

Research Protocol

Title	Dense cranial electroacupuncture stimulation plus body acupuncture for adolescents with psychological distress: a randomized controlled trial with observations on salivary oxytocin and cortisol changes
Protocol No.	DED202405
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Sample Size	148 participants
Study period of Each Subject	8 weeks
Duration of Entire project	About 3 years (1 May 2025 – 30 September 2027)

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ABSTRACT

Objective: Dense cranial electroacupuncture stimulation plus body acupuncture (DCEAS+BA) is a novel acupuncture mode that may have particular efficacy in the treatment of adolescents with psychological distress. The objectives are: (1) to compare the efficacy of DCEAS+BA and minimal acupuncture stimulation (MAS) as a control in treating anxiety and depressive symptoms, and improving sleep quality; and (2) to detect the effects of DCEAS+BA on salivary levels of oxytocin and cortisol and correlates with clinical improvement.

Hypothesis to be tested: DCEAS+BA could alleviate anxiety, depression, and other related symptoms of adolescents. The therapeutic effects of DCEAS+BA may be associated with its enhancement of oxytocin and suppression of stress reactions.

Design and subjects: An assessor-blinded, randomized controlled trial will be conducted on 148 adolescents with psychological distress.

Study instruments and intervention: Participants will randomly receive DCEAS+BA (n = 74) or MAS (n = 74) for 2 sessions per week for 8 weeks.

Main outcome measures: The primary outcome will be assessed using the Chinese version of Beck Anxiety Inventory (BAI-C). Secondary outcomes include the Chinese version of the Beck Depression Inventory II (C-BDI-II) for depression, the Zung Self-rating Anxiety Scale (SAS) and the Zung Self-rating Depression Scale (SDS) as additional assessment for anxiety and depression, the Pittsburgh Sleep Quality Index (PSQI) for sleep quality, and the World Health Organization Quality of Life-Brief (WHOQOL-BREF) (HK version) for the general quality of life. Saliva will be collected at baseline and the end of the 8-week study. Salivary oxytocin and cortisol will be analyzed.

Data analysis: A generalized linear mixed-effect model will be applied to compare treatment outcomes over time in the two groups and linear regression will be conducted to examine inter-correlations between clinical improvement and changes in biomarker levels. Subgroup analysis will be conducted to identify specific effects of DCEAS+BA.

Expected results: DCEAS+BA could produce greater improvement in psychological distress than MAS in adolescents. Multiple correlations between clinical outcomes and salivary oxytocin and cortisol would be identified.

1. BACKGROUND

Psychological distress of adolescents in Hong Kong: Psychological distress refers to a state of emotional suffering characterized by symptom(s) including anxiety, depression, and stress, and is often accompanied by somatic symptoms such as insomnia, headache, and lack of energy (APA,2022). As subclinical symptoms or clinical diagnoses, anxiety and depression are often core symptoms in psychological distress. They are highly comorbid and are often accompanied by physical and sleep problems. Psychological distress is particularly prevalent in adolescents and can be a precursor or features of various psychiatric disorders (Li et al., 2020; Lun et al., 2018). One epidemiological study conducted in Hong Kong local university students has revealed that approximately 70% of students complained of some degree of depression and 54% of anxiety; nearly 9% had moderately severe to severe depressive symptoms and 6% had severe anxiety (Lun et al., 2018). Two most recent large-scale surveys respectively conducted in Hong Kong and China further revealed about 17% of children and youths probably had a mental problem (Chen et al., 2023; Li et al., 2022). This has become more severe during and post COVID-19 pandemic (Ho & Lee, 2022). However, psychiatric disorders of youths are often underdiagnosed and vastly undertreated, because the condition of most young patients is mild and they may fear drugs' side effects which sometimes are a greater risk than the disease itself. This has led to particularly low acceptability for medication interventions in adolescents. The development of novel non-pharmacological treatment strategies for youth is therefore greatly desired.

