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Study Protocol

Effects of acupuncture in treating insomnia due to spleen-stomach disharmony syndrome and its influence on intestinal microbiome: Study protocol for a randomized controlled trial

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ABSTRACT

Background: Insomnia is a common complaint that is closely related to gastrointestinal symptoms, which is consistent with the traditional Chinese medicine classical theory of "stomach disharmony leading to restless sleep." Acupuncture is an effective complementary and alternative medicine therapy to improve gastrointestinal function and restore the normal sleep-wake cycle. However, studies on the effectiveness of acupuncture for insomnia due to spleen-stomach disharmony syndrome are limited to case reports and few randomized controlled trials; deeper research on its mechanism is still lacking. This randomized controlled trial aims to assess the treatment efficacy of "harmonizing stomach to tranquilize mind" acupuncture for insomnia and its influence on the intestinal microbiome.

Methods/design: This is a randomized, single-blind, parallel-group study. Sixty eligible patients with insomnia due to spleen-stomach disharmony syndrome will be randomly divided into two groups (1:1 allocation ratio). The intervention group will use "harmonizing stomach to tranquilize mind" acupuncture, and the control group will receive sham acupuncture. Participants will receive 5 acupuncture treatment sessions per week for 4 consecutive weeks. The Pittsburgh Sleep Quality Index will be used to evaluate the clinical efficacy of acupuncture treatment by making assessments at baseline, the end of treatment and the end of the follow-up. High-throughput 16S ribosomal ribonucleic acid gene sequencing will be performed to detect changes in the intestinal microbial composition before and after treatment.

Discussion: The results of this trial are expected to confirm that "harmonizing stomach to tranquilize mind" acupuncture can effectively relieve insomnia and alter the intestinal microbiome.

Trial registration: Chinese Clinical Trials Registry: ChiCTR1800017092.

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1. Introduction

Insomnia is a common complaint that can present independently, but frequently occurs in association with one or more comorbid illnesses [1]. A clinical study found that 68% of functional dyspepsia subjects reported having sleep disturbances [2]. In addition, many studies have shown that sleep disturbances and gastrointestinal symptoms interact and aggravate each other [3–5]. The co-existence of insomnia with gastrointestinal symptoms can be a big burden for both patients and the health-care system as evidenced by its effects on quality of life, psychological state, work productivity and economic domains [6–8]. Although effective pharmacologic and psychological treatments for insomnia are available, their uses are limited due to adverse effects and feasibility [9–11]. Thus, an increasing number of patients with insomnia are turning to complementary and alternative medicine such as acupuncture.

As a major component of traditional Chinese medicine (TCM), acupuncture has been used in the treatment of insomnia in China

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for thousands of years. "Harmonizing stomach to tranquilize mind" acupuncture has been widely accepted for the treatment of insomnia by ancient and modern acupuncturists [12]. According the theory of TCM, individuals with insomnia due to spleen-stomach disharmony syndrome typically have symptoms of indigestion, such as gas and bloating, in addition to sleep disturbance. Systematic reviews indicate that acupuncture could be effective against both insomnia and gastrointestinal disorders [13,14]. Furthermore, it has been shown that the composition of the intestinal microbiome can be altered through acupuncture, based on spleen and stomach differentiation [15,16]. However, whether changing the human intestinal microbiome through acupuncture intervention can help mediate sleep disorders has not yet been studied.

There has been an explosion of interest in the study of microorganisms inhabiting the gastrointestinal tract and their impact on host health and physiology [17]. Notably, the latest research showed that intestinal microbiome not only interacts with gastrointestinal diseases, but also with sleep disturbances [18–20]. Both animal and human studies found differences in the composition of microbes in subjects with insomnia and those without insomnia [21,22]. Moreover, a study shows that fecal microbiota transplantation is effective for gastrointestinal symptoms as well as insomnia [23]. Thus, the authors speculated that the intestinal microbiome may be associated with the pathogenesis of gastrointestinal disorder-related insomnia.

Considering that acupuncture may relieve insomnia and gastrointestinal symptoms through the intestinal microbiome, in this trial, the sequencing of intestinal microbial communities by the 16S ribosomal ribonucleic acid (rRNA) gene sequencing technique will be used to investigate the mechanism of acupuncture therapy for treating insomnia, and will be paired with sham acupuncture as a control.

