Effect of chrono-acupuncture on insomnia—A randomized controlled trial research protocol

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Abstract

Background: Insomnia is a widespread sleep disorder that significantly affects human health. Insomnia can result in depression, anxiety, and some other symptoms, which present a considerable challenge in clinical practice worldwide[1-2]. Traditional Chinese Medicine(TCM) offers an alternative approach to treating insomnia, and chrono-acupuncture stands out as a promising therapy in this regard. By administering acupuncture at specific time points, chrono-acupuncture aims to regulate the body's biological rhythms, as well as the flow of Qi and blood, potentially providing relief from insomnia symptoms. By leveraging the principles of TCM and the targeted timing of acupuncture stimulation, researchers hope to uncover new insights into the treatment of insomnia and potentially offer an alternative or complementary approach to managing this challenging sleep disorder.

Objective: The purpose of this study is to evaluate the therapeutic effect of acupuncture on insomnia at different hours based on Xuanfu theory and Ying-Wei theory. At the same time, it aims to study the difference between acupuncture and drug treatment of insomnia.

Methods: This protocol is a single-center, randomized, controlled trial. 105 eligible subjects will be included in this protocol, and the insomnia patients will be randomly divided into three groups according to the ratio of 1:1:1: control group, 7-9 am acupuncture group and 5-7 pm acupuncture group.

Results: The results of this protocol will be published in the form of a paper in relevant journals after the experiment is completed.

Conclusions: Outcomes
All results will be analyzed before treatment, after the end of the first week of treatment, after the end of the third week of treatment, and after the third week of treatment. From this study, we believe that acupuncture in insomnia patients at 7-9 am is better than acupuncture at 5-7 pm. At the same time, the curative effect of the acupuncture group was better than that of the drug control group.
Primary outcome
PSG: The researchers used PSG to monitor the sleep of the subjects, recording their total sleep time, sleep latency, Rapid Eye Movement(REM) sleep time, wake time, and sleep efficiency.
Secondary outcomes
(1) PSQI: This scale is mainly used to evaluate the subjects' sleep quality, sleep duration, sleep latency, sleep efficiency, sleep disorder, daytime function, sleep medication, and other 7 items. The full score of all items together is 21 points. The higher a subject's score, the worse their sleep quality; On the contrary, the lower his score, the better his sleep quality.
(2) HAMA, HAMD: This scale consists of two subscales of anxiety and depression, including 14 items, and the total score of the items ranges from 0 to 21 points. If a subject scores more than 7 points, it indicates that the subject has symptoms of anxiety or depression.
(3) AIS: This scale includes 8 items, and the total score of the items is between 0 and 24 points. When the total AIS score <4 is classified as no sleep disorder, the total AIS score between 4 and 6 is classified as suspicious insomnia, and the total AIS score >6 is classified as insomnia.

(4) Neurotransmitter levels that need to be evaluated include Brain-derived Neurotrophic Factor(BDNF) and 5-hydroxytryptamine(5-HT). To do this, the researchers will collect 4ml of venous blood from the subjects in the morning, when they are fasting, and use enzyme-linked immune sorbent assay(ELISA) to measure these two indicators. Clinical Trial: Ethics approval was received from the Medical Ethics Committee of the Affiliated Hospital of Traditional Chinese Medicine of Southwest Medical University, Luzhou, Sichuan, China (No. 2022YFS0613-B4). The findings will be disseminated in a peer-reviewed open-access journal and at academic conferences.

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Abstract

Keywords Insomnia, Xuanfu theory, Ying-Wei theory, Chrono-acupuncture

Introduction Insomnia is a widespread sleep disorder that significantly affects human health. Insomnia can result in depression, anxiety, and some other symptoms, which present a considerable challenge in clinical practice worldwide[1][2]. Traditional Chinese Medicine(TCM) offers an alternative approach to treating insomnia, and chrono-acupuncture stands out as a promising therapy in this regard. By administering acupuncture at specific time points, chrono-acupuncture aims to regulate the body's biological rhythms, as well as the flow of Qi and blood, potentially providing relief from insomnia symptoms. By leveraging the principles of TCM and the targeted timing of acupuncture stimulation, researchers hope to uncover new insights into the treatment of insomnia and potentially offer an alternative or complementary approach to managing this challenging sleep disorder.

