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Protocol

Efficacy of acupuncture versus sham acupuncture for postpartum depression disorder: Study protocol for a randomized controlled trial

Yumei Zhou, Haibo Yu, Yuebao Guo, Chen Chen, Xingxian Huang, Yanhua Gou, Jing Du, Yang Zhuoxin*

The Fourth Clinical Medical College of Guangzhou University of Chinese Medicine, No.1 Fuhua Road, Futian District, Shenzhen, 518033 GuangDong, China

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ABSTRACT

Introduction: This trial aims to conduct a randomized controlled trial (RCT) to determine the efficacy of acupuncture for postpartum depression disorder (PPD) and also to explore the potentional mechanism on the effectiveness of acupuncture for PPD.

Methods: A total of 74 patients with PPD will be recruited and randomly allocated to two groups: verum acupuncture group or sham acupuncture group. All patients will receive 24 treatments over an 8-week period. The primary outcome measurement is 17-item Hamilton Rating Scale (17-HAMD), and the secondary outcomes will be assessed using the Edinburgh Postnatal Depression Scale (EPDS) and the changes in gut microbiota. The 17-HAMD and EPDS will be evaluated before treatment and after 1, 2, 4, and 8 weeks of treatments, as well as 20-week follow-up. The fecal sample collections will be performed before and after 8 weeks treatment. The evaluators and data analysis will be blinded to group allocation.

Discussion: There are some RCTs investigating the effects of acupuncture for PPD, however, the quality of evidence is not "high". Therefore, this study will be designed as a high-quality RCT protocol.

Conclusion: The findings of the randomized and sham-controlled trial will demonstrate whether acupuncture therapy is effective and safe for patients with PPD, probably via regulating the gut microbiota dysfunction. These findings may have an important impact on the practice of acupuncture for patients with PPD.

1. Introduction

Postpartum depression disorder (PPD) is the most frequently occurring psychiatric condition among postpartum women [1]. Approximately 10%–15% of parturient women in the United States and other industrialized countries suffer from PPD [2–4] and bear symptoms such as depressed mood, loss of interest, extreme sadness or hopelessness, and sometimes thoughts of causing harm to self and the baby. PPD is a leading cause of suicide among puerperae and significantly contributes to individual and societal burdens owing to its long-term impact on women, children, and family [5,6].

Although multiple factors induce postpartum psychosis, the etiology of PPD is not well understood. Sociodemographic, psychological, and biological risk factors have all been implicated in the development of PPD. For instance, the poorly received social support contributed to elevated mental depression level [7]. Additionally, genetic, behavioral, and social risk factors are also suggested to be related to PPD. Studies have found that postpartum psychosis is a marker of a more familial form of the perinatal period [8]. Moreover, the alterations in the nervous system (NS), immune system, and hypothalamic–pituitary–adrenal axis (HPA) are regarded as the potential contributors to PPD [9,10], although no evidence has been found yet. In recent years, emerging evidence has suggested that gut microbiota plays an important role in psychiatric disorders, such as depression, through the microbiota–gut–brain axis. This axis mediates a bidirectional communication between microbiota and the brain, causing the emotional affect that influences the gut microbiota function and vice versa [11–13]. The microbiota–gut–brain axis is an integrative physiological concept, which consists of bidirectional communication between the signals in the central nervous system (CNS) and the gastrointestinal system

Abbreviations: CNS, central nervous system; CRF, case report form; *DSM*, *Diagnostic and Statistical Manual of Mental Disorders*; EPDS, Edinburgh Postnatal Depression Scale; EDTA, Ethylene Diamine Tetraacetic Acid; HPA, hypothalamic–pituitary–adrenal; 17-HAMD, 17-item Hamilton Rating Scale; IQR, inter quartile range; NS, nervous system; OTUs, operational taxonomic units; PCR, Polymerase Chain Reaction; PPD, postpartum depression disorder; QIIME, Quantitative Insights into Microbial Ecology; RCT, randomized controlled trial

* Corresponding author.
E-mail addresses: zym_star@163.com (Y. Zhou), 001188@gzucm.edu.cn (Y. Zhuoxin).