2. Study design and methods

2.1. Hypotheses

We have two hypotheses: (1) "harmonizing stomach to tranquilize mind" acupuncture can improve sleep quality and other related symptoms of participants with insomnia due to spleen and stomach disharmony syndrome; (2) "harmonizing stomach to tranquilize mind" acupuncture can modify the composition of the intestinal microbiome in participants with insomnia due to spleen and stomach disharmony syndrome.

2.2. Objectives

This study includes three objectives: (1) to compare differences between the intervention group and control group in the Pittsburgh Sleep Quality Index (PSQI); (2) to compare differences between the intervention group and control group in the Gastrointestinal Symptom Rating Scale (GSRS), the Insomnia Severity Index (ISI), the Self-Rating Depression Scale (SDS), the Self-Rating Anxiety Scale (SAS) and the State-Trait Anxiety Inventory (STAI); (3) to compare the composition of intestinal microbiome between the intervention group and control group.

2.3. Design and setting

This is a randomized, single-blind, parallel-group study that conforms to the *Standard Protocol Items: Recommendations for Intervention Trials guidelines* [24]. This clinical trial has been reviewed and approved by the Sichuan Regional Ethics Review Committee on TCM/Medical Ethics Committee of Affiliated Hospital of Chengdu University of TCM (number: 2018KL-041). This trial will be carried out in Affiliated Hospital of Chengdu University of TCM. Sixty patients will be randomly divided into the intervention group and the control group (1:1 allocation ratio). The flowchart of the trial is detailed in Fig. 1. The schedule of the trial is detailed in Table 1.

2.4. Participants

2.4.1. Recruitment

We will recruit participants using two strategies. One is to recruit participants from the outpatient acupuncture department of Affiliated Hospital of Chengdu University of TCM. The other is to recruit participants through advertisements posted in the public status or a chat group using WeChat (WeChat, Version: 6.7.3, Tencent, Shenzhen, China). Individuals who have trouble with sleep and have gastrointestinal symptoms at the same time, will be able to contact the researchers with the provided phone number, if they are interested in being considered for the trial. On the first visit, participants will be introduced to the study in detail and informed about the potential benefits and possible risks of this study. Patients who meet the inclusion criteria and are interested in participating and providing informed consent will proceed with the trial.

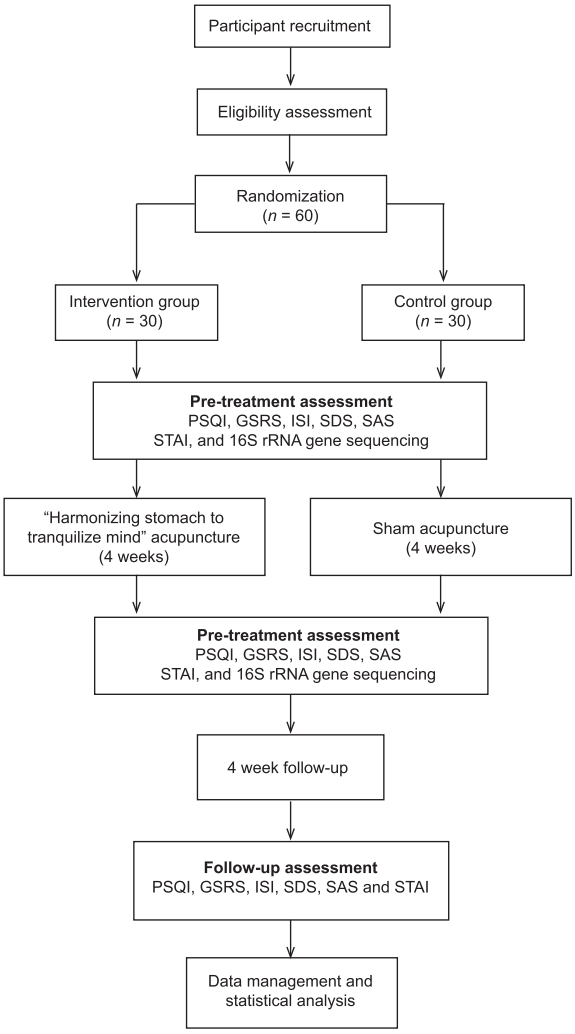

Fig. 1. Trial flowchart. PSQI: Pittsburgh Sleep Quality Index; GSRS: Gastrointestinal Symptom Rating Scale; ISI: Insomnia Severity Index; SDS: Self-Rating Depression Scale; SAS: Self-Rating Anxiety Scale; STAI: State-Trait Anxiety Inventory; rRNA: ribosomal ribonucleic acid.