Electroacupuncture trigeminal nerve stimulation plus body acupuncture: A large body of evidence well confirms the benefits and effectiveness of acupuncture in the treatment of depression, anxiety, and various related disorders. Recently, we further developed a novel acupuncture mode called 'dense cranial electroacupuncture stimulation plus body acupuncture (DCEAS+BA)', where "dense" represents the use of many acupoints mainly located on the forehead. DCEAS is a novel nerve stimulation that is established based on neuroanatomical rationales (Zhang et al., 2012), i.e., electrical stimulation is conducted on many forehead acupoints that are innervated by the first branch of the trigeminal nerve. We have demonstrated the effectiveness of DCEAS in the treatment of major depression, post-stroke cognitive impairment, insomnia, obsessive-compulsive disorder (OCD), and chemotherapy-induced cognitive impairment (see below). Previous studies also have revealed that transcutaneous trigeminal nerve stimulation is effective in reducing depression, anxiety, attention-deficit/hyperactivity disorder (ADHD) and epilepsy (Cook et al., 2016; DeGiorgio et al., 2013; McGough et al., 2019; Schrader et al., 2011). Compared to adults, adolescents are more sensitive to peripheral nerve stimulation, producing robust neuromodulatory effects. These have led to the hypothesis that DCEAS+BA may have particular effects in alleviating adolescents' psychological distress.

Salivary oxytocin and cortisol of youths with psychological disorders: Saliva collection is highly acceptable for children and adolescents. Salivary oxytocin and cortisol are the two most compelling biomarkers for screenings, diagnoses, and predicting treatment outcomes in children and adolescents with psychiatric disorders. Children and adolescents with social disorder and emotional distress had significantly lower levels of salivary oxytocin and higher cortisol levels (Chen et al., 2019; Gordon et al., 2008; Kohlhoff et al., 2022; Lebowitz et al., 2016). Electroacupuncture and acupuncture suppressed salivary cortisol levels while anxiety symptoms were alleviated (Amorim et al., 2022; Huang et al., 2012). The oxytocin system plays an important role in the therapeutic mechanisms of acupuncture in the treatment of pain disorders and autism (Su & Pei, 2021; Yang et al., 2007). These studies suggest that salivary oxytocin and cortisol could serve as valid biomarkers for predicting the treatment outcomes of acupuncture.

2. OUR RELATED PREVIOUS STUDIES AND OTHERS

Over the past years, we have completed a series of clinical trials evaluating the efficacy of acupuncture, with particular DCEAS+BA in patients with cognitive impairment, depression, and insomnia (Table 1). However, our previous studies of DCEAS mainly focused on the general population with psychiatric disorders and aged subjects with stroke, and only estimated clinical efficacy, but did not explore associations with specific biomarker profiles. In particular, it is not yet validated in young subjects with psychological distress.

Table 1. Our major clinical studies on acupuncture treatment of psychiatric disorders

	Major indication	Participants	ACP modes	Treatment session and duration
Zhang et al., 2009	Obsessive compulsive disorder (OCD)	29 participants: 10 in ACP and 9 in waitlist controls	DCEAS	12 sessions (5 sessions per week).
Zhang et al., 2012	Major depressive disorder	73 participants: 35 in sham ACP and 38 in DCEAS	DCEAS	9 sessions in total (3 sessions per week for 3 weeks).
Man et al., 2014	Post-stroke depression	43 participants: 23 in DCEAS, 20 in sham control	DCEAS	3 sessions per week over 4 weeks
Zhang et al., 2020a	Chemotherapy-induced cognitive impairment	93 participants: 46 in DCEAS+BA, 47 in MAS	DCEAS+BA	2 sessions for 8 weeks
Zhang et al., 2020b	Post-stroke depression	91 participants: 45 in DCEAS+BA and 46 in MAS.	DCEAS+BA	3 sessions per week over 8 weeks
Zhang et al., 2021	Chemotherapy-associated Insomnia	28 participants: 13 in ACP group vs. 15 in wait-list control group.	EA on some DCEAS	2 sessions weekly for 6 weeks.
Wong et al., 2021	Major depression	70 participants: 50 in ACP group, 20 in control group.	MA on some DCEAS+BA	2 sessions weekly for 3 weeks.
Yeung et al., 2021	Insomnia	140 participants: ACP group and sham control group (n = 70)	Some DCEAS+BA	twice weekly for 4 weeks,
Cao et al., 2022	Post-stroke insomnia	144 participants: ACP and sham control (n = 72 each)	Some DCEAS acupoints and body points	3 sessions per week for 4 weeks, 4 weeks of followed-up.