Methods This protocol is a single-center, randomized, controlled trial. 105 eligible subjects will be included in this protocol, and the insomnia patients will be randomly divided into three groups according to the ratio of 1:1:1: control group, 7-9 am acupuncture group and 5-7 pm acupuncture group.

Results: The results of this protocol will be published in the form of a paper in relevant journals after the experiment is completed.

Strengths and limitations of this study

1.This study applies acupuncture therapy to deeply analyze the relationship between Xuanfu theory and chrono-acupuncture and insomnia on the basis of conventional western medicine treatment.
2.Guided by the Ying-Wei theory of TCM, this study compares the difference of acupuncture effect between 7-9 a.m. and 5-7 p.m., providing scientific and objective evidence-based basis for the clinical treatment of insomnia.
3.In this study, the evaluation of the efficacy of insomnia is multi-dimensional, This study not only uses various scales, but also uses relevant laboratory indicators, which can verify the evaluation of the efficacy of acupuncture on insomnia from multiple levels.
4.This study is a single-center study, with participants enrolled in only one hospital, which is weak in representativeness, thus reducing the external validity of this study.

1. Introduction

Insomnia is a prevalent sleep disorder affecting a significant portion of the population, with rates ranging from 7.5% to 30% globally and a combined prevalence of 15% in our country[4][3]. The condition not only impacts sleep but can also lead to various health issues such as anxiety, depression, and cardiovascular problems, significantly affecting patients' quality of life and posing a burden on society[2][1]. While drug therapy is commonly used for clinical intervention[5]. Despite the success of methods like clinical acupuncture and ear point pressure in treating insomnia, there is no established standard for Traditional Chinese Medicine(TCM)treatments. However, TCM approaches like acupuncture have gained popularity due to their safety and effectiveness without side effects. Acupuncture works by stimulating specific points in the body to regulate biological rhythms, movement of Qi and blood, organ functions, and the balance of Yin and Yang to address insomnia[6].

This protocol aims to explore the effectiveness of time-selected acupuncture based on Xuanfu theory and Ying-Wei theory, which provide essential frameworks in TCM for understanding insomnia. Xuanfu theory and Ying-Wei theory offer a solid foundation for understanding and treating insomnia. Xuanfu theory focuses on the heart as the center of spiritual activity, suggesting that blockages in this area contribute to insomnia, while Ying-Wei theory emphasizes the role of Qi and blood in guiding bodily functions and the development of diseases, with imbalances leading to insomnia. By combining these theories with the practice of chrono-acupuncture, this study seeks to explore the effectiveness of this approach in curing insomnia. Through the application of chrono-

acupuncture based on Xuanfu theory and Ying-Wei theory, this protocol aims to investigate how these techniques can influence the symptoms and severity of insomnia. By selecting specific acupoints to target Xuanfu and administering acupuncture at different times based on Ying-Wei theory, this protocol seeks to compare the efficacy of acupuncture treatment with conventional drugs for insomnia. Various scales and laboratory indicators will be used to evaluate the outcomes, aiming to provide a more precise and effective treatment strategy for insomnia based on the principles of TCM.

2. Methods

2.1 Research Design

The purpose of this study is to evaluate the therapeutic effect of acupuncture on insomnia at different hours based on Xuanfu theory and Ying-Wei theory. At the same time, it aims to study the difference between acupuncture and drug treatment of insomnia.

2.2 Research setting

The researchers will use the statistical software SPSS25.0 to generate random numbers and group them on cards. The cards are then randomly placed in sealed, opaque envelopes with integers from 1 to 105 written on the surfaces. Patients with insomnia who passed the screening will be assigned the corresponding serial number of envelopes according to the order of visit, and they will be randomly assigned to the 7-9 am acupuncture group, the 5-7 pm acupuncture group, and the control group in a ratio of 1:1:1.