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through the NS, endocrine system, and immunological system [14]. Previously, Zheng et al. found that Actinobacteria in gut microbiota increased in patients with major depressive disorder; nevertheless, Bacteroidetes decreased [15]. Another study reported that some operational taxonomic units (OTUs) in the Bacteroidetes phylum positively correlated with depression, while other OTUs showed a negative correlation [16]. In addition, targeted modifications in the gut microbiota have been proved to be beneficial to correct autism spectrum disorder–related behavioral abnormalities [17]. Thus, the dysbiosis of gut microbiota might be a contributory factor in the development of depression.

Parturient women with PPD require long-term professional treatment programs. Antidepressants are commonly used as the initial treatment for moderate or severe depression. However, such treatments often induce an increased risk of adverse events, including diarrhea, headache, fatigue, and gastrointestinal intolerance. More importantly, women often prefer to stop pharmacological antidepressants worrying about drug transmission through breast milk to the baby [18]. Hence, effective and safe therapies for PPD are urgently needed.

Acupuncture has been applied to treat psychiatric disorders for thousands of years in China. In recent decades, acupuncture has been increasingly adopted as a promising treatment option for depression in Western countries. A cross-sectional study reported that depression ranked second among the full spectrum of acupuncture indications, and acupuncture has been a popular alternative for mental health management in the United States [19]. A randomized controlled trial (RCT) showed that electropuncture was effective for PPD, and the symptom remission after 4-week electroacupuncture treatment was 44% [20]. Another clinical trial also suggested that acupuncture might alleviate symptoms of patients with mild PPD with the HAMD scores decreasing significantly after the therapy [21]. In addition, a systematic review of 9 trials involving 653 women suggested that acupuncture was safe and effective for patients with PPD [22]. Therefore, clinicians prefer acupuncture to treat PPD.

How does acupuncture work on depression? Many researchers have studied its mechanism from different aspects. Recently, researchers found that acupuncture had a modulatory effect on gut microbiota as a result of improving the energy metabolism disequilibrium. Xu et al. [23] found that Bacteroidetes in the stool samples of obese patients decreased and intestinal microbial balance was restored after acupuncture. Bacteroides can suppress intestinal fasting-induced adiposity factor that promotes adiposity [24]. Another study [25] reported that electroacupuncture altered bacteria diversity and metabolic genes of the intestinal flora of obese mice to establish a new balance, which was suggested to be the new target for electroacupuncture treatment against obesity. Additionally, as complementary and alternative therapies, acupuncture or moxibustion has been shown to improve irritable bowel syndrome [26] by adjusting gut microbiota, or modulating plasmatic metabolites of hypertension [27]. However, whether regulating the human gut microbiota through acupuncture intervention can help the patients with PPD has not yet been studied.

Therefore, the aim of this study was to design a prospective RCT of women with PPD so as to investigate the effectiveness and safety of acupuncture, as well as explore its possible mechanism.

2. Methods/design

2.1. Study design

This study will recruit 74 patients with PPD, which will be randomly allocated to the verum acupuncture group or sham acupuncture group at a ratio of 1:1. The protocol has been conducted abiding by the Standard Protocol Items: Recommendations for Interventional Trials and Standards for Reporting Interventions in Clinical Trials of Acupuncture guidelines [28,29]. The flow diagram of the study procedure is shown in Fig. 1.