ACP, acupuncture; **DCEAS**, dense cranial electroacupuncture stimulation; **BA**, body acupuncture; **MAS**, minimum acupuncture stimulation; **EA**, electroacupuncture

There have been several lines of evidence suggesting that younger subjects with psychiatric disorders could achieve earlier responses and may be more efficacious for nerve and brain stimulation compared to older adults (Cotovio et al., 2022; Zhang et al., 2012). There also exists an age difference in the analgesic effects of electroacupuncture between adult and juvenile rats (Yamashita et al., 2021). On the other hand, meta-analysis and recent trials suggested that acupuncture and electroacupuncture may have more benefits in treating anxiety disorders and insomnia, and produce comparable efficacy in improving anxiety disorders as compared to anxiolytics (Amorim et al., 2022; Li et al., 2022; Yang et al., 2012; Yeung et al., 2012; Zhang et al., 2023). This has led to the anticipation that youths with psychological distress could achieve better outcomes on DCEAS+BA compared to other

subpopulations. Therefore, we further propose this trial to examine the efficacy of DCEAS+BA and its associations with salivary oxytocin and cortisol in adolescents with psychological distress. As youths with psychiatric disorders are vastly undertreated, are often reluctant and even decline to take the medications due to fear of the drugs' side effects, the validation of the efficacy of DCEAS+BA provides an effective and acceptable option for this special subpopulation. The exploration of the associations with biomarkers will help us gain more insights into the mechanisms of DCEAS.

3. HYPOTHESIS AND AIMS TO BE TESTED

The working hypothesis of this proposed study is that DCEAS+BA could alleviate anxiety, depression, and other related symptoms of adolescents. The therapeutic effects of DCEAS+BA may be associated with its enhancement of oxytocin and suppression of stress reactions. To test this hypothesis, an assessor-blinded, randomized controlled trial will be designed to achieve the two aims: (1) to compare the efficacy of DCEAS+BA and minimal acupuncture stimulation (MAS) as a control in alleviating anxiety and depressive symptoms, and improving sleep quality; and (2) to detect the effects of DCEAS+BA on salivary levels of oxytocin and cortisol and correlation with clinical improvement.

4. PLAN OF INVESTIGATION

4.1. Outline

This is an assessor-blinded, randomized, MAS-controlled trial. A total of 148 adolescents aged 15-22 years experiencing mild to moderate anxiety or depressive symptoms will be recruited. They will be randomly assigned to DCEAS+BA (n = 74) and MAS (n = 74) for 2 sessions per week for 8 weeks, followed by a 4-week post-treatment observation period. A post-intervention visit will be held at week 12. The primary outcome will be assessed using the Chinese version of the Beck Anxiety Inventory (BAI-C). The secondary outcomes include the Beck Depression Inventory II (C-BDI-II) for depression, the Zung Self-Rating Anxiety Scale (SAS) and the Zung Self-Rating Depression Scale (SDS) as additional assessments for anxiety and depression, the Pittsburgh Sleep Quality Index (PSQI) for sleep quality, and the World Health Organization Quality of Life-Brief (WHOQOL-BREF) (HK version) for the general quality of life. Assessment will be carried out every 4 weeks till week 12, while saliva will be collected at baseline and at the end of the 8 weeks. Salivary oxytocin and cortisol will be analyzed. A generalized linear mixed-effect model will be applied to compare treatment outcomes over time in the two groups and linear regression will be conducted to examine intercorrelations among clinical improvement and changes in biomarker levels. Subgroup analysis will be conducted to identify specific effects of DCEAS+BA.