2.3 Patient recruitment

Three strategies for recruiting insomnia patients are presented in this study, with the first strategy recruiting participants from both outpatient and inpatient populations in the Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University. Second, the researchers will distribute printed posters in public clinics and nearby communities to recruit potentially qualified protocol subjects. Third, the researchers will advertise the study through the Internet, and social software, to attract as many subjects as possible. Participants are recruited and enrolled by three researchers throughout the study.

2.4 Randomization and allocation concealment

Eligible patients will be randomly divided into three groups at a ratio of 1:1:1. The list of random numbers, generated by computer software and kept in opaque and sealed envelopes, will be kept by independent custodians to ensure the secrecy of the distribution. The patients' screening serial numbers will be printed on the outside of the envelope, and the group name will be printed on the inside. The researchers will first verify that each subject meets the eligibility criteria, and if so,

they will be asked to sign a written informed consent. The researchers then will open sealed envelopes containing each subject's assigned serial number. In the event of any errors or omissions in randomization, the researchers would generate a new randomization sequence from the sequence number in the question and apply it to subsequent patients from there.

2.5 Blinding

Considering the significant differences in treatment and the specificity of acupuncture, the researchers will conduct a strict random assignment of participants to ensure the integrity of this study. In addition, the therapeutic effect evaluation and statistical analysis of this study will be blind, and the operation involving the blind method will be carried out by a person who does not know the grouping situation, to achieve the purpose of separating the treatment operator, the therapeutic effect evaluator and the statistical analyst, to avoid the bias caused by subjective impression.

2.6 Inclusion criteria

Subjects who meet all of the following criteria will be included in the study:

(1) Meet the diagnostic criteria of insomnia in China Guidelines for Diagnosis and Treatment of Insomnia (2017) by the Chinese Sleep Research Society.

(2) The age of 18-75 years old, regardless of gender, both men and women.

(3) Conscious, clear thinking, and able to communicate with language.

(4) The patient agrees to participate in the project and signs the informed consent.

(5) The total score of the Pittsburgh Sleep Quality Index (PSQI) was ≥6 points.

2.7 Exclusion criteria

If the subjects meet one of the following criteria, they will be excluded:

(1) There is a taboo against acupuncture.

(2) alcohol, drug abusers, and drug addicts.

(3) Serious mental disorders that affect sleep, such as schizophrenia, bipolar disorder, paranoid mental disorder, schizoaffective disorder, epileptic mental disorder, and severe mental retardation.

(4) Pregnancy, breastfeeding, and pregnancy planning.Patients who have received other acupuncture treatments in the past month.

(5) Participants who are participating in clinical trials of other professional insomnia interventions will influence the outcome of this study.

(6) Have severe gastrointestinal diseases (such as indigestion, gastrointestinal obstruction, gastric and duodenal ulcers) and other gastrointestinal diseases that affect drug absorption.

(7) Patients with hepatic and renal insufficiency, severe neurological diseases, cardiovascular diseases, endocrine system diseases (such as diabetes, hyperthyroidism, etc.), autoimmune diseases,

and other contraindications.

(8) Accompanied by sleep apnea, restless leg syndrome, and other sleep disorders.

(9) Shift workers and/or frequent flyers across time zones.

(10)Subjects deemed unsuitable for the study by the clinical investigator.

2.8 Withdrawal or dropout criteria

(1) Those who do not meet the inclusion criteria and are included.

(2) Those who are unable to determine the efficacy, or whose data is incomplete and affects the evaluation of efficacy or safety.

(3) Although it meets the inclusion criteria and has been included, no treatment related to this trial has been performed.

2.9 Procedure

The recruitment team for this study will conduct a preliminary screening of potential participants based on inclusion criteria, exclusion criteria, and exclusion criteria. Eligible participants willing to participate in this trial will be required to sign a written informed consent. All eligible participants will then be randomly assigned to the 7-9 am treatment group, the 5-7 pm treatment group, and the control group. After randomization, subjects will have a baseline period, and then they will complete questionnaires on the PSQI, Hamilton Anxiety Scale(HAMA), Hamilton Depression Scale (HAMD), and Athens Insomnia Scale (AIS) scales within 24 hours. At the same time, the researchers will collect 4ml of venous blood from the subjects while they are fasting. Polysomnography(PSG) will also be used to monitor the sleep status of the subjects. They will then receive acupuncture treatment in their group for 3 weeks. Data will be collected before treatment, after the first week of treatment, after the third week of treatment, and at the third week after treatment.

