14 (7)	8 (7; p = .099)	0.795
PAC-QOL	31 (9)	22 (8; p = .041)*	1.072
Bristol Investigator	2 (1)	3 (1; p = .167)	0.677

Table 2: *The mean constipation outcomes from the 4-week control-period were compared to those 2-weeks after 4-weekly treatments with the OMM-sequence for constipation in PD (Post-OMM) by repeated measures within-subject ANOVA (n = 6). The Cohen's d effect size categories were: ≤ 0.2 = Small, ≤ 0.5 = Moderate, ≥ 0.8 = Large for the change in outcome measure Post-OMM. Abbreviations: WCCSS: Wexner's Cleveland Constipation Severity Scoring System; PAC: Patient Assessment Constipation; SYM: Symptoms; QOL: Quality of Life; Bristol Investigator: Bristol stool scale (dry, hard stool = 1; goal = 3 - 4; diarrhea = 7) rating of photos by the investigators; SD: Standard Deviation; 95%; *p ≤ .050.*

The Pre-OMM genera SDI, 2.714 (SD ± 0.442), slightly decreased after treatment to 2.702 (SD ± 0.274; p = .940; d = 0.034). The Pre-OMM mean number of families within the phylum Firmicutes, 19 (SD ± 2), was significantly decreased to 16 (SD ± 2; p = .043; d = 1.177) Post-OMM (Table 3). The only family within Firmicutes to significantly change Post-OMM was Ruminococcaceae, which increased by 87.65% (p = .050). Significant changes in genus Roseburia (p = .033; d = 1.109), Intestimonas (p = .035; d = 0.627), and Anaerotruncus (p = .004) were found Post-OMM (Table 4). The Pre-OMM normalized abundances of Roseburia significantly negatively correlated with UPDRS scores. The Pre-OMM normalized abundances of Intestimonas significantly positively correlated with UPDRS scores. Participant samples only contained one family from the phylum Verrucomicrobia.

Bacterial Phyla (n = 6)	Baseline Mean Number of Families within this Phylum (SD)	Post-OMM Mean Number of Families within this Phylum (SD) (p-value*)	Effect Size of OMM Treatment (d)
Firmicutes	19 (2)	16 (2) (p = .043*)	1.177
Actinobacteria	6 (2)	5 (1) (p = .140)	1.044
Verrucomicrobia	1 (1)	1 (p = 1.000)	

Table 3: *The Pre-OMM mean (standard deviation (SD)) number of families within phyla from the 4-week control-period were compared to Post-OMM by repeated measures within-subject ANOVA). *p ≤ .050.*

Genera (n = 6)	Mean Normalized Abundance			Significant pre-OMM correlations (rho, p-value)
	Pre-OMM (95% CI)	Post-OMM (95% CI) p-value	Effect Size (d)	
Roseburia (Family Lachnospiraceae)	24,255 (11589, 36921)	50,369 (17806, 82931) .033*	1.109	• Hoehn and Yahr (r_s = -.883, p = .020) • Mobility, Gait, and Posture composite score (r_s = -.829, p = .042) • 3.3 (r_s = -.886, p = .019) • 4.6 (r_s = -.956, p = .003) • Part 3 and 4.6 (r_s = -.899, p = .015)
Intestimonas (Family Unclassified Clostridiales)	2,567 (322, 4811)	1,443 (18, 2868) .035*	0.627	• Total UPDRS (r_s = .829, p = .042) • Hoehn and Yahr (r_s = .971, p = .001) • 3.10 (r_s = .926, p = .008) • 3.13 (r_s = .841, p = .036) • Mobility, Gait, & Posture composite score (r_s = .886, p = .019) • 4.6 (r_s = .837, p = .038) • Part 3 and 4.6 (r_s = .928, p = .008)