Table 1
Trial schedule.

Item	Baseline		Treatment phase				Follow-up
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 10
Enrolment							
Eligibility screen	×						
Informed consent	×						
Medical history		×					
Randomization		×					
Interventions							
"Harmonizing stomach to tranquilize mind" acupuncture (n = 30)			×	×	×	×	
Sham acupuncture (n = 30)			×	×	×	×	
Assessments							
PSQI		×				×	×
GSRS		×				×	×
ISI		×				×	×
SDS		×				×	×
SAS		×				×	×
STAI		×				×	×
16S rRNA gene sequencing		×				×	
Trial evaluation							
Patient's compliance			×	×	×	×	×
Adverse events			×	×	×	×	

PSQI: Pittsburgh Sleep Quality Index; GSRS: Gastrointestinal Symptom Rating Scale; ISI: Insomnia Severity Index; SDS: Self-Rating Depression Scale; SAS: Self-Rating Anxiety Scale; STAI: State-Trait Anxiety Inventory; rRNA: ribosomal ribonucleic acid.

2.4.2. Inclusion criteria

Patients will be enrolled in this trial if they meet the following criteria: (1) aged 18–65 years old; (2) meet the Western medicine diagnostic criteria of nonorganic insomnia (sleep latency over 30 min; and/or wake up at night more than 2 times per night; and/or the total sleep time < 6 h; duration of symptoms is more than 1 month) [25]; (3) meet the TCM diagnostic criteria of insomnia (conform to the differentiation standard of disharmony between spleen and stomach) [26,27]; (4) PSQI score > 7 points; (5) the patient signed the informed consent and voluntarily participated in the study.

2.4.3. Exclusion criteria

Patients meeting the following criteria will be excluded: (1) patients with severe primary diseases in cardiovascular system, pulmonary system, liver, kidney and hematopoietic system; (2) with systemic diseases such as pain, fever, cough, surgery, etc.; (3) with obvious headache, migraine or history of head trauma; (4) insomnia caused by alcohol abuse and/or psychotropic substance abuse and dependence (including sleeping pills); (5) patients have taken antibiotics, glucocorticoids, immunosuppressive agents or Chinese medicine within last 1 month; (6) patients have taken medicines to treat insomnia at least 2 weeks before entering the study; (7) patient is pregnant or lactating; (8) patient has participated in other clinical trials within 1 month.

2.5. Interventions

In the intervention group, participants will be treated with 0.25 mm × 40 mm disposable sterilized needles (Huatuo brand, Suzhou, China) applied to Baihui (GV20), Zhongwan (CV12) and bilateral Zusanli (ST36) acupoints (Fig. 2) by inserting and manipulating the needle to achieve Deqi (5 sessions per week for 4 consecutive weeks). Needles will be left in place for approximately 30 min.

In the control group, participants will receive shallow needling at sham GV20, sham CV12 and bilateral sham ST36 (sham acupoints located away from the real GV20, CV12 and ST36 about 10 mm). Specifically, 0.25 mm × 40 mm needles will be inserted vertically about 3–5 mm into sham acupoints without manipulation.

Throughout the trial, the participants will be treated separately to prevent communication. Face-to-face adherence reminder sessions will take place at the initial fecal kit dispensing and each treatment session thereafter. This session will include: (1) the importance of following the study guidelines for adherence to acupuncture and discouraging participants from taking any additional treatments for insomnia; (2) instructions about collecting fecal samples, including usage of fecal kit, weight or volume of fecal sample, storage and transport; (3) importance of calling the clinic if experiencing problems possibly related to the study, such as adverse symptoms or lost fecal kit. All acupuncture treatment conforms to the *Revised Standards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA*, Table 2) [28].

