Efficacy and Safety of the Chinese Herbal Formula Hewei Jiangni Recipe for NERD With Cold-heat Complex Syndrome: Study Protocol for a Double-blinded Randomized Controlled Trial

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Efficacy and safety of the Chinese herbal formula Hewei Jiangni recipe for NERD with cold-heat complex syndrome: study protocol for a double-blinded randomized controlled trial

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Abstract

Background

Proton pump inhibitor (PPI) is effective for the treatment of non-erosive gastroesophageal reflux (NERD), but long-term use of PPI is prone to have complications and recurrence after withdrawal. Traditional Chinese medicine (TCM) can relieve the symptoms of reflux and improve the quality of life. The purpose of this study is to evaluate the safety and efficacy of Hewei Jiangni recipe (HWJNR) in the treatment of NERD with cold-heat complex syndrome, clarify the mechanism of HWJNR on NERD based on the correlation analysis of intestinal flora and metabolites.

Methods

This is a single-center, randomized controlled, double-blind, double-simulation clinical trial in which 72 eligible participants with NERD and TCM syndrome of intermingled heat and cold will be randomly allocated in the ratio of 1:1 to two groups: TCM group and western medicine group. The TCM group will receive HWJNR with Omeprazole Enteric-coated Tablets simulator, while the western medicine group will receive Omeprazole Enteric-coated Tablets with HWJNR simulator. Each group will be treated for 8 weeks. The primary outcome is the score of gastroesophageal reflux disease (GERD) health related quality of life questionnaire (GERD-Q). Secondary outcomes include SF-36 quality of Life scale, patient reported outcomes (PRO) self-rating scale score, syndrome score of TCM, and adverse events. Mechanistic outcome is the correlation analysis of intestinal flora and metabolites from healthy individuals and NERD participants before and after the treatment respectively.

Discussion

The goal of this trial is to investigate the efficacy and safety of HWJNR in the treatment of NERD with cold-heat complex syndrome, and to study the composition structure and metabolite expression profile of intestinal flora in patients with NERD through 16SrRNA sequencing and metabonomic correlation analysis of fecal flora, which makes us identify the dominant links of treatment and reveal the potential mechanism of HWJNR.

ChiCTR2000041225. Registered on 22 December 2020

http://www.chictr.org.cn/showproj.aspx?proj=66217

Keywords: Non-erosive gastroesophageal reflux disease, Randomized controlled trial, Hewei Jiangni recipe, Chinese herbal medicine, Study protocol

Administrative information

Note: the numbers in curly brackets in this protocol refer to SPIRIT checklist item numbers. The order of the items has been modified to group similar items (see http://www.equator-network.org/reporting-guidelines/spirit-2013-statement-defining-standard-protocol-items-for-clinical-trials/).

Title {1}	Efficacy and safety of the Chinese herbal formula Hewei Jiangni recipe for NERD with cold-heat complex syndrome: study protocol for a double-blinded randomized controlled trial
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Introduction

Background {6-7}

Non-erosive gastroesophageal reflux (NERD) refers to the reflux disease with typical of gastroesophageal reflux symptoms and no mucosal injury under endoscope, also known as endoscopic negative reflux disease[1]. The incidence of gastroesophageal reflux disease (GERD) is increasing yearly based on the epidemiological investigation[2], which has a great impact on the quality of life. The incidence of NERD accounts for about 70% of GERD[3]. Therefore, the effective management of NERD is critical to human health.

Proton pump inhibitor (PPI) is the first-line drug for the treatment of reflux disease[4]. However, it may lead to the deterioration of nocturnal symptoms and complication in some people[5][6][7]. The efficacy of PPI in the treatment of NERD is relatively rare compared with erosive esophagitis. Studies have shown that there is a tendency to relapse after drug withdrawal[8]. Due to repeated attacks and side effects of long-term acid suppression, NERD has increasingly become an important and thorny problem in digestive system research.

From the perspective of Traditional Chinese medicine (TCM), the stomach belongs to dryness and Yang soil, and majority of them belong to excess and heat, while the spleen belongs to dampness and Yin soil, and most of them belongs to deficiency and cold. With the changes of living standard and dietary structure in the crowd, more and more patients with NERD are characterized by mixed syndrome of intermingled heat and cold according to clinical observation and research of TCM syndrome. They not only suffer from acid reflux, heartburn, dry mouth, bitterness and other syndromes

of excess heat, but also syndrome of deficiency cold, such as aversion to cold and loose stools. The main features of cold-heat complex syndrome are as below: 1)burning discomfort behind the sternum or epigastric part; 2)acid regurgitation or vomiting clear water; 3)dull pain in the epigastrium, like warming and pressing. The general principle of TCM treatment is pungent dispersing and bitter descending, harmonizing stomach and descending adverse qi[9].

