Clinical Research Protocol

Project Name: Study of the Therapeutic Effects of Naohuan Dan and Idebenone in Treating Mild Cognitive Impairment With Kidney Deficiency and Phlegm Stasis

Bidding unit: Sun Yat-sen Memorial Hospital, Sun Yat-sen University

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1 Program Summary

This study conducted a retrospective analysis of 64 patients with mild cognitive impairment of the kidney deficiency phlegm and stasis type who underwent treatment with Naohuan Dan combined with Idebenone. The patients' cognitive function was evaluated using the Mini-Mental State Examination (MMSE) and the Montreal Cognitive Assessment (MoCA) before and after treatment. Daily living abilities were assessed using the Activities of Daily Living (ADL) scale, depression status was evaluated using the Geriatric Depression Scale (GDS), and the severity of Traditional Chinese Medicine (TCM) syndrome was assessed using the TCM diagnostic scale. Peripheral blood from the patients was also collected for analysis of neuron-specific enolase (NSE) and inflammatory factors. The aim was to evaluate the safety and feasibility of using Naohuan Dan combined with Idebenone for the treatment of mild cognitive impairment of the kidney deficiency phlegm and stasis type.

2 Introduction

Mild cognitive impairment (MCI), a transitional state between aging and dementia, is clinically characterized by a decline in cognitive function[1], but daily living abilities remain unaffected or mildly impaired. Previous studies have shown that 50% of MCI patients will progress to dementia within 3-4 years of onset, and the proportion of individuals progressing to dementia increases with age and disease duration[2]. The exact pathogenesis of MCI is not yet fully understood, and there is a lack of effective clinical treatments[3]. Cognitive impairment in MCI patients can severely impact their quality of life and burden families and society.

MCI can be divided into amnestic and non-amnestic types, with amnestic MCI mainly characterized by memory impairment and non-amnestic MCI mainly characterized by cognitive impairments such as language, visual-spatial recognition, and executive function[4]. Main neurological and psychiatric symptoms include anxiety, depression, apathy, and sleep disturbances. A meta-analysis using a random-effects model showed that the cumulative incidence rate of dementia for MCI

patients aged 65 and above after 2 years was 14.9%. As the aging of the population accelerates in China and life expectancy gradually increases, the incidence rate of MCI is on the rise.

MCI belongs to the category of "forgetfulness", "fondness for remembering", and "dementia" in traditional Chinese medicine, with the pathological basis of marrow decline, brain consumption, and loss of function of the nerve machinery. It is a syndrome of virtual and actual deficiency, and the condition is complex. At present, there are several single herbs in traditional Chinese medicine that can improve mild cognitive impairment (MCI), such as ginkgo biloba, acorus calamus, ginseng, and polygala[5], which have effects on inhibiting neuronal apoptosis, promoting neuronal metabolism, and reducing amyloid deposition, thereby improving cognitive function. A meta-analysis[6] found that oral administration of Ginkgo biloba leaf extract preparations for MCI treatment is clinically effective and can effectively prevent the incidence of dementia, but its safety still needs further exploration. In addition, single herbs have the drawback of having a single target point and cannot effectively improve cognitive function in MCI. Chinese medicine compound formulas have multi-target pharmacological effects and multi-level actions. Haifeng Wang[7] found that the TCM for invigorating the kidney and activating blood circulation, which is composed of several Chinese herbs, can improve the executive function and memory of MCI patients, and its mechanism of action may be related to strengthening the body's ability to resist oxidative stress and reducing the damage of free radicals to neurons. Lijing Yang[8] found that the Chuanpu Yizhi Tang compound formula can reduce the levels of serum Hcy and AchE, improve the cognitive function and daily life ability of MCI patients, and has good clinical efficacy. In addition, traditional Chinese medicine techniques such as acupuncture, traditional exercises, and massage have shown certain efficacy in treating MCI, which is worth further exploration and promotion.

Based on the above research background, we believe that the combination of Naohuan Dan combined with Idebenone has great application prospects for the treatment of Kidney Deficiency Phlegm Stagnation Type MCI. To this end, we have

designed a clinical study to evaluate the efficacy and safety of the combination of Naohuan Dan combined with Idebenone for the treatment of MCI, by assessing relevant cognitive function rating scales and TCM syndrome scores. This study aims to provide valuable information for the integration of TCM and Western medicine in the treatment of MCI.