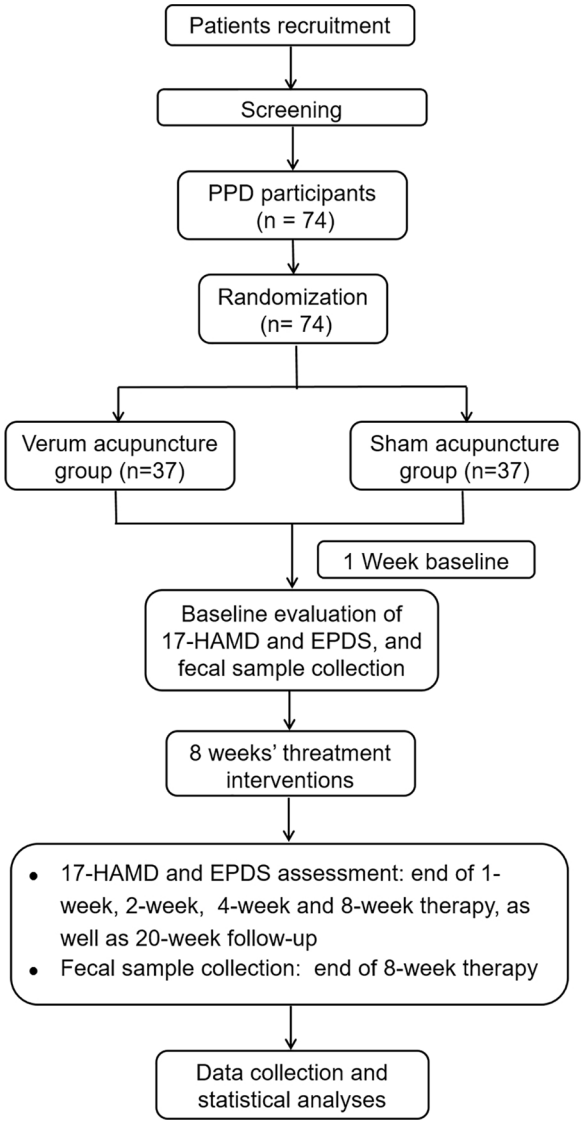

Fig. 1. Flowchart of the study design. This study will recruit 74 patients with PPD, who will be randomly allocated to the verum acupuncture group (VAG) and sham acupuncture group (SAG). Each patient in the VAG or SAG group will receive verum or sham acupuncture treatment, respectively. For all patients with PPD, fecal samples will be collected at baseline and after 8-week treatment, and 17-HAMD and EPDS assessments will be evaluated at baseline and after 1-week, 2-week, 4-week and 8-week treatments, and after 20-week follow-up. The effects and mechanism of acupuncture on treatment for PPD will be analyzed after data collection. EPDS, Edinburgh Postnatal Depression Scale; 17-HAMD, 17-item Hamilton Rating Scale.

2.2. Participants and recruitment

Participants meeting the inclusion criteria of PPD will be recruited mainly through outpatient clinics, advertisements, online or offline (such as newspaper, poster, and websites), and Wechat public account of Shenzhen Traditional Chinese Medicine Hospital and its surrounding communities. If a subject is interested in the research, she will be invited to consult the researchers and accept the screening. Informed consent form will be obtained from all eligible participants before randomization. The schedule of enrolment, intervention, and assessments is shown in Fig. 2.

2.3. Randomization and allocation concealment

After completing screening and formally entering the study, the

	STUDY PERIOD					
	Baseline	Treatment phase				Follow-up
TIMEPOINT	-1 ~ 0 week	1-week of treatment	2-week of treatment	4-week of treatment	8-week of treatment	Follow-up (20th week)
ENROLMENT:						
Eligibility screen	×					
Informed consent	×					
Randomization	×					
INTERVENTIONS						
Verum acupuncture group (n=37)		×	×	×	×	
Sham acupuncture group (n=37)		×	×	×	×	
ASSESSMENTS:						
17-HAMD	×	×	×	×	×	×
EPDS	×	×	×	×	×	×
FAECES COLLECTION:	×				×	
PARTICIPANTS SAFETY:						
Adverse events		×	×	×	×	×

Fig. 2. Study schedule for data collection. EPDS, Edinburgh Postnatal Depression Scale; 17-HAMD, 17-item Hamilton Rating Scale.

participants with PPD will be randomly assigned to the verum acupuncture and sham acupuncture groups at a ratio of 1:1 before therapy intervention. The randomization allocation numbers will be generated by an independent statistician using the Strategic Applications Software (SAS, version 9.1.3, SAS Institute Inc, NC, USA). The sequential numbers will be written on cards and sealed in an opaque envelope by another independent assistant. The assistant will allocate the patients according to their random drawing numbers sealed in the opaque envelope, and inform acupuncturists. Therefore, the concealment ends once the researchers open the envelop and know the allocation.

