Effects of acupuncture on Parkinson's disease-related constipation and roles of gut microbial ecology: study protocol for a randomized controlled clinical trial

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Study protocol

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Abstract

Background: Motor disturbances and non-motor manifestations, notably constipation, stand as primary factors influencing the quality of life in individuals with Parkinson's disease (PD). Recent microbiological research increasingly links PD with the gastrointestinal environment, highlighting the crucial role of gut microbiota. Clinical studies suggest acupuncture may alleviate motor impairments and associated non-motor issues, like constipation, in PD patients. However, limited research on underlying mechanisms necessitates further evidence-based investigation for comprehensive validation. This study aims to investigate whether acupuncture improves the clinical symptoms of patients with Parkinson's disease-related constipation (PDC) by modulating the balance of intestinal microecology.

Methods: This single-blind randomized controlled clinical trial enrolls 84 patients with PDC, randomly allocated in a 1:1:1 ratio to real acupuncture group (RA), sham acupuncture group (SA), and waitlist control group (WG). Treatments will span four weeks, with primary outcomes measured through changes in the Unified Parkinson's Disease Rating Scale (UPDRS). Secondary outcomes include the Stool diary, the Non-Motor Symptoms Scale (NMSS), 30-meter walking test, fecal 16S rDNA gene sequencing, serum Lipopolysaccharide (LPS) and Lipopolysaccharide-binding protein (LBP) levels. The adherence and adverse events will also be recorded. Participants will be followed until week 16, and statistical analyses will encompass all allocated individuals.

Discussion: The outcomes of this study are anticipated to substantiate the efficacy and underlying mechanisms of acupuncture as a complementary treatment for PDC. The study holds the potential to furnish robust clinical evidence, thereby contributing to the establishment of novel guidelines for the treatment of PDC.

Trial registration: The Research Ethical Committee at the First Affiliated Hospital of Guangzhou University of Traditional Chinese Medicine authorized this trial. The trial is registered with the Chinese Clinical Trials Registry (ChiCTR) under registration number ChiCTR2400082060, registered on March 20, 2024. (https://www.chictr.org.cn/showproj.html?proj=200177)

1. Background

Parkinson's disease (PD) is a prevalent neurodegenerative disorder(1) with treatment challenges yet to halt its long-term progression(2, 3). Globally, PD affects approximately 3.9% of individuals(4), with notable prevalence in densely populated regions like Asia(5), including China where it ranges between 1–2%(6). Characterized by four primary motor symptoms—bradykinesia, rigidity, resting tremor, and postural instability—PD results primarily from the loss of nigrostriatal dopaminergic neurons(7–9). Besides motor symptoms, PD presents various non-motor symptoms (NMS) like constipation, urine incontinence, cognitive impairment, fatigue, mood disturbances, and sleep issues. Constipation, often preceding motor symptoms, is the most frequent NMS(10–12). Prevalence estimates of PD-related constipation (PDC) range from 46.83–59.6%(12), with PD patients three times more likely to experience constipation, exhibiting increased severity and harder stools(13). PD significantly impacts patients'

quality of life and correlates with serious health issues such as delirium, adverse drug reactions, syncope, falls, fractures(14), and notably higher hospitalization rates compared to age-sex matched control group(15, 16).

The intestinal microecological balance significantly influences host health, although its specific impact on gastrointestinal function in PD remains underexplored(17). Hypothetical mechanisms based on prior experimental evidence suggest potential relationships. Recent findings highlight the significance of gut microbiota-derived metabolites like short-chain fatty acids (SCFAs), endotoxin, and neurotransmitters (glutamate, serotonin, γ-aminobutyric acid - GABA) in influencing the gut-brain axis and the risk of neurodegenerative diseases(18–25). The composition, diversity of gut microbiota, and the integrity of the gut-blood barrier significantly relate to PD, affecting the progression by potentially inducing systemic inflammation(26). A hypothesis suggests that elevated endotoxin plus aggregable α-synuclein results in neurodegeneration(27). Lipopolysaccharides (LPS) and other bacterial neurotoxins traverse the intestinal wall, entering the bloodstream and compromising the intestinal epithelial barrier(23, 28). This influx of bacterial LPS into the bloodstream triggers the production of inflammatory cytokines via nuclear factor-kB (NF-kB) and Toll-like receptor 4 (TLR4), leading to systemic inflammation(28, 29). Studies have demonstrated elevated LPS absorption in blood samples of individuals with PD, resulting in markedly elevated systemic concentrations of LPS-binding protein (LBP)(29, 30). Further microbiome analysis holds potential to enhance accuracy, elucidate relationships, and clarify underlying mechanisms(31).

Acupuncture, a popular complementary and alternative medicine approach, is recommended as therapy for PD patients based on level B evidence(32, 33). Recent meta-analyses have shown that acupuncture in conjunction with conventional medication exhibits a notable positive impact on movement function in PD patients(34, 35). Animal studies corroborate these findings, suggesting acupuncture's potential in alleviating PD symptoms(36, 37). Studies in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced PD mice indicate that acupuncture may enhance motor function and protect dopaminergic neurons by regulating intestinal microbial imbalance and inhibiting neuroinflammation(38, 39). Clinical research also suggests a connection between gut microbiota and the protective effects of scalp-abdominal electroacupuncture in PD patients(40). Our hypothesis suggests that acupuncture can effectively alleviate both motor and non-motor symptoms in PDC patients, potentially related to improvements in constipation and mediated through intestinal flora regulation. The outcomes of this study are anticipated to substantiate the efficacy and underlying mechanisms of acupuncture as a complementary treatment for PDC.