Table 4: *The mean normalized abundance of genera of Firmicutes in Pre-OMM control-period fecal samples (95% CI) compared to Post-OMM (95% CI) with p-values of within-subject repeated measures ANOVA. The effect sizes (Cohen's d) of the change in normalized abundance of genera post-OMM were calculated. Cohen's d categories: 0.2 = small, 0.5 = moderate, 0.8 = large. The significant, non-parametric, two-tailed Spearman's rho correlations of Pre-OMM genera to UPDRS, *p ≤ 0.05. Measure of PD severity quantified by each UPDRS subscale: Part 3 Motor Examination; 3.3 Rigidity; 3.10 Gait; 3.13 Posture; Part 4: Motor Complications; 4.6 Painful Off-State Dystonia. Abbreviations: UPDRS: Unified PD Rating Scale.*

The normalized abundances (95% CI) of phyla content shifted after four weekly treatments with the OMM-sequence (Figure 2). Significant changes in phyla included Actinobacteria 2.2% (p = .040; d = 0.845) and Verrucomicrobia 8.5% (p = .024; d = 0.675) Post-OMM (Table 5). The UPDRS Total score significantly positively correlated with mean pre-OMM Euryarchaeota r_s = .895 (p = .016), Fibrobacteres r_s = .963 (p = .002), and Synergistetes r_s = .841 (p = .036). The H&Y severity significantly negatively correlated with Actinobacteria r_s = -.859 (p = .028) (Table 6). Verrucomicrobia significantly positively correlated with the Mobility, Gait and Posture composite score r_s = .834 (p = .039) and with 4.6 Painful off-state dystonia score severity, alone, r_s = .870 (p = .024). Of the constipation scales, the PAQ-QOL significantly correlated with Actinobacteria r_s = -.878 (p = .021) and Bacteroidetes r_s = .867 (p = .025). Phylum Proteobacteria did not significantly change Post-OMM or correlate with measures, however, Sutterellaceae increased by 84.19% (p = .03) Post-OMM.

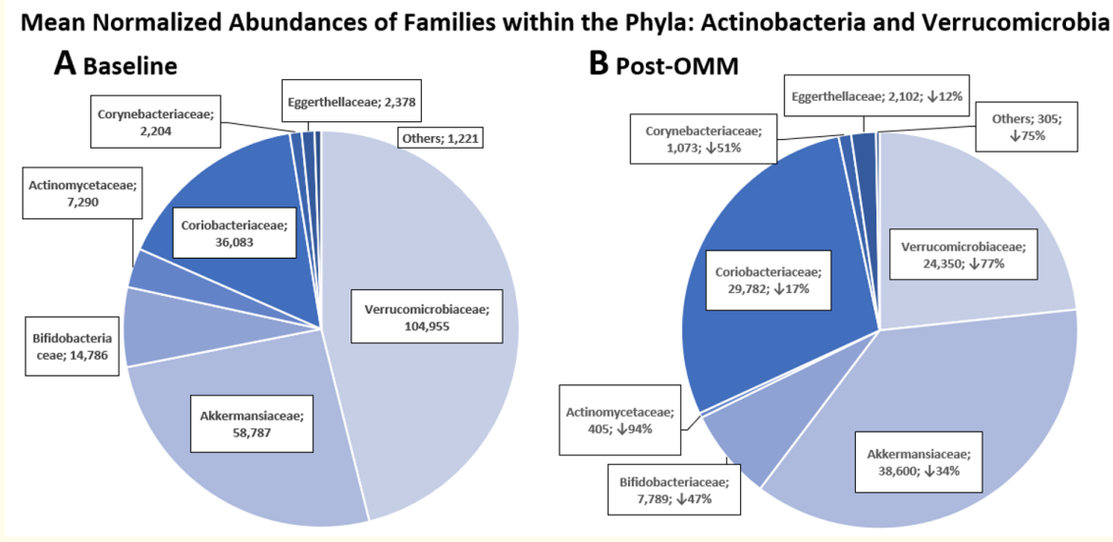

Figure 2: *Distribution of families within the phyla Actinobacteria and Verrucomicrobia. A: mean normalized abundance of each family at baseline. B: Mean normalized abundance of each family Post-OMM, and the percent change in normalized abundance from baseline (Others: Atopobiaceae, Bogoriellaceae, Brevibacteriaceae, Microbacteriaceae, Mycobacteriaceae, Nocardioidaceae, Propionibacteriaceae).*