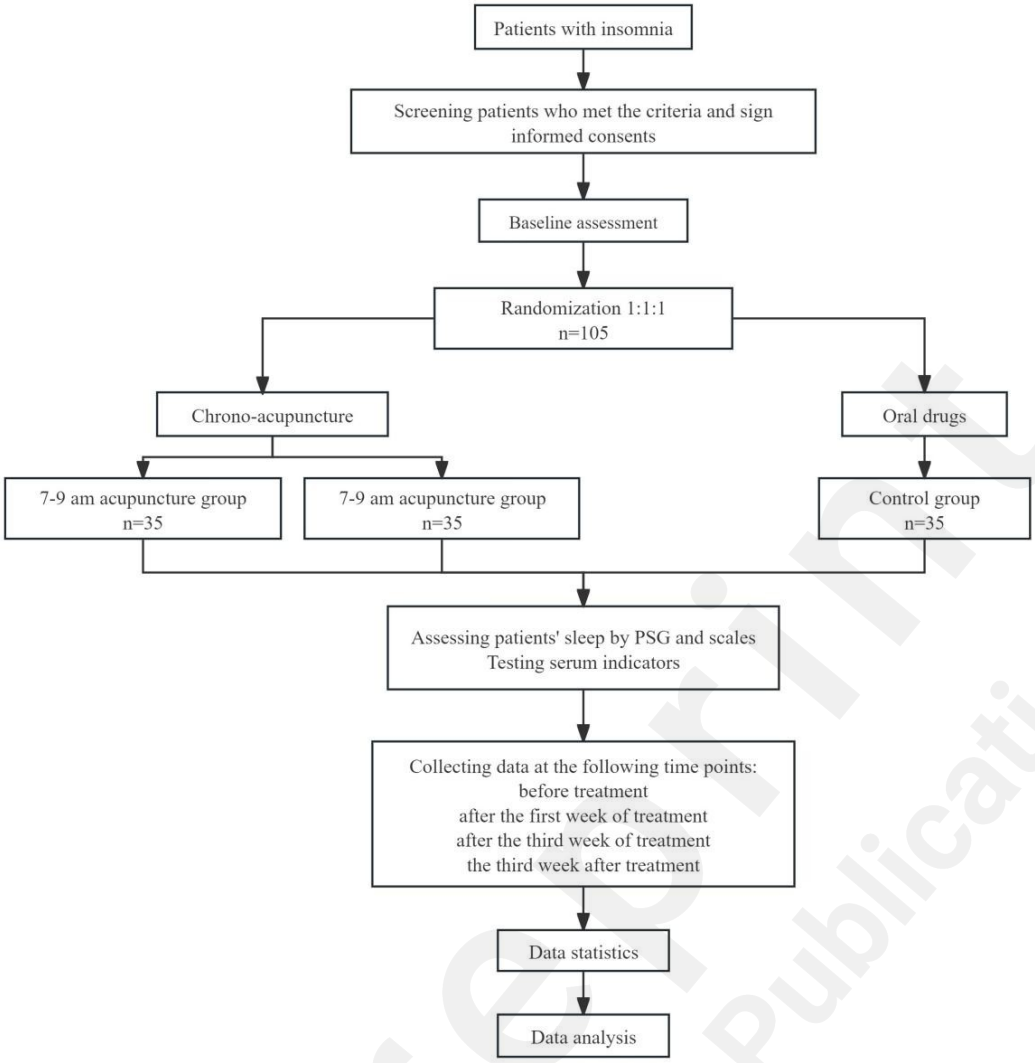

Figure1.Study flowchart. The scales include PSQI, HAMA, HAMD, and AIS. The serum indicators include BDNF and 5-HT.