2. Methods

2.1 Trial design

This single-blind randomized controlled clinical trial will involve PDC patients divided into three groups: real acupuncture group (RA), sham acupuncture group (SA) and waitlist control group (anti-Parkinson drugs only, WG). The RA will receive anti-Parkinson drugs combined with real acupuncture during the

observation period, SA will receive anti-Parkinson drugs combined with sham acupuncture, while the WG will solely receive anti-Parkinson drugs. All enrolled patients will be instructed to adhere to their initial dosage of anti-Parkinson drugs throughout the trial. Any necessary adjustments to medication dosages to alleviate symptoms during the trial will be meticulously recorded on the case report form. Repeated assessments of outcome measures will be conducted prior to and following the 4-week intervention period, as well as upon completion of the follow-up at week 16.

The treatment effect, placebo effect, total effect, and follow-up effect of acupuncture on PDC will be evaluated by comparing the RA, SA, and WG. Additionally, the study aims to observe whether the effect of acupuncture is associated with the regulation of gut microbial ecology balance. Figure 1 showed the Consolidated Standards of Reporting Trials (CONSORT) flow diagram.

The study protocol has been approved by the Ethics Committees at the First Affiliated Hospital of Guangzhou University of Chinese Medicine in accordance with the Declaration of Helsinki guidelines. Registration has been completed in Current Controlled Trials (ChiCTR2400082060), registered on March 20, 2024. (https://www.chictr.org.cn/showproj.html?proj=200177). This study will follow the CONSORT reporting guideline and the Standards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA) guideline for the designing and reporting of this trial.

2.2 Study setting and recruitment

A target sample of 84 participants will be recruited in the acupuncture outpatient at the First Affiliated Hospital of Guangzhou University of Chinese Medicine in China. To recruit potential patients, recruitment advertisements will be posted on WeChat, acupuncture outpatient, inpatient systems, and other official platforms. To enlist potential patients, recruitment advertisements will be disseminated through various channels including social media, the acupuncture outpatient department and other official platforms. The advertisements will include concise descriptions of the inclusion criteria, potential benefits for eligible patients, and contact information for the researcher. All prospective participants expressing interest in the study will be invited to the hospital to undergo comprehensive screening tests administered by a neurology expert. Baseline data and physical examination information of eligible participants who voluntarily participate in this clinical trial will be promptly documented. The recruiting time will start in May 2024 and run until June 2025. All participants will sign informed consent forms in writing.

2.3 Diagnostic Criteria

The revised clinical diagnostic criteria for PD by the International Association of Dyskinesia in 2015 will serve as the diagnostic criteria(9). Currently, there isn't a universally recognized diagnostic standard for PDC. Previous studies often combined the diagnostic criteria for PD and constipation(41, 42). In this study, patients diagnosed with idiopathic PD will be included, regardless of their current anti-Parkinson medication, if they meet the Rome IV criteria(43). These criteria involve having fewer than three spontaneous bowel movements (SBM) per week over the past three months, with a symptom duration of at least six months.

2.4 Inclusion and exclusion criteria

Participants will be recruited based on specified exclusion criteria detailed in Table 1.

2.5 Sample Size.

The sample size calculation was based on the primary outcome measure, which is the difference in UPDRS scores before and after treatment. After a 4-week acupuncture treatment, the mean (SD) difference in UPDRS scores for patients receiving real acupuncture combined with anti-Parkinson drugs was − 5.75 (8.865), for those receiving sham acupuncture combined with anti-Parkinson drugs was − 2.25 (7.534), while patients receiving anti-Parkinson drugs only had a mean difference of 2.25 (4.573). A statistical power of at least 90% was chosen to detect a two-sided significance level of 5%. With an initial sample size of 66 patients (22 per group), accounting for a 20% dropout rate, the final sample size targeted for inclusion is 84 patients (28 per group).

2.6 Randomization and concealed allocation

Participants will be randomly assigned to three groups at a 1:1:1 ratio: real acupuncture plus anti-Parkinson drugs, sham acupuncture with anti-Parkinson drugs and anti-Parkinson drugs only. A statistician not involved in the trial will generate the randomization sequence using SPSS Statistics 22.0 (IBM SPSS Statistics Inc., Chicago, USA). The allocation sequences will be concealed within sealed, sequentially numbered, opaque envelopes managed by an independent third party, ensuring confidentiality. The clinical research coordinator will contact the third party to obtain the participant's random number and group assignment when eligibility is confirmed.

2.7 Blinding

Single-blind method will be used in this study. Following the information from the envelope, each participant will be randomly assigned in a 1:1:1 ratio to RA, SA or WG. Patients in RA and SA, outcome evaluators, statistical analysts will be blinded, and acupuncture operators and patients with WG will not be blinded. To prevent bias, outcome assessors, data collectors, the statistician conducting the analysis, and patients in both RA and SA groups will maintain blinding to group assignments throughout the trial. Acupuncturists will be instructed to refrain from disclosing the patient’s group assignment to the trial's data collectors at any point. The blinding acupuncture device used in SA has obtained the national utility model patent certificate (patent number: ZL 202121352221.7). The customized blinding needles are produced by a manufacturer with relevant qualifications (Guangzhou Suixin Medical Equipment Co., Ltd.). The sham acupuncture device is depicted in Fig. 2. The masking effect of this sham needle device has been elucidated in previous study(44). The tools used for patients in both RA and SA groups will be identical in shape. Additionally, the duration of acupuncture and the acupoints used for both groups will be the same. To maximize the blinding effect, all patients in both groups will wear eye masks throughout the treatment to ensure successful implementation of blinding.