Phylum	Mean Normalized Abundance		% Change (P-value)	Effect Size (d)
	Pre-OMM (95% CI)	Post-OMM (95% CI; p-value)		
Actinobacteria	61096 (42002, 88870)	39496 (28285, 55150; p = .040)*	2.2% (.001)	0.845
Bacteroidetes	354826 (273992, 459508)	305406 (262022, 355973; p = 1.000)	10.2% (< .001)	0.496
Euryarchaeota	40758 (18318, 90691)	15862 (7899, 31852; p = .160)	2.9% (< .001)	0.712
Fibrobacteres	71 (43, 117)	267.0 (127, 562; p = .080)	0.0% (< .001)	0.676
Firmicutes	480735 (415254, 556542)	599474 (537265, 668886; p = .080)	1.1% (< .001)	1.274
Lentisphaerae	1803 (427, 7625)	1639 (607, 4426; p = 1.000)	0.2% (.130)	0.047
Proteobacteria	26230 (20936, 32863)	31048 (14733, 65428; p = 1.000)	0.9% (.032)	0.208
Verrucomicrobia	111313 (68898, 179840)	28076 (13237, 59547; p = .024)*	8.5% (< .001)	0.675
Synergistetes	18047 (-9966, 46060)	4851 (-2780, 12481; p = .175)	1.3% (.175)	0.675
Deinococcus-Thermus	0 (0, 0)	5 (-7, 17; p = .363)	0.0% (.363)	0.577
Fusobacteria	4 (-6, 14)	7 (-11, 26; p = .720)	0.0% (.720)	0.237

Table 5: *The mean normalized abundance of phyla in control-period fecal samples (95% CI) compared to Post-OMM (95% CI; p-value of within-subject repeated measures ANOVA) with the percent change across sessions (p-values of Bonferroni pairwise comparison). Effect size Cohen's d categories were: ≤ 0.2 = small, ≤ 0.5 = moderate, ≥ 0.8 = large for the change in abundance of phyla. *p ≤ .050.*

Phylum	UPDRS Total	H&Y	4.6	Mobility Gait and Posture	PAQ-QOL
Actinobacteria	-.270 (.604)	-.859 (.028)*	-.502 (.310)	-.464 (.354)	-.878 (.021)*
Bacteroidetes	-.243 (.643)	.347 (.501)	-.144 (.786)	-.106 (.842)	.867 (.025)*
Euryarchaeota	.895 (.016)*	.494 (.320)	.390 (.444)	.736 (.096)	-.128 (.810)
Fibrobacteres	.963 (.002)*	.523 (.287)	.441 (.381)	.789 (.062)	-.143 (.788)
Proteobacteria	.541 (.268)	-.246 (.638)	-.292 (.575)	.058 (.225)	-.426 (.400)
Synergistetes	.841 (.036)*	.452 (.368)	.385 (.451)	.704 (.118)	-.175 (.740)
Verrucomicrobia	.481 (.334)	.645 (.167)	.870 (.024)*	.834 (.039)*	-.129 (.807)
Firmicutes	-.155 (.769)	-.592 (.216)	-.180 (.734)	-.348 (.499)	-.678 (.139)

Table 6: *Spearman's rho correlations (p-value) of Pre-OMM control faecal sample microbiota to constipation and PD severity and symptoms scales. Measure of PD severity quantified by each UPDRS subscale: Part 1: Non-Motor Aspects of Experiences of Daily Living; Part 4: Motor Complications. Abbreviations: UPDRS: Unified PD Rating Scale; PAC: Patient Assessment Constipation; SYM: Symptoms.*

** p ≤ .05; ** p < .01.*

UPDRS questions Mobility, Gait, and Posture {2.11 Getting out of bed, a car, or a deep chair (0 - 4); 2.12 Walking and balance (0 - 4); 2.13 Freezing (0 - 4); 3.3 Rigidity (0 - 4); 3.9 Arising from chair (0 - 4); 3.10 Gait (0 - 4); 3.11 Freezing of gait (0 - 4); 3.12 Postural stability (0 - 4); 3.13 Posture (0 - 4)); and 4.6 Painful off-state dystonia}.