2.4. Blinding

The included patients with PPD will accept verum acupuncture therapy or sham acupuncture therapy. Owing to the particularity of acupuncture operation, acupuncturists will be not blinded and clearly know the detailed therapy schemes before the treatment starts. However, participants with PPD will be blinded to group allocation. They only know that they will undergo one of two kinds of acupuncture schemes provided in this study, but not the operation details of their treatments. At the end of the whole study, blind evaluation will be performed. The patients with PPD will be asked to guess the acupuncture type they receive. Additionally, both evaluators and statisticians will be also blinded to reduce risk of bias.

2.5. Study participants

2.5.1. Diagnostic criteria

The diagnostic criteria of PPD will refer to the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-IV*) [30], defined and proposed by the American Psychiatric Association. If the patients meet five or more of the following symptoms for at least 2 weeks, of which the first and second items are essential, they will be diagnosed with PPD by the psychiatrist: (1) low mood and depressive emotion; (2) lack or loss of interest in activities and no sense of pleasure; (3) significant weight gain or loss; (4) poor sleep quality, insomnia, or lethargy; (5) psychomotor excitement or retardation; (6) a feeling of fatigue or weakness; (7) a feeling that life is meaningless, self-accusation, or self-guilt; (8) decline in cognition or difficulty in concentrating; and (9) recurrent thoughts of death.

2.5.2. Inclusion criteria

The participants who meet with all of the following requirements will be considered for enrolment: (1) diagnosed with PPD by the psychiatrist; (2) onset of disease within 12 months after delivery; (3) the scores of 17-HAMD from 8 to 24; (4) aged 20–49 years; and (5) signed informed consent form for volunteering to participate in this study.

2.5.3. Exclusion criteria

Patients with any one of the following items will be excluded from this trial: (1) bipolar disorder (diagnostic criteria according to *DSM-IV*) or serious mental diseases such as schizophrenia; (2) dysnoesia or having difficulty in understanding the content of the questionnaire due to brain diseases or other reasons, or incapable of effective interview; (3) pregnancy; (4) the score of item “suicide” in 17-HAMD scale is more than 2 (with clear suicidal plan or intent, or attempts at suicide); and (5) have committed suicide within 1 year.

2.6. Interventions

Participants with PPD recruited in the study will be randomly assigned to one of two treatment groups: verum acupuncture group or sham acupuncture groups.

2.6.1. Verum acupuncture group

The patients with PPD in the verum acupuncture group will receive acupuncture treatment within 1 week after baseline measurements. Acupuncture operation will be conducted by acupuncturists with at least 3 years of clinical experience. The therapy strategy will be set out on the basis of traditional Chinese medicine and previous clinical research. First, the selected acupoints are Baihui (GV20), Yintang (EX-HN3), Zhongwan (CV12), Qihai (CV6), Guanyuan (CV4), Neiguan (PC6), Shenmen (HT7), Hegu (LI4), Sanyinjiao (SP6), and Taichong (LR3). The locations of acupoints according to the World Health

Table 1
Localization of acupoints selected in this trial.