2.10 Interventions

The researchers participating in the trial are all licensed Chinese medicine or integrative medicine practitioners, and they will receive training on standard practices before the trial. The acupoints selected for this study were identified according to the acupoint location method published by the World Health Organization. The patients in the 7-9 am treatment group and the 5-7 pm treatment group received acupuncture treatment at 7-9 am and 5-7 pm respectively. The following acupoints will be selected for the treatment of insomnia in this study: DU20, GB20, EX-HN3, PC6, BL62, KI6, and LV3. Specific operation: The patient takes a supine position and naturally stretches the limbs flat to form a comfortable position, and the researchers use medical alcohol cotton balls to sterilize the acupuncture points that need to be acupuncture, and then use one-time acupuncture for the above seven points, the researchers will use the technique of nourishing deficiency and purging to ensure that the patients' acupuncture site appears acid, numbness, swelling and other reactions.

The subjects were treated for 30 minutes at a time, then they will be treated with acupuncture five times a week, with two days off, for three consecutive weeks, and will be followed up by the researchers on the third week after the treatment. Control group: Subjects will be given drug treatment (3mg Dexzopicron tablets, produced by Chengdu Kanghong Pharmaceutical Group Co., LTD.), subjects will be told to take the drug orally 3 hours before going to bed every day, the treatment cycle is also three weeks, they will also be followed up at the third week after the completion of treatment.

2.11 Outcomes

All results will be analyzed before treatment, after the end of the first week of treatment, after the end of the third week of treatment, and after the third week of treatment. From this study, we believe that acupuncture in insomnia patients at 7-9 am is better than acupuncture at 5-7 pm. At the same time, the curative effect of the acupuncture group was better than that of the drug control group.

2.11.1 Primary outcome

PSG: The researchers used PSG to monitor the sleep of the subjects, recording their total sleep time, sleep latency, Rapid Eye Movement(REM) sleep time, wake time, and sleep efficiency.

2.11.2 Secondary outcomes

(1) PSQI: This scale is mainly used to evaluate the subjects' sleep quality, sleep duration, sleep latency, sleep efficiency, sleep disorder, daytime function, sleep medication, and other 7 items. The full score of all items together is 21 points. The higher a subject's score, the worse their sleep quality; On the contrary, the lower his score, the better his sleep quality.

(2) HAMA, HAMD: This scale consists of two subscales of anxiety and depression, including 14 items, and the total score of the items ranges from 0 to 21 points. If a subject scores more than 7 points, it indicates that the subject has symptoms of anxiety or depression.

(3) AIS: This scale includes 8 items, and the total score of the items is between 0 and 24 points. When the total AIS score <4 is classified as no sleep disorder, the total AIS score between 4 and 6 is classified as suspicious insomnia, and the total AIS score >6 is classified as insomnia.

(4) Neurotransmitter levels that need to be evaluated include Brain-derived Neurotrophic Factor(BDNF) and 5-hydroxytryptamine(5-HT). To do this, the researchers will collect 4ml of venous blood from the subjects in the morning, when they are fasting, and use enzyme-linked immune sorbent assay(ELISA) to measure these two indicators.

2.12 Sample size calculation

Each group in this study will consist of 35 subjects, based on the following: The minimum

sample size for general clinical trials is 30, and 15% of subjects are expected to drop out in this study. The study consisted of three groups with a total of 105 participants.

2.13 Statistical analysis

The researchers stored the patients' data in an Excel spreadsheet to form a database. All data will be analyzed by the researchers using SPSS25.0 statistical software. General data, clinical indicators, laboratory indicators, and levels of various cytokines were statistically analyzed. The t-test will be used for measurement data conforming to the normal distribution, the paired sample t-test will be used for intra-group analysis, and the independent sample t-test will be used for inter-group analysis. The non-parametric test will be used for measurement data that does not meet the normal distribution, the Wilcoxon signed rank sum test will be used for intra-group analysis, and the Mann-Whitney U test will be used for inter-group analysis. $P<0.05$ means that the difference is statistically significant.

2.14 Safety observation

The researchers will record the adverse events such as hematoma, infection, needle delay, broken needle, bent needle, and fainting needle in the acupuncture site of patients during treatment, record the treatment methods and prognosis in the table, and finally complete the safety evaluation.