3. Interventions

Four experienced physicians, each with over 3 years of clinical practice, will administer the treatments. All physicians will undergo standardized training to ensure consistent procedures. Throughout the study, all enrolled patients will be instructed to adhere to their initial dosage of anti-Parkinson drugs throughout the trial. Any necessary adjustments to medication dosages to alleviate symptoms during the trial will be meticulously recorded and calculated whether the equivalent dose of levodopa changed as well.

Acupuncture procedures in both the RA and SA groups will be conducted using a specialized acupuncture tool comprising a base adhering to the skin and a sleeve. In particular, this acupuncture tool features two types of bases: one is hollow, and the other is sealed by the adhesive layer. Specifically, the special acupuncture tool will be first affixed to the skin. In the acupuncture group, acupuncture needles will be inserted through the cannula and pierced into the skin via the perforated base to achieve the acupuncture effect. However, in the control group, a special placebo needle will be inserted through the cannula and pressed against the adhesive layer and the skin, with slight pressure applied to mimic a placebo effect similar to acupuncture. Before the commencement of our study, patients in both RA and SA groups will be informed that they may receive either "less painful acupuncture" (sham needles) or "traditional Chinese acupuncture" (real needles). Given that the sham acupuncture employed in this study can induce comparable sensations to acupuncture without actual skin penetration, it serves as an effective blinding tool. In this manner, we aim to achieve a single-blind effect on the patients to assess the placebo effect in acupuncture. Figures 2 depict the structure of the sham acupuncture needle.

3.1 Real acupuncture Group (RA)

Participants in the RA will receive 12 treatment sessions, three times a week (Tuesday, Wednesday, and Friday), over a 4-week period, maintaining their original dosage of anti-Parkinson drugs. Each acupuncture session will last for 30 minutes. Acupuncture will focus on specific acupoints within the Shen-regulation acupoint set, with the use of the Intestinal three-needle. The Shen-regulation acupoint set includes key acupoints such as Sishenzhen (comprising GV 21, GV 19, and bilateral points 1.5 cun next to GV 20), GV 24 (Shenting), GV 29 (Yintang). The Intestinal three needles consist of ST 25 (Tianshu), CV 4 (Guanyuan), and ST 37 (Shangjuxu). Prior clinical investigations have established the efficacy of these acupoint sets in mitigating symptoms in PD patients(41, 44). Figure 3 illustrates the selection and location of these acupoints.

Following routine disinfection, the base of the real acupuncture needle will be affixed to the skin. Acupuncture will be conducted using disposable, sterilized stainless steel needles (Tianxie, Suzhou Medical Appliance Factory, Suzhou, China; 0.25 × 25 mm, 0.25 × 40 mm). Acupoints Sishenzhen, GV 24, and GV 29 will be treated with 0.25 × 25 mm acupuncture needles featuring a 15-degree angle. For bilateral ST 25 (Tianshu), CV 4 (Guanyuan), and ST 37 (Shangjuxu), 0.25 × 40 mm acupuncture needles with a 90-degree angle will be employed. After inserting the needles into the corresponding acupoints, twisting them to stimulate qi flow until the patient experiences the de qi sensation (a combination of numbness, soreness, and heaviness). All needles will be retained in place for 30 minutes post-qi arrival.

3.2 Sham acupuncture Group (SA)

Participants allocated to the SA group will receive twelve 30-minute sessions of acupuncture using sham acupuncture needles. These sessions will occur on Tuesday, Wednesday, and Friday, three times a week for four weeks. Participants will also maintain their original dosage of anti-Parkinson drugs throughout the trial. If dosages need to be increased or decreased during the trial to relieve symptoms, the change will be recorded on the case report form.

The acupoints utilized for patients in the SA group are identical to those in the RA group, comprising the Shen-regulation acupoint set and the Intestinal three needles (Fig. 3). After routine disinfection, sham acupuncture needle base will be pasted on the skin. the acupuncture operators quickly tapped the top of the tube to make the needle go downward. Acupuncture will be administered using specialized disposable, sterilized flat-headed stainless steel needles. The angle of insertion for each acupoint will mirror that used in the RA group. As the sham acupuncture utilized in this study can induce similar sensations to acupuncture without actually penetrating the skin, it serves as an effective blinding tool.

3.3 Waitlist control group (WG)

The WG will solely receive anti-Parkinson drugs throughout the observation period. After a 4-week waiting period, patients in SA and WG will have the option to receive the same compensatory treatment for 4 weeks as those in RA.

4. Outcome

The assessment of results primarily involves evaluating the severity of PD and constipation. Outcome measures will be gathered during pre-intervention, post-intervention, and follow-up assessments, with data collected in the "ON" medication state at each time point. And patients will maintain stool diaries, documenting daily records. Clear instructions will be provided to ensure that patients with PD adhere to their regular medication schedule throughout the study, maintaining the "ON" medication state.

The primary outcome will be the change in the Unified Parkinson's Disease Rating Scale (UPDRS) score from baseline to weeks 4 and 16. Secondary outcomes include the Stool diary, Non-Motor Symptoms Scale (NMSS) score, 30-meter walking test, fecal 16S rDNA gene sequencing, and levels of serum Lipopolysaccharide (LPS) and Lipopolysaccharide-binding protein (LBP). Any adverse events during treatment will be recorded, and treatment safety will be assessed. Participant follow-up will continue until week 16. Statistical analysis will encompass all allocated individuals. Refer to Table 2 for the schedule of recruitment, intervention, and assessment.