Acupoint	Localization
Baihui (GV20)	7 cun above the middle of the posterior hairline, on the middle of the top of the head
Yintang (EX-HN3)	On the forehead, between the brows
Zhongwan (CV12)	On the anterior median line, 4 cun superior to the umbilicus
Qihai (CV6)	On the anterior median line, 1.5 cun caudal to the umbilicus
Guanyuan (CV4)	On the anterior median line, 3 cun caudal to the umbilicus
Neiguan (PC6)	2 cun proximal to the processus styloideus radii, between the tendons of the palmaris longus and the flexor carpi radialis
Shenmen (HT7)	In the wrist, the ulnar end of the transverse striation of the carpal palmar, and the radial depression of the flexor tendon of the ulnar carpal
Hegu (LI4)	On the highest point at m. interosseus dorsalis
Sanyinjiao (SP6)	3 cun proximal to the medial malleolus
Taichong (LR3)	Between metatarsal I and II, just distal to the caput

Organization Standard Acupuncture Locations are shown in Table 1 [31]. Second, the detailed intervention methods are as follows: patients with PPD will lie in the prone position, and then the acupuncturists sterilise the skin around the acupoint regions with 75% alcohol pads. Subsequently, disposable, single-use sterilized acupuncture needles (Product type: HuanQiu, Suzhou, China; 0.3 mm × 40 mm/ 0.3 mm × 75 mm; C-160630) will be inserted into the selected acupoints with specified angle and depth. For Baihui (GV20) and Yintang (EX-HN3) acupoints, the needles will be inserted horizontally at ~0.5–0.8 cun to induce a sensation of soreness (de qi). For the Zhongwan (CV12), Neiguan (PC6), Shenmen (HT7), Hegu (LI4), Sanyinjiao (SP6), and Taichong (LR3) acupoints, the needles will be inserted vertically to a depth of ~0.8–1.0 cun. For the Qihai (CV6) and Guanyuan (CV4) acupoints, the needles will be inserted obliquely at ~1.0–1.2 cun. Acupuncturists will rotate and lift the needles for 30 s to achieve de qi. Third, the two groups of acupoints, Baihui (GV20) and Yintang (EX-HN3) as well as Zhongwan (CV12) and Qihai (CV6), will be connected to electrical stimulation (0.30 mm diameter, 40 mm length, Hwato brand, Suzhou Medical Appliance Factory), respectively. The parameters will be set as continuous wave and low frequency of 2 Hz for 30 min. The acupuncturists will adjust the intensity based on the tolerance of the patients. The other acupoints will be stimulated manually by rotating or lifting the needles to evoke needle sensation every 10 min. Fourth, the treatment period will be 8 weeks, and the treatment frequency will be three times per week, that is, in total 24 times acupuncture treatments over 8 weeks.

2.6.2. Sham acupuncture group

The patients with PPD in the sham acupuncture group will receive nonpenetrative acupuncture treatment with placebo needle. It consist of a needle handle, a needle body, a blunt tip, and an adhesive pad [32]. The blunt tip in the placebo needle is similar to the Streitberger needle [33].

The trained acupuncturists will lightly placed the placebo needle (0.30 mm diameter, 25 mm length, Hwato brand; Suzhou Medical Appliance Factory, Suzhou, China) on the skin at the acupoints without penetrating the skin or stimulating deep tissues. The acupuncturists pretend to rotate and lift the needle handle to aid in blinding the patients with PPD. The placebo needle treatment has been confirmed to be a useful placebo control with weak physiological effects [34]. Other details, including acupoints, treatment duration, and frequency of treatment for patients in the sham acupuncture group will be consistent with those in the verum acupuncture group.

2.6.3. Concomitant medications

Patients will be instructed not to take any additional treatment throughout the trial, such as other complementary treatments (moxibustion, berbal medicine, massage, and so on) or medications. In case of severe conditions, other treatments will be adopted under psychiatrist's directions, for instance antidepressant drugs or mind care. All the relevant information will be recorded in detail in the CRFs for further analysis. In addition, during the trial, patients having other diseases along with PPD will be requested to record the disease name, name of the drug, dose, date, and exact time of the medications used, which should then be reported to the researchers.