The normalized abundances of the Actinobacteria families Coriobacteriaceae and Corynebacteriaceae (Table 7) and genera Collinsella, Corynebacterium, and Gordonibactera (Table 8) were significantly, negatively correlated with severity of UPDRS and/or subscores. Eggerthella was significantly positively correlated to Part 1: Non-motor aspects r_s = .975 (p = .005), 2.11 Getting Out of Bed r_s = .928 (p = .008), and 3.13 Posture r_s = .841 (p = .036). Bifidobacterium was significantly positively correlated with Part 1 r_s = .975 (p = .005).

Actinobacteria **Families (n = 6)**	UPDRS						PAC-SYM
	Total	Part 1[a]	Part 4	2.12	3.10	3.11	
Bifidobacteriaceae	0.257 (.623)	0.975 (.005) **	0.145 (.784)	0.232 (.658)	-0.093 (.862)	0.169 (.749)	0.257 (.623)
Coriobacteriaceae	-0.657 (.156)	-0.051 (.935)	-.841 (.036)*	-0.725 (.103)	-0.926 (.008)**	-.845 (.034) *	0.143 (.787)
Corynebacteriaceae[b]	-1.000 (<.001)**	-0.632 (.368)	-.975 (.005)**	-1.000 (<.001) **	-0.791 (.111)	-0.447 (.450)	-0.600 (.285)

Table 7: *Spearman's rho correlations (p-value) of Pre-OMM control faecal sample families (within the phylum Actinobacteria) to constipation and PD severity and symptoms scales. * p ≤ .05; ** p < .01. [a]: Self-report measure scored in 5 of 6 participants; [b]: This bacterial family was found in 5 of 6 participants. Unified PD Rating Scale (UPDRS) subscale: Part 1: Non-Motor Aspects of Experiences of Daily Living; Part 4: Motor Complications; 2.12 Walking and Balance; 3.10 Gait; 3.11 Freezing of Gait. Abbreviations: PAC: Patient Assessment Constipation; SYM: Symptoms.*

Actinobacteria Genera (n = 6)	UPDRS								WCCSS	SYM+ QOL
	Total	Part 1	Part 4	2.11	2.12	3.10	3.11	3.13		
Bifidobacterium	.257 (.623)	.975 (.005)**	.145 (.784)	.551 (.257)	.232 (.658)	-.093 (.862)	.169 (.749)	.377 (.461)	.314 (.544)	-.257 (.623)
Collinsella	-.829 (.042)*	-.462 (.434)	-.928 (.008)**	-.406 (.425)	-.870 (.024)*	-.926 (.008)**	-.845 (.034)*	-.899 (.015)*	-.600 (.208)	-.314 (.544)
Corynebacterium[a]	-1.000 (<.01)**	-.632 (.368)	-.975 (.005)**	-.872 (.054)	-1.000 (<.01)**	-.791 (.111)	-.447 (.450)	-.800 (.104)	-.800 (.104)	-.700 (.188)
Eggerthella	.771 (.072)	.975 (.005)**	.580 (.228)	.928 (.008)**	.696 (.125)	.494 (.320)	.507 (.305)	.841 (.036)*	.657 (.156)	-.200 (.704)
Gordonibacter[a]	-.900 (.037)*	-.316 (.684)	-.975 (.005)**	-.667 (.219)	-.900 (.037)*	-.949 (.014)*	-.671 (.215)	-.900 (.037)*	-.900 (.037) *	-.500 (.391)
Varibaculum[a]	-.100 (.873)	.949 (.051)	-.300 (.624)	.462 (.434)	-.205 (.741)	-.053 (.933)	.447 (.450)	.410 (.493)	-.100 (.873)	-.900 (.037)*