4.1 Baseling

Demographic information, comprehensive medical history, including medication details (especially laxatives and anticholinergic medications), smoking habits, and body mass index, will be documented. The food frequency questionnaire (FFQ) will be employed to assess the patients' diet over the last 2 months, enabling the calculation of the average daily intake of total energy, fat, protein, carbohydrate,

and dietary fiber. This method is designed to minimize the influence of dietary structure on outcome measures, including gut microbiota. Physical activity will be evaluated at baseline to minimize its influence on disease progression and the improvement of constipation symptoms. Anti-parkinsonian medication before intervention will be noted and converted into a daily levodopa equivalent dose. Constipation severity will be assessed using the Stool diary. Additionally, the use of laxatives, massage, and other non-acupuncture treatments will be recorded.

4.2 Primary outcomes

A thorough evaluation of disease severity in included patients before and after treatment will be conducted using the UPDRS. Widely utilized in clinical settings and scientific trials for PD (45, 46), the UPDRS has demonstrated responsiveness to changes in disease progression, motor fluctuations, and intervention outcomes. To ensure consistent and standardized application of the scale, a teaching videotape is provided, serving as a valuable asset to enhance inter-rater reliability(47). Outcome assessors will have a uniform training based on the teaching videotape.

4.3 Secondary outcomes

4.3.1 Severity of the constipation

The severity of constipation will be assessed using a stool diary, recorded from baseline (week − 1) to the end of follow-up (week 16). Following the initial baseline visit, participants will undergo a one-week pre-intervention phase (Week − 1) during which they will be trained to maintain a baseline stool diary. This diary captures data on the number of spontaneous bowel movements (SBM), stool consistency (evaluated using the 7-point pictorial Bristol Stool Scale [7 = hard lumps; 6 = lumpy sausage; 5 = cracked sausage; 4 = smooth sausage; 3 = soft lumps; 2 = mushy; 1 = watery]), and the use of laxatives. Only patients who successfully completed the baseline constipation diary will be included in the treatment, and they will continue to maintain the constipation diary until the end of follow-up (week 16).

4.3.2 Motor symptoms and non-motor symptoms

The assessment of motor symptoms will involve a comprehensive evaluation utilizing UPDRS Part III and objective data referencing the 30-meter walking test. The 30-meter walking test entails the patient walking 30 meters in a straight line at a consistent speed, repeated three times. Recorded data will include walking time and the number of steps taken, facilitating the calculation of the patient's average stride length and step count.

Non-motor symptoms will primarily be evaluated utilizing the NMSS, a healthcare professional-completed scale that categorizes the severity (mild, moderate, and severe) and frequency (every day, several times a week, once a week, and rarely) of 30 different non-motor symptoms across 9 distinct domains(48). The NMSS is recommended by the MDS and other expert societies for holistic assessment of non-motor symptoms and their burden(48, 49).

4.3.3 Intestinal flora detection

All participants will follow electronic video instructions and a brochure providing guidance on optimal stool collection methods to ensure sample integrity and prevent contamination. (Supplementary Video 1). Stool collection will utilize fecal DNA preservation tube (CW2654) (Kang Wei Century, China), which allows preservation at room temperature for a minimum of 60 days and long-term storage at -80℃. Stool samples will be collected at home by participants within 1 week before treatment initiation and within 1 week after completing 12 acupuncture sessions. The samples will be transported to the hospital as soon as possible and submitted to the researcher, who will freeze them immediately in a -80℃ freezer.

Fecal flora genomic DNA extraction will use the OMEGA Soil DNA Kit (M5635-02) following strict instructions. The quantity and quality of genomic DNA will be measured using a NanoDropNC2000 spectrophotometer and agarose gel electrophoresis. Bacterial 16S rRNA gene V3-V4 region PCR amplification library will be performed with forward primer 338F (5 '-ACT CCT ACG GGA GGCAGC A-3') and reverse primer 806R (5 '-GGA CTA CHV GGG TWT CTA AT-3'). Recovered PCR products will undergo fluorescence quantification with the Quant-iT PicoGreen dsDNA Assay Kit and be mixed accordingly. Sequencing libraries will be prepared with the TruSeq Nano DNA LT Library Prep Kit from Illumina, and quality inspection will be done using Agilent High Sensitivity DNA Kit. Qualified libraries will be quantified with the Promega QuantiFluor system and the Quant-iT PicoGreen dsDNA Assay Kit. Finally, qualified libraries will be mixed for high-throughput sequencing on Illumina MiSeq/NovaSeq platforms.

4.3.4 Serum measures of endotoxin exposure

Serum LPS and LBP will be measured both before and after acupuncture treatment. Endotoxin will be quantitative measured in serum by Human Endotoxin Test kit (MM-1309H1) (Meimian, Jiangsu, China). LBP will be measured using the Human LBP ELISA Kit (MM-1104H1) (Meimian, Jiangsu, China). All procedures will adhere to the manufacturer's instructions. Initially, the absorbance of each sample will be measured at 450 nm. Subsequently, a standard curve will be constructed, with absorbance plotted on the ordinate and the corresponding standard concentration on the abscissa. A regression equation derived from the standard curve will be utilized to determine the concentration of LPS and LBP in each sample.

4.3.5 Safety Evaluation

During acupuncture sessions, practitioners will meticulously document adverse reactions such as dizziness, broken needles, hematoma, and infection. If a participant withdraws due to adverse reactions, the dropout reason and the last treatment time will also be documented, and any completed assessment items will be recorded. Severe adverse events had to be reported to the principal investigator and the data and safety monitoring board within 24 hours after their occurrence. Dropouts resulting from adverse reactions will be included in the statistical analysis of adverse reactions. Adherence will be evaluated based on the completion of acupuncture sessions.