2.7. Microbial composition analysis

2.7.1. Fecal sample collection

Fecal samples will be performed at baseline and after 8-week treatment. Fecal samples will be collected in a sterile plastic cup and immediately stored in a −20 °C freezer after defecation. The samples will be transported at −80 °C storage to the laboratory of Shenzhen Hospital of Chinese Medicine within 1 week after defecation. During the transportation, foam incubator and ice bags will be used to avoid fecal sample thawing and being contaminated. In the case of hospitalized patients, the collected samples will be placed directly in the refrigerator at −80 °C.

2.7.2. DNA extraction and 16S ribosome RNA V4 region sequencing

DNA extraction will be carried out on the basis of the MOBIO PowerSoil® DNA Isolation Kit 12888-100 protocol. Before use, DNA is stored at −80 °C in Tris-EDTA (Ethylene Diamine Tetraacetic Acid) buffer solution. The unique fusion primers will be designed according to the universal primer set, 515 F (5′-GTGYCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACNVGGGTWTCTAAT-3′), as well as barcode sequences, to augment the V4 region of the 16S rRNA gene and add barcode sequences. The Polymerase Chain Reaction (PCR) mixtures contained 1 μL of each forward and reverse primer (10 μM), template DNA (1 μL), 4 μL of dNTPs (2.5 mM), 10× EasyPfu Buffer (5 μL), 1 μL of Easy Pfu DNA Polymerase (2.5 U/μL), and double distilled water (1 μL) in a 50 μL reaction volume.

Thermal cycling are as follows: an initial degeneration at 95 °C for 5 min, 30 cycles of degeneration at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 40 s, with a final extension step at 72 °C for 4 min. Amplicons from each sample will be run on an agarose gel. The expected band size for 515f-806 r is ~300–350 bp. The amplicons will be quantified with Quant-iT PicoGreen dsDNA Assay Kit (ThermoFisher/Invitrogen; cat. no. P11496), according to the manufacturer's protocol. The amplicon library for high-throughput sequencing on the Illumina MiSeq platform will be combined with an equal amount and subsequently quantified (KAPA Library Quantification Kit KK4824), according to the manufacturer's protocol.

2.7.3. Profiling of 16S rRNA gene sequencing data

The raw sequences will be processed using the Quantitative Insights into Microbial Ecology 1.8.0 pipeline1 to concatenate reads into tags according to the overlapping relationship. Then, reads belonging to each sample will be separated with barcodes, and low-quality reads will be removed. The processed tags will be clustered into the OTUs at the commonly used 97% similarity threshold. The OTUs will be assigned to taxa by matching with the Greengenes database (Release 13.8) 2. A

phylogenetic tree of representative sequences was built. Alpha and beta diversity analyses will be performed. Distances will be calculated with R (3.3.1, flexmix package).

2.8. Outcome measures

The following outcomes will be assessed by independent assessors, who will be blinded to the randomization. 17-item Hamilton Rating Scale (17-HAMD) and Edinburgh Postnatal Depression Scale (EPDS) will be evaluated at 6 timepoints (at baseline, the end of 1-week, 2-week, 4-week, 8-week intervention and the end of a extro 12-week follow-up). Microbial composition analysis with 16S rRNA gene sequencing will be performed at the baseline and the end of the 8-week of intervention (Fig. 2).

2.8.1. Primary outcome

The primary outcome measurement is the change of 17-HAMD from baseline to end of 8-week treatment, which will be used to analyze the clinical efficacy of acupuncture on patients with PPD.

17-HAMD scale is a clinician-administered measurement with 17 items to evaluate the severity of depressive symptoms [35]. The total score of 17-HAMD ranges from 0 to 52. In general, scores of 0–7 is considered normal with no indication of depression; "7 < score ≤ 17" indicates mild depression; "17 < score ≤ 24" indicates moderate depression; "> 24" indicates servere depression [36].