4.2. Subjects

4.2.1. Inclusion criteria: Subjects will be eligible for this study if they: (i) are aged 15-22 years; (ii) are experiencing anxiety as evidenced by a BAI-C score of at least 8 (no symptoms = 0-7; mild = 8-15; moderate = 16-25; severe = 26-63), and/or a C-BDI-II score of at least 14 (no symptoms = 0-13, mild = 14-19; moderate = 20-28; severe = 29-63); (iii) have symptoms that have persistent for more than 3 months; (iv) are able to provide informed consent for participation, and complete treatments and questionnaires as scheduled; and (v) have no suicidal ideation, as evidenced by “no” answers or a score of <3 for all questionnaires of the Columbia-Suicide Severity Rating Scale (C-SSRS).

4.2.2. Exclusion criteria: Subjects will be excluded if they: (i) have unstable systemic medical conditions that may limit their participation in the study, for example, severe liver, cardiovascular, or kidney impairment; gastrointestinal or endocrine dysfunction; malignancy; autoimmune disease; acute

infectious disease; or any conditions with unstable vital signs or require intensive hospitalization; (ii) have bipolar or psychotic disorder (e.g. schizophrenia); (iii) have a history of brain injury or surgery; (iv) have alcohol abuse or substance abuse; (v) are pregnant or in lactation, or intend to conceive; (vi) have heart pacemaker or other metal devices implanted in the body; (vii) have concurrent or use of drug treatment for anxiety/depression in the previous 6 months; (viii) have treatment with acupuncture or brain stimulation in the previous 6 months; (ix) have a history of epilepsy; (x) have a condition of bleeding tendency, or coagulopathy, or currently receiving anti-coagulant treatment; or (xi) involved in other interventional clinical studies in the last 3 months or any relevant condition potentially interfering with study evaluation.

4.2.3. Estimation of sample size: The improvement in anxiety symptoms will serve as the primary outcome (see below). We hence referred to controlled trials of acupuncture treatment of anxiety disorders for the sample estimation. However, most of these trials used anxiolytics and antidepressants as controls (Li et al., 2022; Yang et al., 2021). We therefore additionally referred to our two previous trials that evaluated the efficacy of DCEAS on major depressive disorder and DCEAS+BA on post-stroke depression with sham acupuncture and MAS as control (Zhang et al., 2012, 2020b). The estimation is based on the following formula,

$$n = \frac{2}{(M/SD)^2} \times C_{p,power}$$

where n is the number of subjects required in each arm; M is the mean difference between the two groups. SD is the standard deviation. $C_{p,power}$ is equal to 7.9 when the two-tailed level of α and power ($1-\beta$) are set at 0.05 and 80%, respectively. The sample size estimation was based on 3 representative trials of acupuncture for anxiety disorders (Che et al., 2015; Fan et al., 2014; Zhou et al., 2015) and our two trials for depression (Zhang et al., 2012, 2020b). At a highly conservative level, it is expected that DCEAS could produce a significantly greater improvement than controls in reducing the severity of anxiety and depression, with a standard deviation (SD) equal to approximately 200% of the mean difference (M). It then requires 74 subjects per arm with an assumed dropout rate of 15%. We propose to recruit a total of 148 subjects ($n = 74$ for each group).

4.3. Methods

4.3.1. Screening, randomization, and blindness: The screenings and recruitment will be carried out by Chinese medicine practitioners (CMPs), research assistants, physicians and psychiatrists, and via advertisement and social media. The recruitment also will be conducted on the local university campus. The randomization sequence will be produced in advance with a block of 2, 4 or 6, and a ratio of 1:1 of DCEAS+BA and MAS groups. Treatment conditions will be blind to clinical assessors and data analysts (see below).

4.3.2. Acupuncture treatment regimens

4.3.2.1. DCEAS plus body acupuncture: Based on our and other studies as mentioned earlier, the combination of DCEAS and body acupuncture could produce robust effects. For DCEAS, 6 pairs of acupoints are used: Baihui (GV20) and Yintang (EX-HN3), left Sishencong (EX-HN1) and Toulinqi (GB15), right Sishencong (EX-HN1) and Toulinqi (GB15), bilateral Shuaigu (GB8), bilateral Taiyang (EX-HN5), and bilateral Touwei (ST8). All these acupoints are located on the forehead innervated by the trigeminal nerve and could soothe anxiousness, relieve depression, and improve sleep quality according to TCM theory (Zhang et al., Liao et al., 2023) (Table 2). For body acupuncture, the following acupoints are used: Shenmen (HT7), Neiguan (PC6), Zhongwan (CV12), Guanyuan (CV4), Zusanli (ST36), Sanyinjiao (SP6), and Taichong (LV3). These points could calm the mind, tranquilize the spirit, and modulate visceral functions.