2.6. Microbial composition analysis

2.6.1. Collection of fecal samples

A total of 120 fecal samples will be obtained from 60 subjects (before and after treatment) for high-throughput 16S rRNA gene sequencing. The participants cannot receive any antibiotic treatment for at least one month before sample collection. In the 7 days before sample collection, subjects cannot take any food containing probiotics such as yogurt. Fecal samples will be collected by participants at the hospital or home with Longsee Fecalpro Kit (Longsee Medical Technology Co., Ltd. Guangdong, China; approximately 3 g), under detailed written and oral instructions. Each sample will be either frozen immediately at −80 °C or briefly stored in personal −20 °C freezers before transporting to the hospital within 24 h. At the hospital, the samples will be aliquoted for subsequent analysis of intestinal microbial composition.

2.6.2. Microbial sequencing

For assessing the microbial composition of the fecal samples, extracted sample DNA content will be quantified with the Longsee Stool DNA Kit (Longsee Medical Technology Co., Ltd. Guangdong, China). The V3 to V4 variable region sequence of the 16S rRNA gene of bacteria is used as a target, and 334F-806R with a barcode sequence is used as a primer for polymerase chain reaction (PCR) amplification to obtain a PCR product. After PCR products are quantified and library preparation is constructed, high-throughput sequencing will be performed using the Illumina MiSeq platform

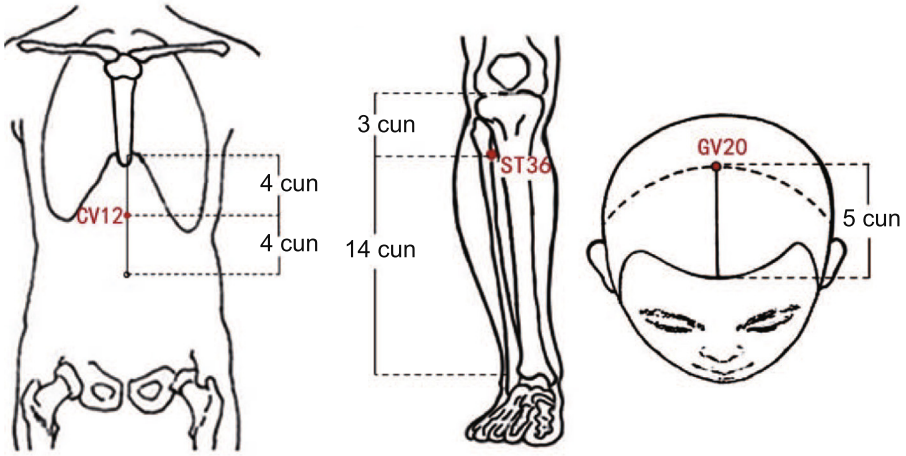

Fig. 2. Acupoints' location.

Table 2
Intervention details by STRICTA Checklist.

Item	Detail	Intervention
Acupuncture rationale	1a) Style of acupuncture	Traditional needle acupuncture
	1b) Reasoning for treatment provided, based on historical context, literature sources, and/or consensus methods, with references where appropriate	Based on the theory of TCM, insomnia is closely related to the spleen and stomach. When looking back to the literature regarding insomnia, it is believed that "stomach disharmony leading to restless sleep" is an important mechanism for the attack of insomnia. This theory is widely accepted in clinical behavior by ancient and modern acupuncturist. Hence, we selected GV20 as the primary acupuncture point; CV12 and ST36 as the subsidiary acupuncture points
	1c) Extent to which treatment was varied	No variation
Details of needling	2a) Number of needle insertions per subject per session (mean and range where relevant)	Four needle insertions per subject per session
	2b) Names (or location if no standard name) of points used (uni/bilateral)	GV20, CV12 and bilateral ST36
	2c) Depth of insertion, based on a specified unit of measurement, or on a particular tissue level	From 30 to 40 mm
	2d) Response sought (e.g., Deqi or muscle twitch response)	Deqi (a composite of sensations including soreness, numbness, distention, heaviness, and other sensations)
	2e) Needle stimulation (e.g. manual, electrical)	Manual stimulation (small, equal manipulations of twirling, lifting, and thrusting)
	2f) Needle retention time	30 min
	2g) Needle type (diameter, length, and manufacturer or material)	0.25 mm × 40 mm disposable sterile needles (Huatuo brand, Suzhou, China)
Treatment regimen	3a) Number of treatment sessions	20 sessions in total
	3b) Frequency and duration of treatment sessions	Five sessions per week for 4 consecutive weeks
Other components of treatment	4a) Details of other interventions administered to the acupuncture group (e.g., moxibustion, cupping, herbs, exercises, lifestyle advice)	Two groups will be educated through sleep hygiene brochure
	4b) Setting and context of treatment, including instructions to practitioners, and information and explanations to patients	All practitioners received a 2-day training session before study initiation
Practitioner background	5) Description of participating acupuncturists (qualification or professional affiliation, years in acupuncture practice, other relevant experience)	All acupuncturists who have a license and at least 2 years of acupuncture practice
Control or comparator interventions	6a) Rationale for the control or comparator in the context of the research question, with sources that justify this choice	Sham acupuncture
	6b) Precise description of the control or comparator. If sham acupuncture or any other type of acupuncture-like control is used, provide details as for Items 1 to 3 above	Same as intervention group except 2b), 2c) and 2d) 2b) sham acupoints located away from acupoints about 10 mm 2c) From 3 to 5 mm 2d) Without Deqi sensation