3 Research Objectives and Endpoints

3.1 Main purpose

This study evaluated the efficacy of Naohuan Dan combined with Idebenone for treating MCI patients with Kidney-Deficient and Phlegm-Stasis syndrome. Changes in cognitive function, daily living abilities, depression levels, and Traditional Chinese Medicine clinical symptoms were assessed using relevant rating scales, and compared with the efficacy of Idebenone alone, demonstrating the advantages of combining traditional Chinese and Western medicine in treatment.

3.2 Main outcome

The primary outcome measure for the study was the Mini-Mental State Examination (MMSE) scale scores [9], which were used to examine the cognitive function of the participants before and after therapy. A total score of 23-30 is considered to indicate normal cognitive function, 17-22 indicates mild cognitive impairment, 11-16 indicates moderate cognitive impairment, and 10 or below indicates severe cognitive impairment.

The clinical efficacy was evaluated based on the MMSE scale, and the efficacy was calculated by comparing the scores before and after the therapy. The following criteria were used to determine the efficacy: (i) Markedly effective: ≥20% improvement in MMSE score. (ii) Effective rate: ≥12% improvement in MMSE score. (iii) Ineffective: <12% improvement in MMSE score[10]. The total efficacy was calculated as the sum of the markedly effective and effective rates.

3.3 Secondary outcomes

The secondary outcome measures for the study included various scales and serological indicators. The scales used in the study were the Montreal Cognitive Assessment (MoCA) scale, the ADL scale, the Geriatric Depression Scale (GDS) and the TCM syndrome score scale.

The MoCA scale is a widely used screening tool for detecting mild cognitive impairment. It assesses various cognitive domains such as attention, memory, visuospatial skills, language, and orientation[11]. The total score on the MoCA scale is 30, and a score of 26 or higher is considered to indicate normal cognitive function.

The ADL scale is used to assess the participants' ability to perform daily living activities[12]. The total score on the ADL scale is 100 points.

The GDS scale is a self-report assessment tool used to detect and measure the severity of depression in older adults[13]. The total score on the GDS scale is 30 points.

The TCM clinical symptoms of the participants were evaluated using the TCM syndrome score scale[14]. This tool assesses the severity of specific TCM symptoms and divides them into four grades: none, mild, moderate, and severe, with 0, 2, 4, and 6 points (for main symptoms) or 0, 1, 2, and 3 points (for secondary symptoms), respectively. The total score was calculated before and after the therapy to determine the effectiveness of the treatment.

Blood samples were collected from the participants before and after the therapy via cubital venipuncture. Enzyme-linked immunosorbent assay (ELISA) was used to determine the serum levels of neuron-specific enolase (NSE), interleukin-8 (IL-8) and tumor necrosis factor-alpha (TNF-α) using the Reader M3 multi-function microplate reader.

3.4 Safety Index

Before and after treatment, the patient's elbow vein blood is collected for blood routine, urine routine, stool routine, and liver and kidney function tests. The patient's electrocardiogram is also monitored, and any adverse reactions after medication are observed and treated accordingly.

4 Research Population

4.1 Inclusion criteria

The following inclusion criteria were used: (1) diagnosis in line with Chinese medicine and Western medicine; (2) suitable for age 55-85 years old, gender is not limited; (3) a score between 21-26 on Mini-Mental State Examination (MMSE), 1 point for primary school education; (4) agree to take part in this experiment and sign the informed consent form.

4.2 Exclusion criteria

The following exclusion criteria were used: (1) patients with severely impaired heart, liver, kidney and other functions; (2) patients with neurological diseases that affect brain function and cognitive impairment; (3) patients with severe depression and mental illness; (4) patients with poor compliance and cooperation who failed to complete the study according to the protocol.

4.3 Lifestyle and Precautions

During the course of treatment, if the patient has comorbidities such as hypertension, diabetes and hyperlipidemia, their original treatment plan should be maintained, and appropriate symptom-relieving treatments such as blood pressure-lowering, hypoglycemic and lipid-lowering drugs should be provided. Smoking and drinking should be prohibited during the treatment period, and regular exercise and a light diet should be maintained.

4.4 Recruitment and Retention Strategies

At the outpatient and ward departments of Traditional Chinese Medicine and Rehabilitation of Sun Yat-sen Memorial Hospital, eligible MCI patients with the type of kidney deficiency and phlegm stasis were identified according to the inclusion criteria. After fully communicating trial-related information with the patients, they

were informed of the possible adverse reactions and risks, and asked to sign an informed consent form voluntarily and informed about the trial plan.