Patients experiencing no complete spontaneous bowel movements (CSBMs) for more than three consecutive days or intolerable symptoms, such as severe bloating due to constipation, will be permitted to use emergency medications (e.g., lactulose and glycerin enemas) under medical supervision.

However, the first bowel movement after emergency medication use will not be included in the results. Any adjustments in anti-Parkinson's drug dosages, whether increased, decreased, or modified, will result in patient exclusion. Acupuncture treatment will cease if patients cannot comply with the regimen, experience sudden worsening of their condition during treatment, or require alterations in their anti-Parkinson drug regimen.

5. Statistical Analysis

To ensure data integrity and accuracy, two independent statisticians will perform statistical analyses using SPSS 22.0 (IBM SPSS Statistics Inc., Chicago, USA), establish the database and conduct proofreading for logical consistency. Descriptive analysis will be employed to assess the baseline characteristics of patients in each group. Normality distribution of quantitative variables will be evaluated using the Shapiro-Wilk test, with a P value > 0.05 indicating normal distribution of the quantitative variable. If the result indicates normality, it will be presented as mean (SD), and the t-test will be utilized for comparison between the two cohorts. If the result does not conform to normality, median (IQR) will be reported, and the nonparametric Mann-Whitney U test will be applied for comparison. Qualitative variables will be described by frequency counts and proportions (rates). Group data will be compared using the $\chi 2$ test. If the theoretical frequency is too small, the Fisher exact probability method will be employed, where a P-value < 0.05 will be considered statistically significant. Repeated-measures analysis of covariance (ANCOVA) will be utilized for analyzing changes in UPDRS, stool diary, NMSS and 30-meter walking test. All hypothesis testing will be two-sided, with P < 0.05 considered statistically significant.

For the analysis of intestinal flora sequencing results, taxonomy will be assigned using the Greengenes May 2013 database. Microbial alpha diversity will be assessed on datasets rarefied to equal sequencing depth (37,662) using the Chao1 index of richness, Faith's phylogenetic diversity, and the Shannon index of evenness. Microbial composition will be compared across samples using weighted UniFrac distances and visualized through principal coordinates analysis(50). Differences in microbial composition significance will be evaluated with multivariate Adonis using 100,000 permutations(51). The baseline differential abundance of microbial genera will be determined using multivariate negative binomial mixed models implemented in DESeq2, with bowel habit subtype and sex as covariates(52).

Study participants will be assigned a study ID (pseudonym) for all data collection. Self-administered questionnaires will be directly recorded in REDCap. Participant files will be retained for a minimum of 10 years. Weekly meetings among the study team, clinical partners, and the principal investigator ensure close monitoring of the data. Any adverse events or issues that arise are promptly addressed.

6. Quality control

To enhance quality control throughout the trial process, several measures will be implemented. (1) Prior to commencing clinical research, all researchers will undergo comprehensive training on the trial

protocol, standard operating procedures, and personnel deployment. (2) To ensure the feasibility of the research operation, all participating acupuncturists possess certification with over five years of experience and have mastered the operational procedures of the specialized needle equipment. (3) All personnel responsible for scale assessments will undergo unified training. (4) The instruments, equipment, and reagents to be utilized adhere to stringent quality standards to ensure optimal functionality under normal conditions. (5) To uphold the reliability of our research findings, we implement information feedback at the beginning and mid-term of the project, promptly addressing any encountered issues throughout the research process.

7. Discussion

7.1 Yin-Yang balance and intestinal microecology

Acupuncture, through its regulation of Yin and Yang balance in the body, collaborates with maintaining the equilibrium of intestinal microecology(53). Rooted in the holistic principles of traditional Chinese medicine, this approach considers the internal organs, body surface tissues, and external environmental factors as an interconnected whole. The balance of Yin and Yang is fundamental to human health in this traditional perspective, and its imbalance is considered a basic mechanism in the development of diseases. Similarly, the concept of intestinal microecology, viewed as an ecosystem comprising a multitude of microorganisms and flora, aligns with this holistic understanding. Modern medicine recognizes the critical role of intestinal microecological balance in human health, where disease occurrences often correlate with gut flora imbalances. Certain studies posit a close association between the occurrence and progression of PD and intestinal microecology(26). Clinical investigations substantiate that combining acupuncture therapy with standard anti-Parkinson drugs notably enhances clinical symptoms in PD patients, surpassing the efficacy observed with anti-Parkinson drugs alone and exceeding the placebo effect(41, 44). Consequently, both acupuncture therapy and intestinal microecology underscore the significance of maintaining an overarching balance and exhibit substantial relevance to the context of PD. The adoption of the brain-gut co-regulating acupoint selection protocol, incorporating Intestinal three-needle based on the Shen-regulation acupuncture theory, aims to comprehensively regulate patients with PD and may extend to regulating their intestinal microecology.

7.2 The Mechanism of Acupuncture on PD and Microbiome Dysbiosis

Acupuncture, a widely utilized complementary and alternative medicine approach, has garnered a Level B recommendation as a therapeutic adjunct for PD(32, 33). Globally, medical practitioners increasingly incorporate acupuncture into PD treatment strategies to address both motor and non-motor symptoms. Despite its widespread use, international acknowledgment of acupuncture's efficacy in PD remains limited, primarily due to the predominant publication of evidence-based literature in Chinese journals, often characterized by varying quantity and quality. Numerous animal studies underscore acupuncture's potential in mitigating PD-associated neuronal apoptosis in the striatum(54), normalizing brain

functional connectivity(36), and reducing lipid peroxide levels in dopaminergic neurons, thereby protecting against oxidative damage(37).