2.15 Quality control

To ensure the real reliability of the research results and reduce the deviation of the final data of the research, the following points are required:

(1) The researchers participating in the research must have a master's degree or above, and they must have obtained the professional qualification certificate, and then they will receive unified training organized by professionals. Through training, researchers must master how to screen eligible subjects according to inclusion and exclusion criteria and ask subjects to sign informed consent. At the same time, they must have good communication skills with patients.

(2) This study will include as few researchers as possible (< 4) to increase the psychological acceptance of the subjects.

(3) Efficacy evaluators will receive training on the standard completion of the Case Report Form(CRF)form.

(4) Before the final data analysis, the data statistical analyst must check the omissions and errors in the preparation and entry of the data; In the whole process of the study, the principle of separation of acupuncture operator, efficacy evaluator, and data statistical analyst is strictly followed to avoid data deviation and ensure the authenticity of the results.

2.16 Management of Adverse Events

Researchers will record and report adverse events that occur during the study at any time, and treat patients in a timely and appropriate manner. Before the start of the trial, the researchers should explain to the subjects the precautions, the requirements of the trial, etc., and report any changes during and after the treatment. In the course of the experiment, once the subjects suffer from dizziness, infection, etc., the researchers should immediately treat them accordingly until they completely return to normal. All adverse reactions or adverse events (regardless of whether they are related to the treatment in this study) should be recorded in detail, including the time of occurrence, symptoms, signs, extent, duration, laboratory test indicators, treatment methods, and results, course, follow-up time, etc., and the cause of adverse reactions should be analyzed.

2.17 Ethics

This study will be implemented per the Declaration of Helsinki and approved by the Medical Ethics Committee of the Affiliated Hospital of Traditional Chinese Medicine of Southwest Medical University, Luzhou, Sichuan, China (No. 2022YFS0613-B4). Once recruited, each participant will be asked to voluntarily sign a written informed consent before randomization as acceptance for this study. This research was supported by the special research project of the Science and Technology Department of Sichuan Province. This study has been registered in the Chinese Clinical Trials Registry (identification number: ChiCTR230078363).

3. Discussion

Historical records of insomnia date back thousands of years, with references found in ancient texts like the *Yellow Emperor's Inner Classic* and the *Eighty-one Difficult Issues*. Modern medicine defines insomnia as a sleep disorder marked by recurrent and persistent difficulties in falling asleep and staying asleep[7]. Insomnia is often attributed to lifestyle factors such as external pathogenic influences and emotional disturbances[1-2]. Certain studies suggest that insomnia primarily stems from imbalances in Yin and Yang as well as a disharmony of Ying-Wei[8]. Sleep and wakefulness in individuals rely on the concept of Wei Qi, with the proper circulation of Wei Qi ensuring regular sleep patterns. Hence, the treatment of insomnia should focus on harmonizing Ying and Wei levels. Xuanfu theory in TCM serves as a crucial theoretical framework for addressing insomnia. Ying-Wei theory and Xuanfu theory have become the guide to insomnia treatment. Current insomnia treatments encompass somatic therapies (pharmacological interventions and physical interventions), psychological interventions, and a combination of somatic and psychological approaches, with pharmacological treatments being the most advanced. Common pharmacological treatments for insomnia comprise benzodiazepine receptor agonists, melatonin and its receptor agonists, orexin receptor antagonists, histamine antagonists, as well as anti-anxiety and antidepressant medications.

While these medications extend nighttime sleep duration, prolonged drug use significantly affects daytime function, leading to drowsiness, fatigue, impaired cognition, and other adverse effects[9]-[11]. Numerous studies have demonstrated the efficacy of TCM in treating insomnia, with acupuncture being particularly effective.