Retention strategies: (1) Researchers should maintain one-on-one communication with patients as much as possible. (2) Clear trial-related information should be provided to patients during the first communication, along with a process calendar, and patients should be informed of the next appointment time and reminded through phone calls. (3) Researchers can communicate the current progress with patients at appropriate times to help them fully recognize their contribution to the research. (4) Sufficient work should be done to obtain informed consent during the screening phase and to answer any questions patients may have.

5 Research Design

5.1 Intervention

This retrospective, two-armed, single-center clinical observation evaluates the combination of Naohuan Dan and Idebenone for the treatment of MCI patients with Kidney Deficiency and Phlegm Stasis. Eligible patients were randomized using a random number table into a control group and a treatment group. The control group was given 30mg of Idebenone tablets three times a day after meals, while the treatment group was given Naohuan Dan in addition to Idebenone. The main components of the Naohuan Dan formula are Rehmannia glutinosa 25g, Codonopsis pilosula 20g, Acorus calamus 10g, Polygala tenuifolia 8g, Eucommia ulmoides 15g, and Poria cocos 12g, among other ingredients. The herbal granules were provided by Yifang Pharmaceutical Co., Ltd. The patients in both groups received corresponding treatments for underlying diseases such as hypertension and diabetes if present. One dose of herbal granules was administered daily with warm water in the morning and evening.

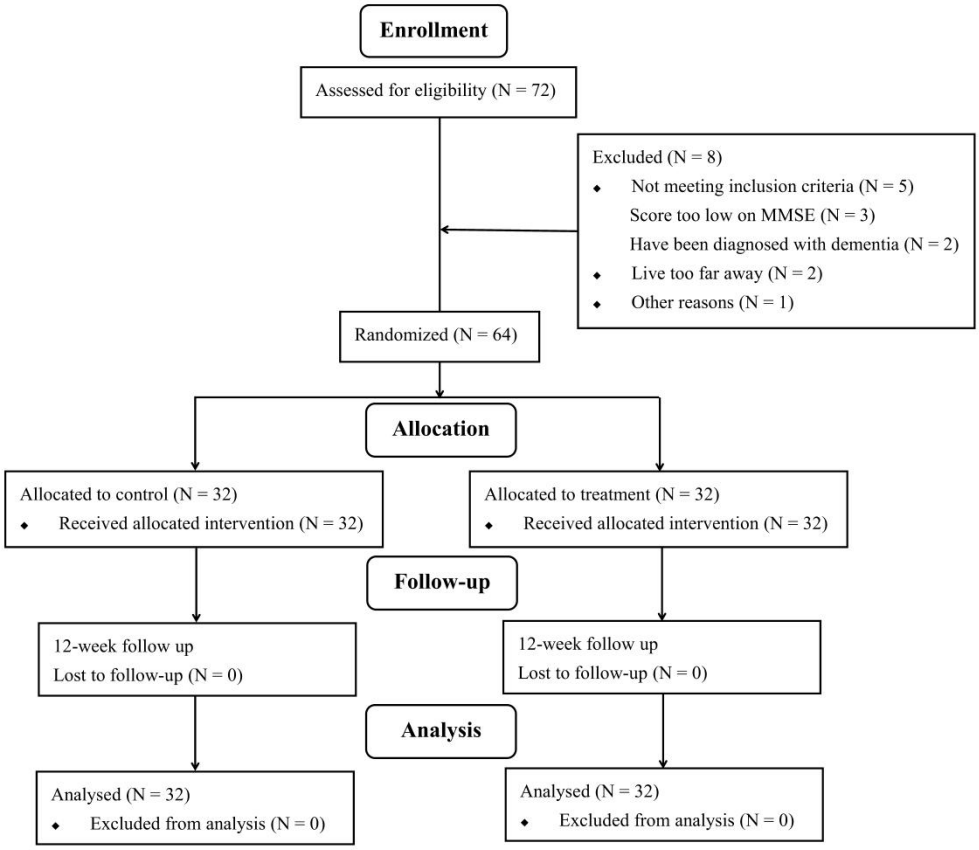

Fig. 1 CONSORT flow chart.