2.8.2. Secondary outcome

(1) Edinburgh Postnatal Depression Scale

The EPDS is a 10-item self-reporting scale considered to have satisfactory validity and reliability for detecting postnatal depression [37]. EPDS includes mood, fun, self-accusation, anxiety, fear, insomnia, coping ability, sadness, crying, and self-injury. Each item contains four levels: 0 score (never), 1 score (occasionally), 2 score (often), and 3 score (always). The EPDS generates scores from 0 to 30; scores more than 13 indicate clinically significant depression. The higher the score, the more severe the depression. If the score of 10th item is more than 0, it indicates that a puerpera may have thoughts of suicide or other disordered behaviors, and needs to be transferred for a consultation to a psychiatric hospital immediately.

(2) Microbial composition analysis with 16S rRNA gene sequencing

The 16S rRNA gene sequencing is a high-thoughput and reliable detection technique to analyze gut microbiota community composition and features, such as OTU classification, alpha diversity, beta diversity, species difference and so on [38].

2.9. Safety assessment

Any adverse events observed during the trial will be assessed and recorded. Some adverse events might be related to acupuncture therapy, such as feelings of sharp pain, bleeding, hematomas around the inserted acupoint region, fainting, or nausea during the acupuncture therapy and follow-up periods. Additionally, other uncomfortable conditions not related to acupuncture, such as cough and headache, will be also recorded in the CRF.

2.10. Data management

The paper CRF will be used to record and manage the raw data of the individual participant. All data should be recorded in a timely and accurate manner by trained clinical researchers. To ensure data accuracy, double-entry and double-check approaches will be adopted while populating the eCRF with data. Two independent researchers blinded to the group allocation will separately enter and check data using the Epidata software. If any inconsistency is identified during the data entry or logic consistency check, the investigators will be contacted for clarification and validation.

Each participant will be marked with a unique identifier code in the data set. After completion of data checking, the final version of the data set will be kept in a locked compact disk. All private information and medical records about participants will be properly protected and kept in specialized cabinets during the whole study. The statistician have direct access to the complete data set after presenting a formal written application to the data administrator.

2.11. Statistical methods

2.11.1. Sample size

This trial is a clinical gut microbiota study, in which at least 10 participants per group are required [39]. According to the new systematic review [39], the sample size of the included six clinical studies ranged from 10 to 63, and the average sample size was 34. Considering a 10% dropout rate, 37 patients with PPD will be included in each group. Finally, 74 patients with PPD will be enrolled in the study to investigate the difference in responses of gut microbiota between verum acupuncture and sham acupuncture in patients with PPD.

2.11.2. Data analysis

The qualified statisticians and biostatisticians will perform statistical analyses. All analyses will be based on the intention-to-treat principle.

(1) Clinical outcome measures

For continuous variables, when normally distributed, the Student t test will be used for between-group differences and paired t test for within-group differences, which will be expressed as mean ± standard deviation. For nonnormal distribution of data, the Mann–Whitney U test for between-group differences and the Wilcoxon test for within-group differences will be used, and data will be expressed as median or inter quartile range (IQR). For categorical variables, the X^2 test will be used for examining the between-group differences, and percentages and frequencies will be presented to describe the effect size.

All clinical data analyses will be performed using the SPSS V.22.0 software. A two-tailed test will be conducted, and a P value < 0.05 was considered statistically significant.

(2) Gut microbiota data

Professional statisticians performed these analyses. The gut microbiota data will be analyzed using the Mothur data analysis platform (www.mothur.org/) and R language platform. Pearson's correlation between the changes in gut microbiota and the improvement in clinical variables will be conducted in each group.