Human beings are in harmony with nature due to the interplay of Yin and Yang influences, resulting in a cyclic physiological balance of Ying and Wei, manifesting as wakefulness during the day and sleep at night. Recent researches indicate that sleep arousal is governed by sensory inputs and sleep-promoting chemicals[12]. Sleep medicine researchers have identified that the wake-promoting system, Non-rapid Eye Movement(NREM) sleep generation system, and REM sleep generation system in the brain collaboratively oversee sleep regulation. Furthermore, the circadian rhythm and sleep homeostasis mechanisms play significant roles in this process[13]. In recent years, with the development of society, the acceleration of the pace of modern life, and the change of lifestyle, people's pressure has been increasing. The phenomenon has resulted in a significant rise in the prevalence of insomnia, making it a prominent medical and public health concern. Insomnia will produce pain, anxiety, depression, fear, and other adverse effects on individuals, but also leads to their cognitive efficiency, psychological and social function, even affects the family, and brings a heavy economic burden to the whole society[14]-[16].

Recent research has revealed that individuals' sleep patterns are influenced not only by external factors but also by their internal genetic rhythms. Natural phenomena include circadian, tidal, lunar, and seasonal rhythms that impact natural organisms[17]-[19]. Influenced by the principles of Yin and Yang, regular human activities follow daily, monthly, yearly, and age-related patterns, with insomnia occurrences also exhibiting similar rhythms[20]-[21]. Therefore, when using acupuncture for insomnia treatment, it is crucial to consider timing, a fundamental aspect of TCM's temporal approach. The *Eighty-one Difficult Issues* contains extensive records on temporal medicine. The theory in TCM delves into the interplay between the environment and human life, exploring the physiological and pathological rhythms of the body such as daily, monthly, and yearly cycles, including seasonal influences on health and disease onset. The significance of TCM's temporal approach to acupuncture lies in its ability to comprehend the temporal patterns of disease occurrence, progression, and transmission, thereby emphasizing preventive care strategies. Chrono-acupuncture is a therapeutic approach developed based on the principles of the *Eighty-one Difficult Issues*. This involves selecting acupoints based on the interplay of Yin-Yang, five elements, and tissue dynamics, serving as a vital link between time, organs, and acupuncture points. Numerous studies have verified that acupuncture following the meridian flow theory can effectively alleviate insomnia symptoms.

Selecting acupoints based on midday and noon meridian flow patterns has a notable therapeutic impact on insomnia, enhancing sleep parameters, and quality, and regulating sleep patterns in insomnia patients. Current research by scholars on chrono-medical diagnosis and treatment of insomnia primarily centers on understanding the pathogenesis and therapeutic approaches related to the daily rhythm of insomnia, albeit lacking depth[22]-[23]. Consequently, there is a need for comprehensive research on time-based acupuncture therapy for insomnia.

The concept of Ying-Wei theory stems from the *Eighty-one Difficult Issues*. Ying Qi and Wei Qi, subtle essences derived from the digestive fluids of the spleen and stomach, serve as the primary sources of energy, with Ying-Yin playing a pivotal role in sleep regulation. Wei Qi corresponds to daytime while Ying Qi aligns with nighttime and Ying-Yin principles. The balance between Ying and Wei energies forms the foundation of sleep. Their interaction follows inherent laws, with Wei Qi transitioning from Yang to Yin, aligning with natural day-night cycles for maintaining normal rest and rejuvenation. An imbalance in Ying and Wei energies leads to sleep disturbances. The Ying-Wei theory serves as a crucial guiding principle in acupuncture practice. Acupuncture plays a significant role in harmonizing Ying and Wei levels, balancing Yin-Yang dynamics, and facilitating the timely entry of Wei Qi into the body in alignment with the circadian rhythm. It is a commonly utilized method for treating insomnia in clinical practice. Therefore, in the treatment of insomnia, focusing on accessing the meridians to enhance the harmony of Ying and Wei and correct any imbalances is crucial for regulating Ying-Yin.

Xuanfu, which first appeared in the *Yellow Emperor's Inner Classic*, is essential for facilitating the flow of Qi and blood, enhancing brain sensitivity. It is considered the most subtle structure in the human body and is distributed widely in the upper and lower parts of the body, serving as the pathway for the movement of Qi in Ying and Wei. Blockages in Xuanfu can hinder the flow of Qi in Ying-Wei, impacting all body functions. Therefore, ensuring the unobstructed flow of Xuanfu is key for maintaining overall health.