Table 8: *Spearman's rho correlations (p-value) of control sample faecal genera (within the phylum Actinobacteria) to constipation and PD severity and symptoms scales. Measure of PD severity quantified by each UPDRS subscale: Part 1: Non-Motor Aspects of Experiences of Daily Living; Part 4: Motor Complications; 2.11 Getting Out of Bed, a Car, or a Deep Chair; 2.12 Walking and Balance; 3.10 Gait; 3.11 Freezing of Gait; 3.13 Posture. Abbreviations: UPDRS: Unified PD Rating Scale; WCCSS: Wexner's Cleveland Constipation Severity scoring system; PAC: Patient Assessment Constipation; SYM: Symptoms; QOL: Quality of life. * p ≤ .05; ** p < .01. [a]: Bacterial genera was found in 5 of 6 participants.*

There were significant correlations of the Pre-OMM outcome measures with families previously found related to PD. Pre-OMM mean Prevotellaceae (phylum Bacteroidetes) abundance significantly negatively correlated with UPDRS (r_s = -0.83; p = .04) and subscore-2.12 Walking and Balance (r_s = -0.81, p = .05). The initial Bristol r_s = .900 (p = .037) and Mobility, Gait and Posture score r_s = -0.464 (p = .432; n = 5) did not significantly correlate with Pre-OMM samples for Prevotellaceae. Of the phylum Firmicutes, Pre-OMM Ruminococcaceae significantly negatively correlated with UPDRS 2.13 (r_s = -0.88, p = .02). Pre-OMM Lactobacillaceae phylum Firmicutes significantly positively correlated with PAC-QOL (r_s = 0.83, p = .04, N = 6), and SYM-QOL (r_s = 0.94, p < .01). Lachnospiraceae significantly positively correlated with UPDRS-Part-3 (r_s = 1.00, p < .01), PAC-SYM r_s = .923 (p = .026; n = 5). Pre-OMM mean Clostridiales XIII phylum Firmicutes significantly positively correlated with UPDRS-Part-3 (r = 1.00, p < .01) and PAC-QOL initial Clostridiales XIII r_s = -1.000 (p < .001; n = 4). WCCSS r_s = -0.955 (p = .045; n = 4). Additionally, initial Clostridiales XIII abundance significantly correlated with Pre-OMM Prevotellaceae r_s = .987 (p = .002; n = 5).

Discussion

Constipation and the gut microbiome may be a measureable prodromal sign of PD that worsens QOL [1-3]. It is unknown if treating CC in individuals with PD alters the gut microbiome. The purpose of this pilot study was to determine the effect of OMM treatment of CC on the gut microbiome among men and women over 40 years old with PD-CC. The hypothesis was that there will be a significant difference in normalized abundance of gut microbiota after four weekly OMM treatments using a pre-defined experimental OMM sequence. By improving CC severity without molecularly perturbing the microbiome, the natural response can be studied.

An OMM sequence used physical exam, assessment and non-invasive, manual treatment of neuromusculoskeletal dysfunctions pertinent to CC in PD for this IRB-approved prospective ABA-design concept study in six subjects with PD-CC. Validated CC instruments were administered during a 4-week control-period (A), 4-weekly OMM-treatments (B) and 2-weeks no-intervention (A). Biweekly stool

samples were assessed for normalized microbiota abundance. This OMM treatment sequence combined techniques to specifically address PD-related dysregulation by the ANS and ENS, pelvic floor muscle dystonia, bradykinesia effects on the gastrointestinal system, and decreased pumping mechanics in thoracic excursion and diaphragm restriction [9,35].