Hewei Jiangni recipe (HWJNR) was created by Professor Junxiang Li of Dongfang Hospital, Beijing University of Chinese Medicine on the basis of inheriting "Tongjiang theory" in the treatment of spleen and stomach diseases. Table1 lists the detailed formula. This prescription was based on the in-hospital preparation ("Hejiang capsule" approval number: Beijing Pharmaceutical Z20160002) of Dongfang Hospital to treat NERD patients with cold-heat complex syndrome specifically. Our research group conducted a randomized controlled study in 2002, which showed that HWJNR could reduce heartburn, acid regurgitation, and other clinical symptoms, and the recurrence after 12 weeks withdrawal was significantly less than that in western medicine group.

Table 1

Formula of HWJNR

Pinyin name	**Latin name**	**Family name**
Huangqin	Radix Scutellariae	Labiatae
Huanglian	Coptidis Rhizoma	Ranunculaceae
Qingbanxia	Pinelliae Rhizoma	Araceae

Ganjiang	Zingiber officinale Rosc.	Zingiberaceae
Zhebeimu	Fritillaria thunbergii Mip.	Liliaceae
Pugongying	Taraxacum mongolicum Hand.-Mazz.	Compositae
Longdancao	Gentianae Radix et Rhizoma	Gentianaceae
Zhishi	Citrus aurantium L.	Rutaceae
Quangualou	Trichosanthis Fructus	Cucurbitaceae
Zhigancao	Glycyrrhiza uralensis Fisch.	Leguminosae

Through the single-center, random, double-blind, double-simulation research, this trail aims to evaluate the efficacy and safety of HWJNR in patients with NERD (cold-heat complex syndrome). In addition, this trail intends to reveal the underlying pathogenesis by observing the effects of changes in intestinal bacteriocyte structure on metabolic ideotypes and functional metabolic small molecules.