Disposable acupuncture needles (0.25 mm in diameter and 25-40 mm in length) are inserted at a depth of 10-30 mm perpendicularly or obliquely into acupoints. Manual manipulation is then conducted to evoke a needling sensation. After that, electrical stimulation is delivered on DCEAS, with continuous waves at 2 Hz through an electrical acupuncture stimulation instrument (Hwato, SDZ-II). The use of low frequency rather than high frequency is because it could modulate biochemical adaptation of the brain more favorably in improving cognitive functions (Han, 2003). The intensities of stimulation are adjusted to a level at which patients feel most comfortable. The stimulation lasts 30 minutes. For body acupoints, no electrical stimulation is delivered. Acupuncture treatment will be conducted twice weekly for 8 weeks. Previous studies have shown significant improvement was observed in 6-8 weeks of acupuncture treatment (Zhang et al., 2012).

4.3.2.2. Minimum acupuncture stimulation (MAS): MAS will serve as controls based on the following two principles: (1) the acupoints chosen are not or are less related to the treated syndromes according to TCM theory; (2) the number of acupoints used and the intensity of electrical stimulation are adjusted to minimum levels at which participants realize that they are receiving acupuncture treatment but the therapeutic effects are least. The rationales for the choice of MAS, rather than sham acupuncture as controls are; (1) MAS provides the lowest-grade stimulation, which resembles similarities with medicinal studies where a low dose is compared to a higher dosage of the drug under investigation (Enck et al., 2010; Mao et al., 2013); (2) it is well documented that sham acupuncture is not a real placebo as it is often as effective as true acupuncture and has different biological effects; (3) the procedure of MAS is much more convenient than sham acupuncture; and (4) our previous studies have well validated reliability and practicability of MAS (Zhang et al., 2020a,b).

The following 6 acupoints will be used for MAS control: bilateral Tongtian (BL07), bilateral Shousanli (LI10) and bilateral Fuyang (BL59). Electrical stimulation will be only performed on bilateral Tongtian (BL07) and the intensity is adjusted to a level at which patients just start feeling the stimulation.

Both the combination acupuncture and MAS treatment will be performed by Chinese medicine practitioners who have had at least 5 years of experience in clinical practice.

4.3.2.3. Termination criteria and post-termination management: To ensure participants' safety, those who cannot tolerate acupuncture treatment or are hospitalized due to any reasons will be discontinued from the study and given individual clinical treatment.

4.3.3. Existing interventions: All participants of the two groups will continue their current interventions and medications as usual. The existing interventions and medications will be recorded and serve as covariates included in the outcome analysis.