The CC measure results are consistent with prior studies on the use of OMM in the adjunctive management of CC with respect to significant improvements in CC measures using this frequency of treatment sessions [9-11]. Determining changes in the gut microbiome after treating CC with OMM is novel. The microbiota found to significantly change Post-OMM demonstrated some Pre-treatment clinical significance in PD severity. The role that the PD-CC microbiome plays in manifestation or progression of PD is not clear. A previous study combined the abundance of Verrucomicrobiacae and bacterial families of Firmicutes with the WCCSS to identify PD patients [6]. In the present study, there was a significant improvement in WCCSS, and there were significant changes in Verrucomicrobiacae abundance and Firmicutes diversity Post-OMM (Specifically, the balance of families and the normalized abundances of Roseburia and Intestimonas). There was a significant shift in the balance of phyla Post-OMM. This intervention may have benefits other than quality of life in CC because the proportions Bacteroidetes, Firmicutes, and Verrucomicrobia found in humans has previously been associated with PD [6-8]. Particular families (Lactobacillacae, Clostridiales Incertae Sedis IV, Ruminococcicae, Enterobacteriaceae) within the phylum Firmicutes are associated with PD-CC [6]. Also, microbial diversity is thought to be healthy in mammals [8,38]. A normalized abundance of Prevotellaceae ≤ 6.5% of the total gut microbiota was also found to have 86.1% sensitivity and 38.9% specificity for PD [6]. Consistent with this, the present results demonstrated a mean normalized abundance of Prevotellaceae of 0.97%. The Pre-OMM reduction of the normalized abundance of Roseburia in those with more severe PD motor impairment significantly increased Post-OMM with a large effect size. The Pre-OMM correlation of normalized abundances of Intestimonas suggested that those with more severe PD motor features had greater abundances that significantly decreased Post-OMM, with a medium effect size. There was a large, significant effect size on the mean normalized abundance of Actinobacteria. There was a medium effect size on the mean normalized abundance of Verrucomicrobia. In this study, Verrucomicrobia was correlated most significantly with UPDRS score 4.6 Painful OFF-State Dystonia. This correlation may involve the impact of dystonia on gait and mobility because inactivity has previously been associated with CC.

The correlation of Firmicutes abundance with PD-CC supports other microbiological studies of PD-CC. Although the overall goal of the OMM-sequence was improving CC, the treatment targets included physiology pertinent to the UPDRS subscores chosen for the composite score for rating Mobility, Gait and Posture. Some of the microbiota that significantly changed Post-OMM had been strongly, significantly correlated with this Mobility, Gait, and Posture composite score. The specific OMM techniques used were not attributable to specific changes in the microbiome. Previous studies have utilized UPDRS subscores to investigate relationships of specific microbial taxon to features of PD; For example, Enterobacteriaceae has been positively associated with the severity of postural instability and gait difficulty on UPDRS [6]. Additionally, this study's results indicated that the Actinobacteria families Coriobacteriaceae and Corynebacteriaceae and genera Collinsella, Corynebacterium, and Gordonibactera were significantly less as severity of the Mobility, Gait, Posture composite score worsened. Eggerthella was significantly positively correlated to Part 1: Non-motor aspects, freezing of gait and posture. Understanding the changes in the gut microbiome during treatments improving CC severity may give insight into the mechanisms underlying constipation and its role in the manifestation of PD.

The results support the hypothesis because the normalized abundances in the gut microbiome significantly shifted with concurrent improvement in the mean Bristol rating and significant improvement in the mean constipation severity and quality of life scores among individuals with PD-CC Post-OMM. There were several limitations to this study, including sample size, single-blinding, and lack of independent-controls receiving face-to-face time to assess effects of the provider-patient interaction, physical exam, and sham. The study design took the small sample size and other limitations into consideration. A time-series, within-group design with repeated measures of CC was

used. Disadvantages from learning effects and regression to the mean that may occur in within-group design studies were mitigated by having the same outcome measure intervals during control-period and intervention-period [40-42]. Secular trends were unlikely given the prevalence of CC in PD, subjects participating at variables times of the year, variable disease severities based on the H&Y, and accounting for potential confounding variables. Effects of within-subject characteristics on measurements were evaluated with disease severity and feature stratification covariates and controlling for potential confounding variables. In this study, subjects were not restricted from their typical symptom management in order to minimize anxiety, health concerns, and comfort. The study was time- consuming for subjects and their caregivers. Significance was reached after seven subjects completed the study. Sample size was too small to confidently calculate Number Needed to Treat or to isolate pharmaceutical and nutraceutical effects. Although significant changes and correlations were identified, the small sample size limits generalizability of the results. Complete blinding was not feasible. The outcomes were measured by one of two physician-investigators and one of two student-investigators.