5.2 Research Calendar

Program	Screening period -7 to -1 day	Enrollment/Base line Visit 1, Day 1	Study Visit 2 7+/-1 day	Study Visit 3 14+/-1 day	Study Visit 4 21+/-1 day	Study Visit 5 28+/-1 day	Study Visit 6 35+/-1 day	Study Visit 7 42+/-1 day	Study Visit 8 49+/-1 day	Study Visit 9 56+/-1 day	Study Visit 10 63+/-1 day	Study Visit 11 70+/-1 day	Study Visit 12 77+/-1 day	Study Visit 13 84+/-1 day
General Information Access	√													
Informed Consent	√													
Medical History	√													
Random Grouping	√													
Medication		√	√	√	√	√	√	√	√	√	√	√	√	√

The MMSE scale	√													√
The MoCA scale	√													√
The ADL scale	√													√
The GDS scale	√													√
The TCM Syndrome score scale	√													√
Lab Test	√													√
Blood routine, Urine routine, Stool routine and Liver and Kidney function	√													√
Electrocardiogram	√													√
Adverse Events and Evaluation	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Complete the Case Report Form	√	√	√	√	√	√	√	√	√	√	√	√	√	√

5.3 Definition of End of Study

When the last patient who met the inclusion and exclusion criteria completed the prescribed course of drug treatment, there were no obvious adverse reactions and events in the last follow-up, and the corresponding data were collected as the end of the study.

5.4 Statistical Analysis

5.4.1 sample size

This study is a retrospective, two-arm study with a fixed sample size and no strict hypothesis testing. A total of 64 participants were enrolled in this study.

5.4.2 Data Analysis Set

Full Analysis Set (FAS) analyzed all cases who were enrolled and received at least

one dose of the study drug, according to the intention-to-treat (ITT) principle. For cases where the entire treatment process could not be observed, the last observed data was carried forward to the final results of the trial (LOCF).

Per-protocol Set (PPS) included all cases that complied with the trial protocol, had good compliance, did not use any prohibited medication during the trial, and completed the required case report form. No data imputation was conducted for missing data. The efficacy of the medication was analyzed for both FAS and PPS.

Safety Analysis Set (SAS) consisted of all enrolled cases who had received at least one dose of the study drug and had safety records available after medication use. This dataset was used for safety analysis.

5.4.3 Statistical Analysis

All data were analyzed using SPSS25.0 statistical software. For continuous variables, median (minimum, maximum) was used for descriptive statistics; mean ± standard deviation was used if the data followed a normal distribution and had homogeneous variance. Paired t-test was used for within-group comparisons, and two independent sample t-tests were used for between-group comparisons. Wilcoxon rank sum test was used for non-normal distributed data. For categorical variables, frequency (percentage) was used for descriptive statistics, and rates or composition ratios were used for comparisons between groups using the χ^2 test. Rank sum test was used for ordinal data. A value of $P<0.05$ was considered statistically significant.

6 Research Intervention

6.1 Intervention

The intervention was administered as follows: (1) The control group received Idebenone (Shenzhen Neptunus Pharmaceutical Co., Ltd., National standard: H10970362, Specification: 30mg) orally, at a dose of 30mg per time, three times a day, after meals for 12 consecutive weeks. (2) The treatment group received Naohuan Dan combined with Idebenone. The main components of traditional Chinese medicine

were Codonopsis Radix 20g (Batch number: 1031653), Radix Rehmanniae Preparata 25g (Batch number: 1050973), Drynariae Rhizoma 15g (Batch number: 0117293), Acori Tatarinowii Rhizoma 10g (Batch number: 1021283), Radix Polygaiae 8g (Batch number: 9086013), Poria Cocos 12g (Batch number: 1051133), etc. The traditional Chinese medicine granules were provided by Guangdong Yifang Pharmaceutical Co., Ltd., at a dose of one per day, delivered with warm water in the morning and evening, and taken orally for 12 consecutive weeks. (3) Basic and symptomatic treatments were given to both groups according to the patient's underlying diseases and complications, such as antihypertensive and hypoglycemic treatments.

6.2 Study Intervention Adherence

Patients who are able to take medication on time and in the correct dosage, attend regular hospital follow-ups, do not take prohibited drugs, and cooperate with medical evaluations during the course of treatment are considered to have good compliance.