Table 2. Major TCM-based therapeutic effects of the acupoints used in the trial

Acupoint	TCM-based therapeutic effects
Baihui (GV20)	Raises Yang, regulates the five Zang's activities, strengthens the brain, and relieves stress-related anxiety, depression, and insomnia.
Yintang (EX-HN3)	Quiets the spirit and clears the mind, widely used to treat insomnia, headache, vertigo, forgetfulness, and agitation.
Sishencong (EX-HN1)	Tranquilize and calm the mind, suppressing sympathetic activities, and are commonly used for insomnia, pain, fatigue and depression.
Toulinqi (GB15)	Reduces headache and dizziness, enlighten the head orifices, rebalance mood, and suppress convulsion and nervousness.
Shuaigu (GB8)	Dispels head cloudiness, reopens the head orifices, relieves pain and agitation.
Taiyang (EX-HN5)	Alleviates headache, dizziness, insomnia, and eye problems.
Touwei (ST8)	Alleviates headache, regulate mood and relieve sinus problems.
Shenmen (HT7)	Calms the mind and tranquilize the spirit, often used for a variety of psychogenic conditions, including insomnia, anxiety and depression.
Neiguan (PC6)	Calms the mind and heart, strongly inhibits sympathetic tone, with cardiovascular dysfunctions, often used for insomnia, fatigue, pain, and depression.
Zhongwan (CV12)	Particularly benefits stress-related digestive disorders, such as stomach pain, heartburn, diarrhea, along with insomnia and anxiety.
Guanyuan (CV4)	Tonifies Qi and supplements Yang, benefits constipation, retention of urination, frequent nocturia and indigestion.
Zusanli (ST36)	Invigorate the spleen, harmonize the stomach, reinforce healthy and original <i>Qi</i> , rebalance the mood.
Sanyinjiao (SP6)	Tonifies Yin and improve vitality and immunity, clam the mood by nourishing the three foot meridians (spleen, kidney and liver).
Taichong (LV3)	Quiets the spirit and stabilizes the mind, harmonizes liver and spleen, controls liver-Qi stagnation-induced hyperactivity.

4.4. Study design

4.4.1. Clinical assessment: The primary outcome will be assessed using the Chinese version of the Beck Anxiety Inventory (BAI-C). The secondary outcomes include the Chinese version of Beck Depression Inventory II (C-BDI-II) for depression, the Zung Self-Rating Anxiety Scale (SAS) and the Zung Self-Rating Depression Scale (SDS) as additional assessments for anxiety and depression, the Pittsburgh Sleep Quality Index (PSQI) for sleep quality, and the World Health Organization Quality of Life-Brief (WHOQOL-BREF) (HK version) for the general quality of life. Adverse events will be systematically recorded. Assessment will be carried out face-to-face at baseline and once every 4 weeks till week 12.

Fatal or life-threatening unexpected AEs will be reported to the regulatory bodies as soon as possible but no later than 7 calendar days after first knowledge by the principal investigator that a case qualifies, followed by as complete a report as possible within 8 additional calendar days. This report would include an assessment of the importance and implications of the findings, including relevant previous experience with the same or similar medicinal products. For other serious, unexpected AEs that are not fatal or life-threatening, they will be reported as soon as possible but no later than 15 calendar days after first knowledge by the principal investigator or that the case meets the minimum

criteria for expedited reporting. For non-serious AEs and serious AEs that are expected, they will be reported in a brief summary at the conclusion of the trial.

4.4.2. Salivary sample collection and oxytocin and cortisol assay: Salivary samples will be collected at baseline and the end of the 8-week study, respectively. On collection day, 2 salivary samples will be collected at 08:00-09:00 and 20:00-21:00, respectively, to meet the cortisol circadian rhythm with peak time at 08:30 and the lowest level at 20:00-22:00. Volume of each sample collected is 3-4 ml and the detailed procedure is described in the assay kit (<https://salimetrics.com/saliva-collection-handbook/>). Oxytocin and cortisol concentrations will be analyzed using enzyme-linked immunosorbent assay (ELISA) kits.

4.4.3. Maintenance of fidelity and consistency: To ensure consistency of procedure and assessment throughout a study period, a training workshop will be conducted at pre-trial. All investigators will receive training. The training manual will be prepared. Trainees are required to obtain satisfactory training outcomes which will be rated by the PI and a senior investigator. For clinical assessment training, an investigator who has extensive experience in clinical assessment will instruct clinical instruments. A depressed patient video will be used for the training workshop. An inter-rater reliability coefficient (k value) of >0.80 would be achieved after the completion of each training workshop. To further ensure consistency and fidelity, the following two additional measures will be taken: (1) all assessments from baseline throughout the endpoint for each individual will be performed by the same assessor, if possible; (2) all sessions of acupuncture treatment of each patient will be conducted by the same CMP, if possible.

4.4.4. Safety assurance and ethical consideration: Case Record Form (CRF) will be used to record all activities and events in the course of the study. Emergency contact numbers (the investigator's mobile number and a hotline number) will be provided in the informed consent for seeking advice.