The choices of outcome measures validated for CC in PD were limited. The PAC-QOL is a validated tool used by healthcare providers and pharmaceutical effectiveness in randomized controlled trials to assess the impact on patients and their families [43]. Manometry directly measures colonic transit time, however, the PD population tends to have higher risk of disabling neurologic, airway, and pulmonary complications during procedures. Prior studies of the interrater reliability for Bristol scores rated by gastroenterology providers was good [44], but agreement among them decreased when distinguishing type 2 from 3, (which represents the boundary between abnormally slow transit with low water content and normal stools), making improvement less clear. The use of the gut microbiome as a predictor or measure of PD-CC may have challenges such as varying geographical regions, setting, microbial analysis methods, medical history, demographics, and diet. The clinical relevance of the shift in microbiome balance to PD severity remains unclear because the UPDRS was not repeated Post-OMM.

Future investigations of the effects of this OMM sequence for adjunctive treatment of CC in PD would benefit from real-time measurements of variables collected in Apps (Ex. FDA MyStudies and lifestyle health diaries) designed to facilitate the input of data directly by patients to address the shortfalls of traditional clinical trials, pragmatic trials, and observational studies [45]. Real-time studies of multiple variables allow for complex analysis via experience sampling methods and ecological momentary assessment [45]. Larger sample size, sham and placebo control groups and repeating the UPDRS would improve generalizability of the results. The effect sizes from this study may be used for future power analysis. In general, the molecular products of the microbiome are vast, including fatty acids, hormones, neurotransmitters, and other bioactive molecules. Considering this, understanding how chronic imbalances of the microbiome affects the human body may be more helpful than short term changes in particular species. Future studies could also include probiotics or microbial products.

Conclusion

The gut microbiota among individuals with PD-CC significantly differed after four weekly treatments with the OMM-sequence aimed at addressing the PD features contributing to CC, including ANS and ENS dysregulation, pelvic floor muscle tone imbalance and dystonia and restricted pulmonary syndrome. There were large effect sizes on constipation severity and quality of life as well as the normalized abundance of Actinobacteria and the diversity of families within Firmicutes. The baseline results strongly, significantly suggest that there was less normalized abundance of Actinobacteria as severity of H&Y and PAC-QOL worsened. Within the phylum Actinobacteria, the baseline normalized abundances of Bifidobacteriaceae and Bifidobacterium had near perfect, significant, positive correlation with worsening severity of UPDRS Part 1: Non-motor aspects of experiences of daily living worsened. Other families and genera of Actinobacteria were strongly, significantly less as severity of Mobility, Gait, and Posture worsened. Within the phylum Firmicutes, there was a large effect size with significantly increased Roseburia as well as moderate effect size with significantly decreased Intestimonas Post-OMM. The baseline

results suggest that Roseburia was strongly, negatively correlated with H&Y and for the Mobility, Gait, and Posture composite subscore, while Intestimonas was strongly, positively correlated with Total UPDRS, H&Y, and the Mobility, Gait, and Posture composite subscore. Verrucomicrobia abundance was significantly decreased in Post-OMM samples, and the effect size was moderate. Pre-OMM Verrucomicrobia significantly, positively correlated with worsened severity of Mobility, Gait, and Posture, particularly, Painful OFF-State Dystonia.

Further studies are necessary to use microbiome analysis in the assessment of CC as a reliable, feasible PD biomarker. However, the significant changes Post-OMM in the normalized abundance of certain microbiota that were significantly correlated with UPDRS Total score, non-motor aspects, Mobility, Gait, and Posture subscore and the constipation measures may guide future research to establish microbiome analysis with assessment of CC to assess effectiveness of this OMM-sequence or other CC management in PD.

Author Contributions

Study conception by JDM, TSL, SCY. Literature review by JDM, TSL. Statistical analysis performed by JDM, HS. Manuscript reviewed by JDM, SCY, LM, HS, TSL.

Conflict of Interest

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