Despite these promising preclinical findings, clinical investigations into the mechanisms underlying acupuncture's efficacy in PD are scarce, with even fewer exploring its impact on the intestinal microbiome in PD patients. A clinical study utilizing scalp-abdominal electroacupuncture to target the gut-brain axis demonstrated significant alterations in the intestinal flora of PD patients post-treatment(40). However, the intricate and multifaceted nature of intestinal microecology(55), influenced by various factors such as diet, age, sex, obesity, physical activity, and antibiotic use, complicates the interpretation of study outcomes. Notably, the study did not comprehensively account for these potential confounders when establishing the baseline(40).

In this research protocol, meticulous consideration has been given to potential confounding factors during baseline establishment. Beyond evaluating changes in the structure of intestinal microecology, the study aims to investigate the impact of acupuncture on key components of intestinal metabolites, specifically LPS and LBP. A preceding randomized controlled trial has already affirmed that acupuncture, when compared to a placebo, effectively alleviates constipation symptoms in PDC patients(41). However, the study lacked comparison with an anti-Parkinson drug-only group, failed to elucidate the exact efficacy of the real and sham needles, and did not further explore the specific mechanism of acupuncture initiation, as only one preliminary analysis was conducted. Consequently, the present study aims to further elucidate the mechanistic underpinnings of acupuncture in the treatment of Parkinson's Disease with Constipation (PDC) by incorporating sham acupuncture and waitlist control groups as designated control groups.

7.3 Investigating Acupuncture's Impact on PDC patients: Brain-Gut Co-Regulation Study

Constipation, intestinal microecological imbalance, and PD may collectively contribute to a reciprocal and deleterious cycle. Constipation, recognized as one of the earliest manifestations of autonomic dysfunction in PD, holds significant clinical relevance(56). The multifactorial etiology of constipation in PD patients encompasses reduced neuronal density in the myenteric ganglion(57), dyssynergic defecation(58), and adverse effects of various anti-Parkinson's drugs, particularly anticholinergics and dopamine agonists(59). A population-based study underscores the escalating risk of Parkinson's disease with increasing constipation severity, emphasizing hazard ratios ranging from 3.3 to 4.2(60).

Constipation instigates an imbalance in intestinal microecology, characterized by fecal accumulation, proliferation of pathogenic bacteria, endotoxin release, mucosal disruption, and compromised barrier function, thereby triggering the release of inflammatory factors(61). This dysbiosis in intestinal microorganisms is intricately linked to PD(62). Observational findings reveal that PD patients experiencing constipation, particularly preceding motor symptoms, exhibit severe axial symptoms and rapid disease progression(63). Cohort studies affirm a significant correlation between constipation severity and the progression of non-motor symptoms, including cognitive impairment in PD patients(64).

Gastrointestinal symptoms further emerge as predictive indicators of cognitive trajectories in de novo Parkinson's disease(65, 66). Recent investigations report motor improvement subsequent to constipation treatment(67, 68). Thus, constipation exhibits a discernible association with various clinical symptoms in PD, underscoring its multifaceted impact on the disease's clinical course.

The selection of acupoints for the brain-gut co-regulating protocol, specifically utilizing Intestinal three-needle in conjunction with the Shen-regulation acupuncture theory, holds strategic significance. This approach aims to systematically investigate the potential correlation between the amelioration of constipation through acupuncture and its impact on motor symptoms and other non-motor symptoms in PD. Additionally, it seeks to elucidate the potential role of intestinal microecology in mediating these observed effects.

The outcomes of this study are anticipated to substantiate the efficacy and underlying mechanisms of acupuncture as a complementary treatment for PDC. The study holds the potential to furnish robust clinical evidence, thereby contributing to the establishment of novel guidelines for the treatment of PDC.

Data collection and management

Case report forms (CRFs) are designed to make data entry and export more convenient. Evaluators will record the detailed personal information and classify the research data of the subject in the CRFs. After the observed recourse end, CRFs will be sorted out in time and imported into the electronic database. To ensure the security and accuracy of databases, the completed paper CRFs will be collected into locked cabinets uniformly. In addition, the electronic database is managed by a third-party person who does not involve in the research process, so the researcher cannot modify the data content. Participants will be identified by a code and their personal information will be hidden and kept strictly confidential.

Trial status: The recruiting time has been starting in May 2024 and run until June 2025.

Abbreviations

Parkinson's disease (PD), Parkinson's disease-related constipation (PDC), real acupuncture group (RA), sham acupuncture group (SA), waitlist control group (WG), the Unified Parkinson’s Disease Rating Scale (UPDRS), the Non-Motor Symptoms Scale (NMSS), Lipopolysaccharide (LPS), Lipopolysaccharide-binding protein (LBP) , non-motor symptoms (NMS) , short-chain fatty acids (SCFAs), spontaneous bowel movements (SBM), the food frequency questionnaire (FFQ)

Declarations

Ethics approval and consent to participate: The study protocol complied with the ethical standards described in the Declaration of Helsinki. All methods of this study were performed in accordance with the relevant guidelines and regulations. The procedures implemented in this study adhered to the International Guidelines for Human Biomedical Research Ethics and the Declaration of Helsinki. The

Research Ethical Committee at the First Affiliated Hospital of Guangzhou University of Traditional Chinese Medicine authorized this trial (approval number: JY2023-154). Furthermore, the study was registered with the Chinese Clinical Trials Registry (registration number: ChiCTR2400082060, registered on March 20, 2024). Any alterations or modifications to the study protocol must be communicated to the Ethics Committee at the First Affiliated Hospital of Guangzhou University of Traditional Chinese Medicine, and reported to the Chinese Clinical Trials Registry. All patients will provide written informed consent, including consent for the collection of their blood and stool samples for this study.