3. Discussion

PPD is a common health concern worldwide in parturient women with severe and far-reaching consequences. It negatively affects patients and their relatives [40], as well as development in children, including delayed cognitive and language development [6]. Antidepressant medication and cognitive behavioral therapy are recommended as the first-line treatments for PPD. However, owing to the side effects of drug treatments, breastfeeding women preferred to receive natural and harmless approaches. As one of the alternative treatments, acupuncture has a long history in China and has gained mounting attention in many Western countries [41]. According to the published systematic reviews and meta-analysis, acupuncture seems to be a promising therapy for PPD [22,42]. However, so far, few well-

designed RCTs provide clear evidence about the efficacy of acupuncture for PPD. In this study, we will conduct a randomized, sham-controlled trial to test the effectiveness of acupuncture in patients with PPD, as well as to explore the potential mechanism.

In this study, the eligibility of PPD patients will be evaluated by professional clinical physicians, and they will be randomly allocated to verum acupuncture group and sham acupuncture group. The treatment protocol has been developed on a basis of the theory of traditional Chinese medicine and previous researches [20,43]. The interventions will be performed by experienced acupuncturists with at least 3 years of clinical experience. The patients, outcome assessors and statisticians will be blinded to group allocations. Data will be analyzed by an independent statistician.

It is critical to choose an appropriate control group in acupuncture trials. In the previous studies, some study had no control group and only made comparison before and after acupuncture treatment [21], or someone had sham-controlled group but with too small sample size [20]. Nowadays, the placebo-controlled acupuncture protocols have been usually performed, including non-acupoint acupuncture [44], superficial acupuncture [45], and needling of acupoints with non-penrtrating needles [46]. However, it is controversial that the specific effect of acupuncture for a long time, and some researchers think that acupuncture works mainly by a placebo effect [47]. In this study, the control group will receive non-penrtrating needles at true acupoints. This sham acupuncture design may produce a smaller nonspecific effect. Although the placebo-controlled acupuncture protocol might be difficult to mask participants, the same control has been successfully been used in other trials [20]. The patients with PPD in different groups will receive acupuncture treatment in different rooms to minimize the interference. Moreover, all patients with PPD in this study will be asked to guess which treatment they have received to verify whether the patients are masked successfully.

In this study, the 17-HAMD and EPDS will be used to assess the changes in depression severity at 6 timepoints (at baseline and after 1-week, 2-week, 4-week, and 8-week treatment, as well as an extra 12-week follow-up), and the microbial composition analysis with 16S rRNA gene sequencing will be conducted before and after 8-week therapy. Thus, one of the strengths of this study is that dynamic evaluation of the condition of patients with PPD is possible at six different timepoints separately, which help obtain a better description of the fluctuations in HAMD and EPDS scores to observe the efficacy of acupuncture. Additionally, the relationship analysis between clinical scales scores and gut microbiota community composition will be beneficial to explore the potential mechanism of acupuncture.

This study also has some limitations. For example, the whole observation duration of 20 weeks (8-week treatment and 12-week follow-up) might have resulted in a higher rate of loss; however, several measures will be taken throughout the study to reduce the expulsion rate.

In summary, this clinical trial will be conducted to demonstrate whether acupuncture therapy is effective for PPD, and also explore its possible mechanism. The hypothesis that the effect of acupuncture on PPD is related to the regulation of the gut microbiota will be tested. The findings of the trial might have an important impact on the practice of acupuncture for patients with PPD.

Ethical approval and consent to participate

This study was approved by the Medical Ethics Committee of Shenzhen Traditional Chinese Medicine Hospital (approval number: Shenzhen Traditional Chinese Medicine Hospital Ethics approval (research) [2018] 81). If the protocol needs to be amended, all materials on the trial will be reported to the Ethics Review Committee, and the amended protocol can be implemented only after consent acquirement. All participants will sign the written consent to participate in the study after being informed in detail about the study procedures.

Consent for publication

Not applicable.

Availability of data and materials

The full data of the study are available upon reasonable request from the publisher.

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Authors’ contributions

ZYM conceived and designed the study, and drafted the original grant proposal and trial protocol. GYB, GYH and YHB designed the statistical analysis. CC, DJ and HXX performed the Clinical Trial Registration. YZX proofread the final manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare no competing interests.

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