Modern researches indicate that the sleep center in the human brain stem regulates the transition between sleep and wakefulness by producing endogenous sleep-promoting substances[24]-[26]. 5-HT is a crucial neurotransmitter that plays a significant role in insomnia[27]-[28]. When the secretion of serotonin is too low, the secretion of neurotransmitters that promote awakening increases, the activity of the hypothalamic-pituitary-adrenal axis significantly increases, and the excitatory state of the human cerebral cortex are no longer inhibited, which will cause insomnia[29]-[31]. Acupuncture can enhance blood supply to the brain stem, increase blood oxygen saturation, regulate the brain's 5-HT delivery system, and boost the production of sleep-promoting substances, thereby improving sleep

quality[32]-[33]. BDNF is one of the most active neurotrophic factors and can stimulate and control neurogenesis, BDNF is more active in the human hippocampus, cortex, and forebrain, and these areas are critical to the human mind, they can be involved in the regulation of sleep-wake homeostasis[34][35][36]. Moreover, acupuncture has been found to influence the expression of brain-derived neurotrophic factors.BDNF is a key factor in neurogenesis and cognitive function[37]. BDNF levels are closely linked to cognitive and mental health disorders[38]. Basic studies have found that when the level of BDNF in awake rats is higher than that in sleep, the level of BDNF will increase after sleep deprivation in rats[39]. Acupuncture has shown promise in increasing BDNF expression, particularly in conditions like insomnia. In animal models of cognitive impairment, acupuncture has been shown to increase the expression of BDNF in the hippocampus[40]. Considering the TCM concept of the twelve-hour cycle, treating patients for insomnia during the balance point of Yin and Yang (5-7 pm) can be beneficial. Acupuncture at specific points during this time frame can help regulate the body's energy channels, promote normal sleep patterns, and enhance overall well-being. Acupoints such as DU20, GB20, EX-HN3, PC6, BL62, KI6, and LV3 are selected based on meridian flow guidance to address insomnia effectively during different time intervals[41]-[45]. Further research is needed to validate the efficacy of acupuncture treatment for insomnia at specific times.

In conclusion, Ying-Wei theory and Xuanfu theory play significant roles in the development of insomnia, while chrono-acupuncture offers a promising approach for treating insomnia. Furthermore, 5-HT and BDNF levels in human serum serve as reliable indicators for assessing insomnia. PSG is a highly effective method for detecting sleep patterns, extensively applied in clinical and scientific sleep medicine research. It offers an objective assessment of patients' sleep quality by monitoring their sleep duration, and efficiency, and identifying instances of hypopnea or sleep apnea. Assessment tools like PSQI, AIS, HAMA, and HAMD are utilized in clinical trials to evaluate sleep quality and insomnia. Hence, drawing on Xuanfu theory and Ying-Wei theory, this study implemented acupuncture interventions at varying times to investigate the differential therapeutic effects. This approach aims to establish a scientific, evidence-based foundation for treating insomnia clinically and to formulate a more precise, effective, and high-quality treatment strategy. This endeavor holds immense importance in advancing the clinical utilization and scholarly progress of acupuncture for insomnia treatment.

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Conflict of interest statement All authors have no conflicts of interest to disclose.

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Abbreviation: Pittsburgh Sleep Quality Index Score (PSQI). Hamilton Anxiety Scale(HAMA), Hamilton Depression Scale (HAMD).Athens Insomnia Scale (AIS). Polysomnography (PSG). Brain-derived Neurotrophic Factor (BDNF).Enzyme-linked immunosorbent assay (ELISA).Non-rapid Eye Movement(NREM).Traditional Chinese Medicine(TCM).Rapid Eye Movement(REM).5-hydroxytryptamine (5-HT). Case Report Form(CRF)

Ethics and dissemination Ethics approval was received from the Medical Ethics Committee of the Affiliated Hospital of Traditional Chinese Medicine of Southwest Medical University, Luzhou, Sichuan, China (No. 2022YFS0613-B4). The findings will be disseminated in a peer-reviewed open-access journal and at academic conferences.

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esign, Investigation, Resources.Dechou Zhang: Methodology, Resources, Supervision, Validation.Xiao Wu: Conceptualization, Methodology, Resources, Supervision, Review.

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Supplementary Files