Consent for publication: Not applicable

Data Availability Statement: The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Competing interests: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author contributions Professor Zhuang had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed to the article and approved the submitted version.

Concept and design: Lei, Li, Fan, Quan.

Acquisition, analysis, or interpretation of data: All authors.

Drafting of the manuscript: Lei, Wu, Lin, Liao, Xu, et, al.

Acupuncture for patients: Lei, Wu, Liao, Liu, Tsang;

Critical revision of the manuscript for important intellectual content: Zhuang, Fan, Zhang, Liu and Leong.

Statistical analysis: Lei.

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Tables

Tables 1-2 is available in the Supplementary Files section.

Figures

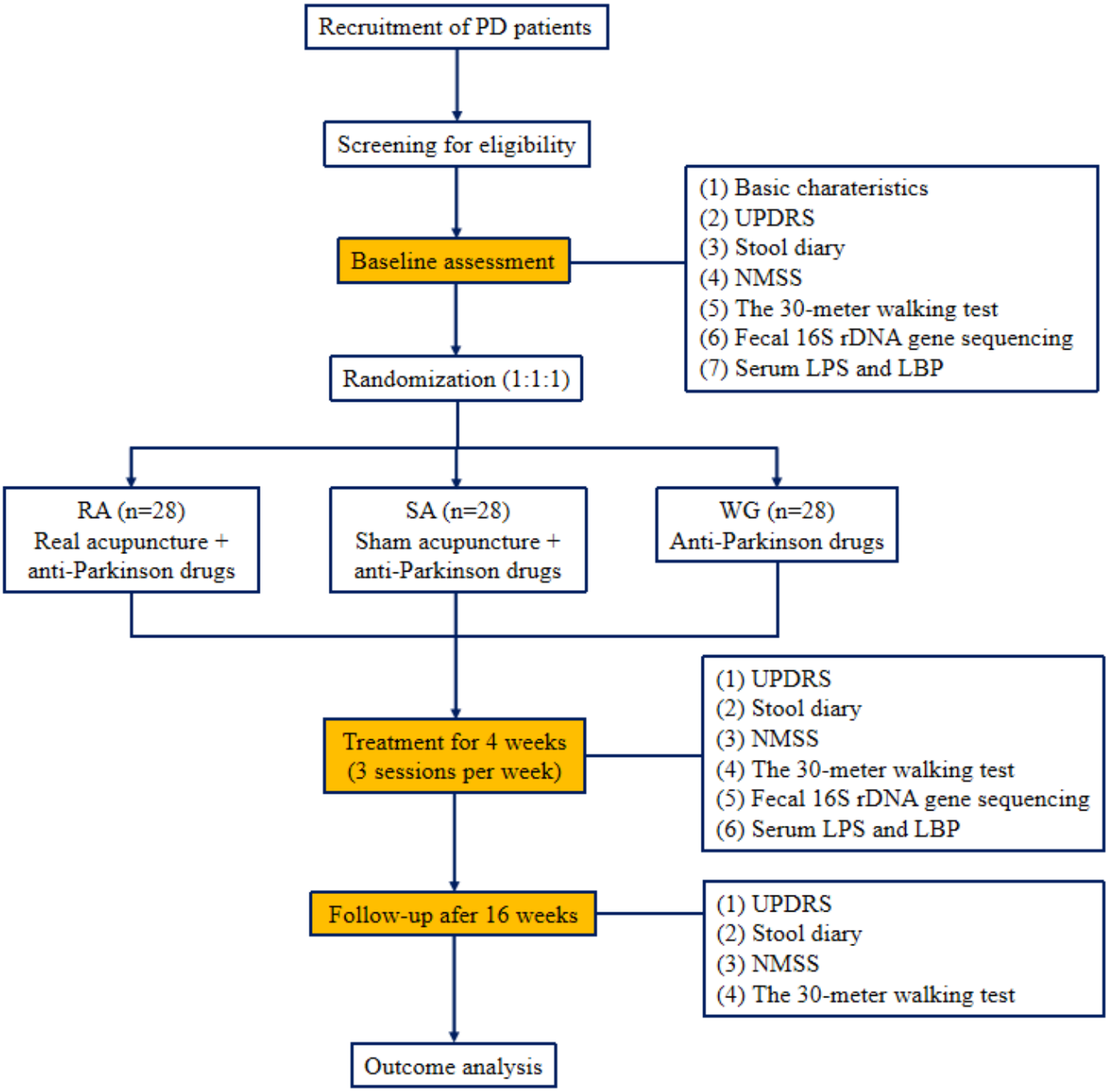

Figure 1

Flowchart of the present study protocol. RA, real acupuncture group; SA, sham acupuncture group; WG, waitlist control group; UPDRS, Unified Parkinson's Disease Rating Scale; NMSS, the Non-Motor Symptoms Scale; LPS (Lipopolysaccharide); LBP (Lipopolysaccharide-binding protein).