The study will be carried out in accordance with the ethical principles of the Declaration of Helsinki' and its subsequent amendment. Ethical approval is being sought and must be obtained before the recruitment starts. The proposed study will be registered in www.clinicaltrials.gov, which is accessible to the public, before the onset of patient enrollment. A full explanation of the study goal, procedures, and potential side effects and risks will be presented to each patient recruited. All participants are required to provide written consent. For participants whose ages are under 18 years, written consent must be provided by their guardians. Informed consent and other documents provided to patients will be written in both English and Chinese. This study is conducted on Chinese patients. Patients would not receive remuneration, but treatments and biochemical assessments directly associated with the clinical trial will be provided at no cost. Upon completing all 12 weeks of the treatment and assessment, an HKD\$500 supermarket voucher will be given to patients to thank them for their support of this study. The issue of confidentiality is a major ethical issue, and will be solved by recording the data in a manner that does not allow the participants to be identified (i.e., using a non-recognizable code for each patient). A review of medical records that have been already recorded as part of clinical care. Therefore, this poses no physical risks.

4.4.5. Record retention: The principal investigator, research team, and ethical review authority are responsible for overseeing this study to get access to, use, and retain subjects' personal data for the purposes of clinical study; and the relevant regulatory bodies can get access to subjects' personal data for the purposes of checking and verifying the integrity of study data and assessing compliance with the study protocol and relevant requirements. The collected personal information and study data will be kept for 5 years after the completion of the study. The collected salivary samples will be destroyed after the completion of the study.

4.5. Data processing and analysis

One biostatistician who is blinded to interventions will be responsible for statistical analysis. The analysis will be carried out on the intention-to-treat (ITT) population, defined as participants who are randomized. A linear mixed-effect model will be applied to compare the primary and secondary outcomes between the two groups. The model will be established using time and group as categorical fixed factors and random intercepts within a scaled identity covariance matrix. Certain demographic and baseline clinical characteristics will serve as covariates. Subgroup analysis will be further conducted based on demographic and baseline clinical variables, in particular different levels and sources of anxiety, depression, and sleep quality, so that particular benefits of the intervention in subpopulation could be determined. Student t-test will be used to detect differences in continuous baseline variables and biomarker levels between the two groups. Categorical baseline variables, including discontinuation and incidence of adverse events will be analyzed using Chi-square (χ^2) test. Linear regression will be conducted to examine inter-correlations between changes in clinical outcomes and biomarker levels. Statistical significance is defined as a two-tailed $P < 0.05$.

4.6. Termination criteria

4.6.1. Premature termination or suspension of study: The study will be completed as planned unless the following criterion are satisfied that require temporary suspension or early termination of the study: new information or other evaluation regarding the safety or efficacy of the procedure that indicates a change in the known risk/benefit profile, such that the risk/benefit is no longer acceptable for subjects participating in the study.

4.6.2. Premature termination or suspension of investigational sites: A study site may be terminated prematurely or suspended if the site (including the investigator) is found in significant violation of protocol or contractual agreement, is unable to ensure adequate performance of the study, or as otherwise permitted by the contractual agreement.

4.6.3. Procedures for premature termination or suspension: In the event that the principal investigator, an institutional review board (IRB) / research ethics committee (REC) or regulatory authority elects to terminate or suspend the study or the participation of an investigational site, a study-specific procedure for early termination or suspension will be provided by the principal investigator; the procedure will be followed by applicable investigational sites during the course of termination or study suspension.

4.6.4. Criteria for discontinuation or withdrawal of a subject: (1) pretreatment event or AE: the subject has experienced a pretreatment event or AE that requires early termination because continued participation imposes an unacceptable risk to the subject's health or the subject is unwilling to continue because of the pretreatment event or AE; (2) major protocol deviation: the discovery post-randomization that the subject failed to meet protocol entry criteria or did not adhere to protocol requirements, and continued participation poses an unacceptable risk to the subject's health; (3) lost to follow-up: the subject does not return to the clinic and attempts to contact the subject are unsuccessful; (4) withdrawal of consent: the subject wishes to withdraw from the study; (5) study termination: the principal investigator, IRB, REC, or regulatory agency terminates the study.