STRICTA: Standards for Reporting Interventions in Clinical Trials of Acupuncture; TCM: traditional Chinese medicine; GV20: Baihui; CV12: Zhongwan; ST36: Zusanli.

(Illumina, San Diego, USA) to obtain base sequence information of the V3 to V4 variable region of the 16S rRNA gene.

2.6.3. Analysis processing

QIIME 2 software (QIIME 2, RRID: SCR_008249, URL: http://qiime.org/) will be used to perform quality control of the sequences and operational taxonomic unit (OTU) clustering. The OTU representation sequence is compared with the Greengenes database (URL: http://greengenes.secondgenome.com/) to identify the corresponding taxonomic unit of the OTU and its abundance information. We will calculate metrics such as the Chao1 estimator, the Abundance Coverage-based estimator, the Shannon diversity index, and the Faith's Phylogenetic Diversity index to evaluate the richness and evenness of bacterial flora in samples. We will use principal component analysis, principal co-ordinates analysis, linear discriminant analysis effect size, analysis of the composition of microbiomes, Kruskal Wallis and other methods to characterize the bacteria in each experimental group. In addition, the Kyoto Encyclopedia of Genes and Genomes Ortholog functional profiles of microbial communities were predicted with the PICURSt software (http://huttenhower.sph.harvard.edu/galaxy/root?tool_id=PICRUSt_normalize).

2.7. Outcome measures

The following outcomes will be assessed by independent outcome assessors. These assessors are trained before participating in the trial and are blinded to the randomization. PSQI, GSRS, ISI, SDS, SAS and STAI surveys will be performed at three time points (the baseline, the end of 4 weeks of intervention and the end of a 4-week follow-up); high-throughput 16S rRNA gene sequencing will be performed at the baseline and the end of the 4 weeks of intervention (Fig. 1).

2.7.1. Primary outcomes

The primary outcome measure in this study is PSQI, consisted of 19 self-rated questions, which are grouped into seven component scores ranging from 0 to 3 each. The seven component scores are then summed to yield a global PSQI score, which has a range of 0 to 21, with higher scores indicating worse sleep quality [29].

2.7.2. Secondary outcomes

The intensity of gastrointestinal symptoms, the frequency of attacks, duration of attacks, and impact on the daily living will be assessed by GSRS, a 15-item self-reported scale. All individual items are scored on a 7-point Likert scale and are subsequently clustered into five domains (abdominal pain, reflux, indigestion, diarrhea and constipation); higher scores indicate more severe symptoms [30,31].

The nature, severity and impact of insomnia will be assessed by ISI, which is a brief self-report instrument measuring a patient's perception of insomnia. The 7 items are rated on a 0 to 4 scale, and the total score ranges from 0 to 28. A higher score indicates more severe insomnia [32].

Emotional states will be assessed by the SDS, SAS and STAI. SAS is a 20-item self-report measure of anxious symptoms. Each of the items is ranked on a 4-point Likert scale. Higher total scores indicate greater levels of anxious symptomatology [33]. SDS is a 20-item self-report tool which was developed to measure depressive symptoms and for depression screening [34]. STAI is a 40-item questionnaire, of which 20 items reflect the current anxiety state called State Anxiety and 20 items relate to background, sustained anxiety, known as the Trait Anxiety. Each item is scored from 1 to 4, with the total score ranging from 20 to 80 for each scale; higher scores indicate increased anxiety [35].

High-throughput 16S rRNA gene sequencing is an intuitive and reliable detection approach to study gut microbial community composition and dynamics [36].