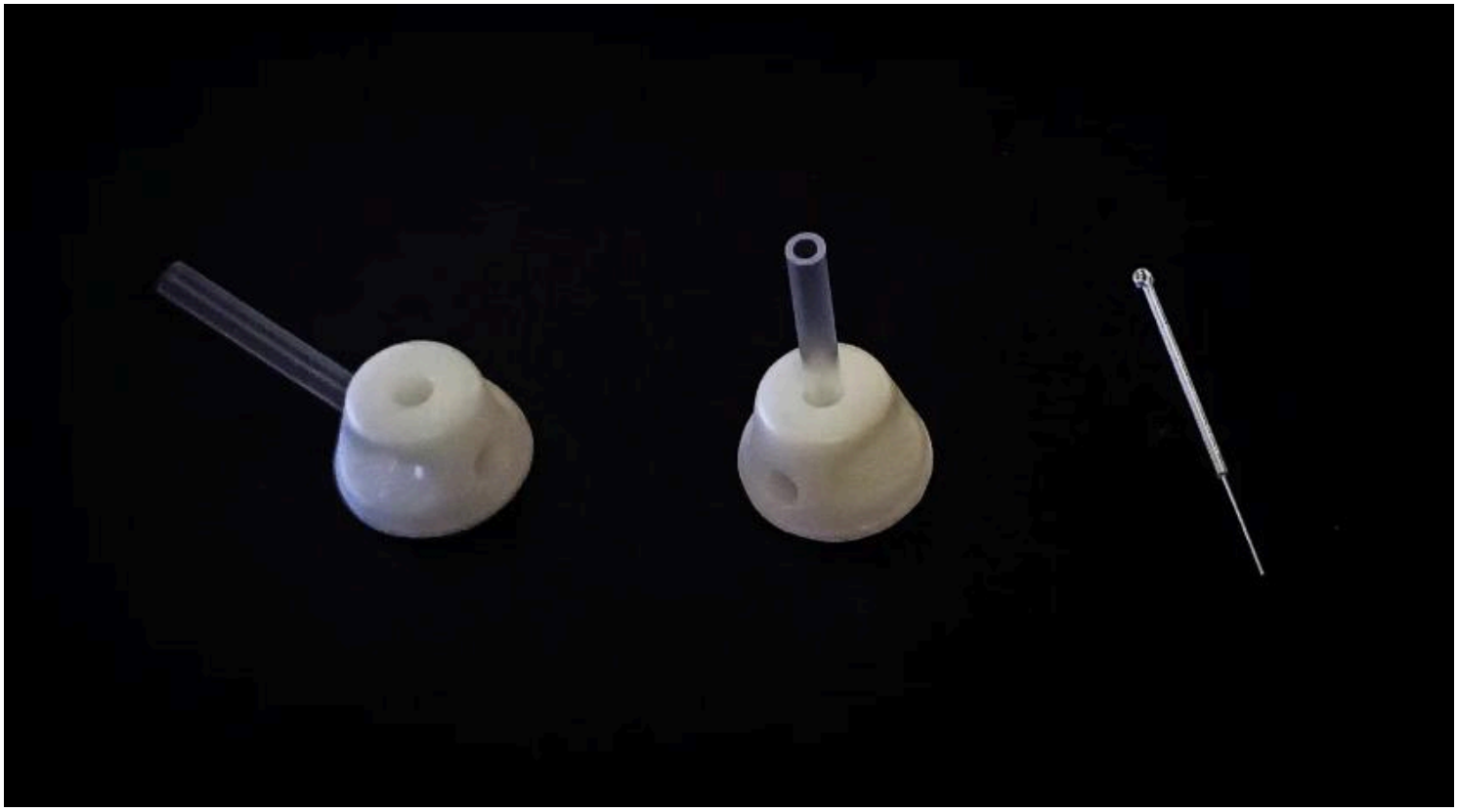

Figure 2

Schematic diagram of the specialized acupuncture equipment.

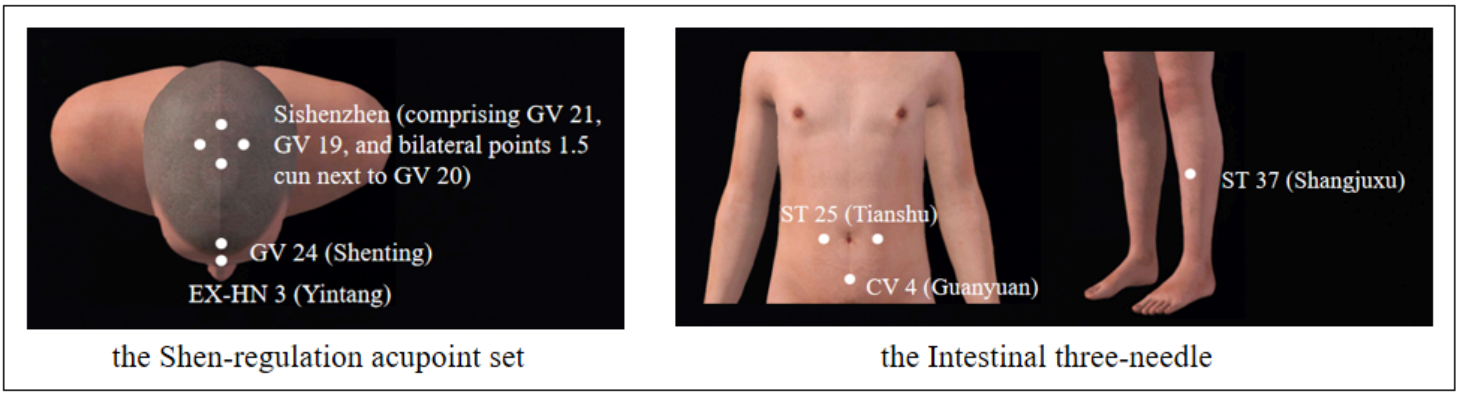

Figure 3

Selection and location of the acupoints for acupuncture.

Supplementary Files

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- Table1.png

- Tabe2.png
- spiritchecklist.jpg
- Video1.mp4