Methods to improve compliance include: (1) providing medication records and instructing patients to record medication intake daily, which can be returned during the next follow-up visit; (2) providing detailed explanations and addressing patient questions during the first medication distribution, while affirming the patient's contribution to research; (3) issuing medication in short-term doses and reminding patients of their next follow-up appointment in advance.

7 Study Intervention Discontinuation/Subject Discontinuation and Withdrawal

7.1 Study Intervention Discontinuation

The enrolled patients may discontinue treatment at any time for any reason or, in case of any adverse event, at the discretion of the investigator. Furthermore, the investigator may stop the treatment of a patient if the patient is deemed unsuitable for treatment, violates the study protocol, or for management and/or safety reasons.

The subject must discontinue treatment but may continue to be monitored in the

study if any of the following occur: 1) the patient or the patient's family requests discontinuation of treatment; 2) an intercurrent illness which interferes with further treatment occurs; 3) the investigator determines that the patient should be withdrawn from the study; 4) poor compliance by the patient; 5) the investigator believes that continuing to administer the investigational drug to the subject would place the subject at unnecessary risk based on the subject's medical condition or personal situation; 6) completion of the treatment as outlined in the protocol.

7.2 Subject discontinuation/withdrawal from the study

If a patient or their family withdraws their informed consent to participate in the study, the enrolled patient must be withdrawn from the study. If a patient withdraws from the study, they will no longer receive treatment or participate in planned visits. If a patient is lost to follow-up, they must also be withdrawn from the study.

7.3 Lost to follow-up

Timely education and reminders should be provided to enrolled patients. The next follow-up appointment should be scheduled during each visit, and the patient's contact information should be recorded to facilitate timely communication, enhance medication adherence and patient follow-up compliance, and reduce data loss due to patient loss to follow-up. If there is data loss due to patient loss to follow-up, it should be excluded from data analysis and appropriately documented.

8 Adverse Events and Unexpected Events

8.1 Adverse event

Adverse events (AE) refer to all adverse medical events that occur in subjects after receiving the investigational drug. They can manifest as symptoms, signs, diseases, or laboratory abnormalities, but are not necessarily causally related to the investigational drug.

The grading of adverse events is as follows:

Grade 1: Mild; asymptomatic or mild symptoms; only clinical or diagnostic manifestations; no intervention required;

Grade 2: Moderate; requires minimal, partial, or non-invasive intervention; restricts appropriate age daily activities;

Grade 3: Severe or significant medical events that require drug treatment, but do not immediately threaten life; hospitalization or prolonged hospitalization; disability; restricts activities of daily living (ADLs);

Grade 4: Life-threatening consequences; requires urgent intervention;

Grade 5: Death related to AE.

8.2 Serious Adverse Event

Serious adverse events (SAEs) are adverse events that meet at least one of the following criteria:

1. Results in death, excluding death related to progression of the disease under investigation.

2. Puts the subject's life at risk ("life-threatening" as defined by the fact that the event places the subject at risk of death at the time of the AE, not necessarily that death is a possible outcome if the condition worsens).

3. Results in permanent or significant disability/incapacity.

4. Other medically important events: defined as an event that endangers the subject or requires medical intervention to prevent any of the above outcomes from happening.

9 Data collection and management

All patient clinical testing reports in this project strictly adhere to the legally prescribed national unit of measurement. The patient testing and follow-up records must be complete, including the date, recorded items, results, and normal value ranges, among other information, and must be signed by relevant personnel. Clinical case data and follow-up data are recorded and collected by trained and professional data

collection and management personnel, and reviewed and confirmed for accuracy by the team leader before being entered into the database.

10 Ethical Requirements

This study follows the "Guidelines for Good Clinical Practice" and "Management Measures for Clinical Trials Initiated by Researchers in Medical Institutions" (trial) regulations, as well as the Helsinki Declaration. Before the trial can begin, the study protocol must be approved by the hospital ethics committee. If revisions to the protocol are necessary during the study, the updated version must be resubmitted to the ethics committee for review, and the researcher can proceed only with their approval.

Every patient who is enrolled in the study must sign an informed consent form. Copies of the informed consent form and contact information for the researcher and ethics committee must be provided to patients upon request. This study will collect clinical data and personal information for scientific research purposes, which may involve patient privacy. All participants and data analysts involved in the study have signed confidentiality agreements and will not disclose any patient information or disease-related information to any unrelated individuals or organizations. The collected patient data will be managed uniformly to prevent personal privacy from being leaked.

11 References

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