4.6.5 Procedures for discontinuation or withdrawal of a subject: The investigator may terminate a subject's study participation at any time during the study when the subject meets the study termination criteria. In addition, a subject may discontinue his or her participation without giving a reason at any time during the study. Should a subject's participation be discontinued, the primary criterion for termination would be recorded. Subjects who discontinue the study during the treatment period will visit the site as soon as possible for clinical assessment if applicable. To ensure participants' safety, those discontinued because of severe side effects or hospitalization due to any reason will be given individual clinical treatment. Discontinued or withdrawn subjects will not be replaced.

4.7. Anticipated results, potential pitfalls and contingency plans

Our previous and preliminary studies have confirmed the efficacy of electroacupuncture trigeminal nerve stimulation in the treatment of depression (Zhang et al., 2010, 2020a,b, 2023). These provide a direct and solid basis for the feasibility and success of the proposed study. We expect that DCEAS+BA could produce superior efficacy over MAS control in improving emotional and sleep symptoms. Multiple correlations between outcomes and biomarkers would be identified.

Related collaborative networks, protocols, and research teams have been well established. It is very unlikely that impassable obstacles will be encountered in the proposed study. Despite this, the completion of the recruitment in a timely manner may be a potential issue. If the recruitment rate cannot reach the goal at the end of the first year, we will seek more local study sites. Meanwhile, we will extend the promotion by distributing leaflets on the university campus, delivering public lectures, and advertising in the newspaper. These promotion measures have substantially accelerated the recruitment rate in our previous trials.

5. EXISTING FACILITIES, PERSONNEL, AND RECRUITMENT STRATEGY

There are about 50-70% of local adolescents who had emotional symptoms and 6%-9% with moderate to severe conditions. These provide sufficient subjects to ensure recruitment in a timely manner. We plan to recruit about 6 participants per month and 148 could be completed in about 24 months.

Table 3. A list of major members of research team

Name	Position and affiliation	Role and duties
Zhang, Zhang-Jin	Professor, Associate Director, School of Chinese Medicine, HKU	PI: Foresee, design, supervise and monitor the entire project.
Cheng, Pak Wing, Calvin	Clinical Assistant Professor, Department of Psychiatry, HKU	Co-I: Provide advice in screening and diagnosis
Chen, Haiyong	Assistant Professor, School of Chinese Medicine, HKU	Co-I: Provide advice in design and data analysis
LO, Kwai Ching	Assistant Lecturer, School of Chinese Medicine, HKU	Co-I: responsible for recruitment, training, and acupuncture treatment.
Chan, Mei Yan	Postdoctoral Fellow, School of Chinese Medicine, HKU	Co-I: responsible for recruitment, training, and acupuncture treatment.

The members of the research team have extensive experience in related studies (Table 3). The PI (Zhang ZJ) is a pioneer who was the first to conduct a systematic evaluation of the efficacy of acupuncture and Chinese medicines in the treatment of various psychiatric disorders. He has directed numerous clinical studies in this field. He will foresee, supervise, and monitor the project. The Co-Is Dr. Cheng PW is a psychiatrist who will provide advice on the diagnosis and treatment. Dr. Chen Hai-Yong, Dr. Chan Mei Yan, and Dr. Lo Kwai Ching have extensive experience in clinical studies and were involved in several clinical trials. They will coordinate the proposed project, organize training workshops, and conduct acupuncture treatment. Two research assistants will be employed to perform acupuncture treatment, clinical assessment, and salivary analysis.

6. FUNDING

The study is funded by the Tung Wah Group of Hospitals.

7. TIMELINE

Time (months)	Key milestone	Deliverable
3 months	Prepare and obtain related documents	Submit the obtained documents
6 months	30 subjects to be recruited	List of subjects
12 months	60 subjects to be recruited	List of subjects
18 months	90 subjects to be recruited	List of subjects
24 months	148 subjects to be recruited	List of subjects
30 months	Salivary sample analysis and data analysis	Submit data analysis results
36 months	Prepare final report and paper(s)	Submit final report.

8. REFERENCES

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