2.8. Safety assessment

Adverse events, such as pain, bleeding, or fainting, will be documented in the case report form throughout the trial. If the adverse event is severe and associated with the trial, the participant will be withdrawn from the study and given appropriate medical care.

2.9. Sample size

According to a previous study [37], the global score of PSQI after treatment in the acupuncture group was 6.9 ± 2.23 and was 10.43 ± 4.34 in the control group. With $\alpha = 0.01$, $1 - \beta = 0.8$, and providing for a two-sided outcome, at least 24 participants will be required for each group, as calculated by Calculators software (URL: http://powerandsamplesize.com/Calculators/). So far, there is no definite sample size calculation for intestinal microbiome research. Reviewing similar studies and the requirements of 16S rRNA gene sequencing research data analysis indicates that at least 25 samples from each group should be analyzed [38]. Allowing for a 20% withdrawal rate, the authors plan to enroll a total of 60 participants with 30 participants in each group.

2.10. Randomization

In order to avoid possible selection bias, the trial statistician will place individual randomization numbers (Research Randomizer, https://www.randomizer.org/) into sealed envelopes. Participants will get one of these envelopes at the first visit to the Chengdu University of TCM. When each patient visits the clinic, acupuncturists will open the envelope and will find the treatment condition to be applied to that patient.

2.11. Blinding

Considering the particularity of acupuncture manipulation, it is difficult to blind acupuncturists in this study. But acupuncturists will be strongly coached not to disclose the allocation status of the participant at any point. The participants, researchers, outcome assessors and statisticians will be blinded to treatment allocation.

2.12. Statistical method

Demographics, baseline characteristics and curative effects of the subjects will be analyzed with different approaches by SPSS 20.0 statistical software (SPSS Inc., Chicago, IL, USA). The qualitative data will be expressed as a percentage or proportion, and will be compared using the chi-square test. The quantitative data will be represented as mean ± standard deviation. Independent sample *t*-test will be applied to compare the effect of acupuncture between intervention group and control group. Repeated-measure analysis of variance will be used to determine differences in the same group at three time points (the baseline, the end of 4 weeks of interventions and the end of 4-week follow-up) and a Bonferroni test will be used for posthoc pairwise comparison. Two-sided tests will be used during the analysis and $P < 0.05$ will be considered a threshold for statistical significance.

3. Discussion

This is a randomized, single-blind, parallel-group study which aims to evaluate the efficacy of acupuncture in treating insomnia

and to investigate how changes in the intestinal microbial composition are linked with treatment outcomes.

According to the theory of TCM, insomnia is closely related to functions of the spleen and stomach. The TCM insomnia literature indicates that "stomach disharmony leading to restless sleep" is an important mechanism for some types of insomnia. As GV20 is located at the vertex of the head, where all the yang meridians meet, treating that point can clear the mind, lift the spirit and strengthen the ascending function of the spleen [39]. CV12 is the front-mu acupoint of the stomach as well as root and knot acupoints of the spleen. ST36, the he-sea acupoint and lower-he-sea acupoint of the stomach, is usually used in combination with CV12. This pair of points is frequently used in clinic, with the functions of harmonizing the stomach and strengthening the spleen, as well as promoting digestion and elimination. Thus, the acupuncture technique of "harmonizing stomach to tranquilize the mind" is specifically used in the treatment of insomnia.

The control-group design in studies evaluating the efficacy of acupuncture intervention remains methodologically challenging. Sham acupuncture aims to blind the participants and to control for nonspecific placebo effects. However, it is possible that sham acupuncture may produce clinical effectiveness. This methodological limitation needs to be improved in future studies.

In the present trial, besides using the PSQI, GSRS, ISI, SDS, SAS and STAI to assess the clinical curative effect across a range of response dimensions, GSRS will be introduced to assess the clinical curative effect. In addition, we choose 16S rRNA gene sequencing to assess changes in the intestinal microbial composition.

In conclusion, the results of this study are expected to demonstrate that "harmonizing stomach to tranquilize mind" acupuncture is effective in relieving insomnia and altering intestinal microbiome. We expect the results will further support the use of acupuncture to treat insomnia, thus providing reliable evidence for future research and practical recommendations for clinical practice.

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Competing interests

The authors declare that they have no competing interests.

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