Methods/design

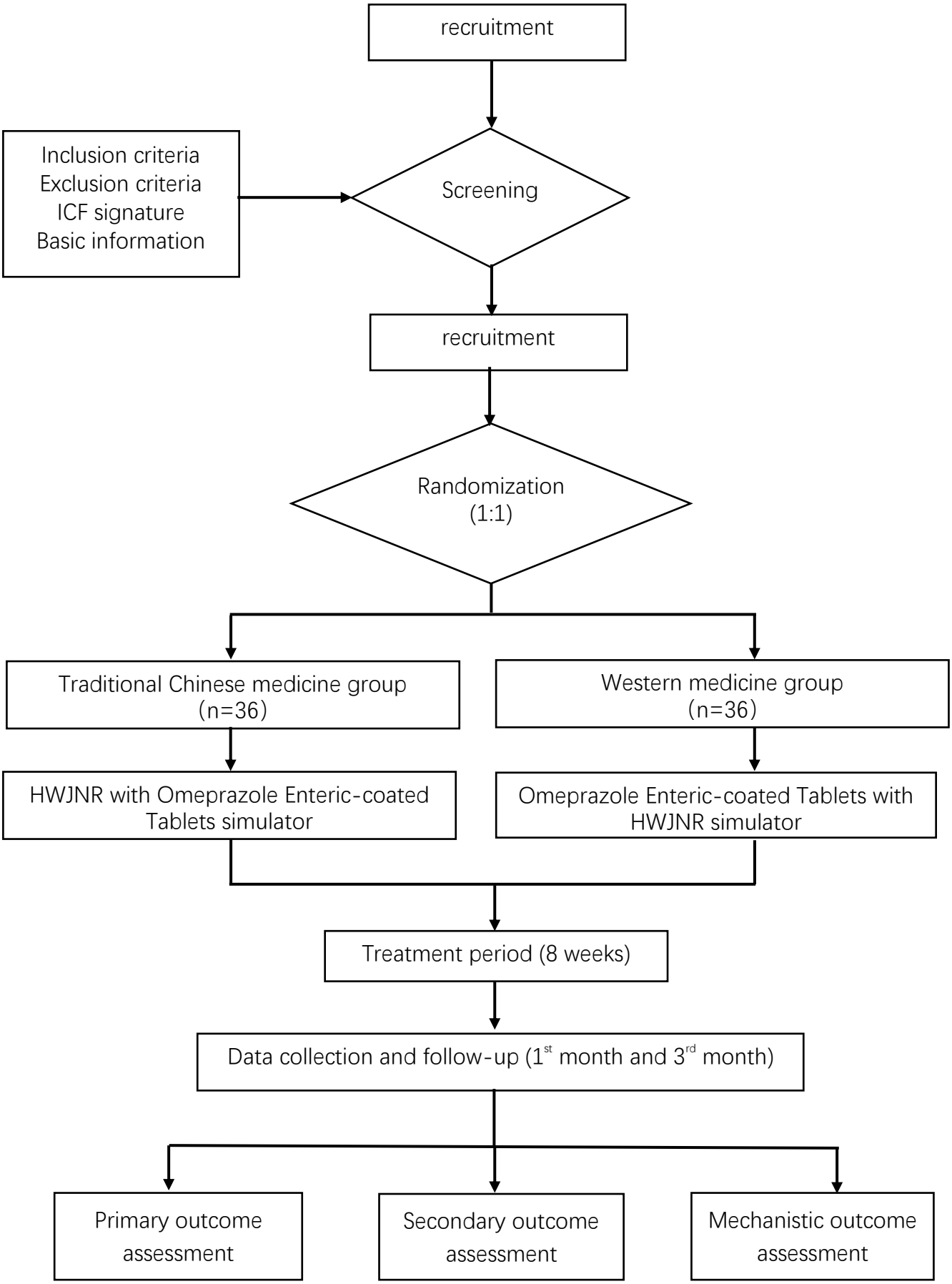
Study design and setting {8-9}

This is a single-center, double-blind, double-simulation, randomized controlled trial (RCT), which will be conducted in Dongfang Hospital, Beijing University of Chinese Medicine. The study is conducted in accordance with the Declaration of Helsinki (Edinburgh 2000 version). The final protocol (Version 2.0, 11 November 2020) of this trial has been approved by Ethical Committee of Dongfang Hospital, Beijing University of Chinese Medicine (Version number: JDF-IRB-2020033602). In addition, this trial has been registered in the Chinese Clinical Trial Registry (No. ChiCTR2000041225,

registered on 22 December 2020).

We aim to enroll 72 patients with NERD over a 2-year period (Date from November 2020 to December 2022) from the department of gastroenterology in Dongfang Hospital, Beijing University of Chinese Medicine. Eligible patients will be randomized at a ratio of 1:1 to either TCM group (HWJNR with Omeprazole Enteric-coated Tablets simulator) or western medicine group (Omeprazole Enteric-coated Tablets with HWJNR simulator) for 8 weeks treatment. At end of this trial, the primary outcome and the secondary outcome will be evaluated. We will obtain peripheral venous blood, urine and stool from NERD patients before and after treatment. This study involves 3 site visits (week 0, week 4, and week 8) and 10 call visits (weekly call during the treatment period and follow-up at month 1 and month 3). The process of the study is schematically shown in Figure 1. And we will additionally recruit 10 healthy controls.

Figure 1


recruitment
Inclusion criteria
Exclusion criteria
ICF signature
Basic information
Screening
recruitment
Randomization
(1:1)
Traditional Chinese medicine group
(n=36)
Western medicine group
(n=36)
HWJNR with Omeprazole Enteric-coated Tablets simulator
Omeprazole Enteric-coated Tablets with HWJNR simulator
Treatment period (8 weeks)
Data collection and follow-up (1st month and 3rd month)
Primary outcome assessment
Secondary outcome assessment
Mechanistic outcome assessment

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Participants

We aim to recruit patients with NERD who have either typical gastroesophageal reflux symptoms (such as acid reflux, heartburn, etc.) or no typical gastroesophageal reflux symptoms but with 24-hour impedance-pH monitoring suggested that they are related to gastroesophageal reflux. The symptoms above are not less than three months, and the gastroscopy show non-erosive gastroesophageal reflux. The inclusion criteria of TCM refers to the diagnosis of cold-heat complex syndrome in the "consensus on Integrated traditional Chinese and Western Medicine diagnosis and treatment of gastroesophageal reflux Disease" issued by the Digestive Professional Committee of the Chinese Society of Integrated traditional Chinese and Western Medicine in 2017 (Table 2). The Inclusion criteria of western medicine is based on the consensus of experts on gastroesophageal reflux disease issued in 2014 (Table 3). And we will also recruit 10 healthy subjects for control.

Table 2 Diagnostic criteria for cold-heat complex syndrome of NERD

Category	Symptoms or signs
Main symptom	1.Burning discomfort in the retrosternal or epigastric region. 2.Anti-acid or spitting clear water. 3.Dull pain in the epigastric stomach, like warming and pressing. 4.Fasting stomachache, relieved by eating.

Minor symptoms	1.Loss of appetite; 2.Fatigue; 3.Thin stools; 4.Lukewarm hands and feet.
Tongue	Red tongue, White fur
Pulse condition	Weak pulse
The main symptom 1 or 2 is necessary. Patients with two main symptoms+ either of the other one or two minor symptoms +appropriate tongue and pulse condition can be diagnosed as cold-heat complex syndrome	

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Table 3 Inclusion criteria of NERD

1. Those who have the typical clinical symptoms of NERD, such as heartburn, reflux, etc., persist or recur for more than three months, and the above symptoms have a negative impact on the quality of life of the patients. 2. Those who have atypical or extraesophageal symptoms, such as epigastric pain, abdominal distension, belching, non-cardiogenic chest pain, pharyngitis, cough, asthma or foreign body sensation in the throat, which persist or recur for more than three months, the above symptoms have a negative impact on the patient's quality of life: mild symptoms ≥ 2 days in a week, or moderate and severe symptoms ≥ 1 days in a week. 3. Reflux diagnostic questionnaire (RDQ) scale symptom score ≥ 12), which is for diagnosis of reflux diseases in the China gastroesophageal reflux Research

Cooperation Group.

4. Those who no esophageal mucosal lesions and Barrett esophageal manifestations by gastroscopy.

5. 24-hour esophageal impedance-PH monitoring: impedance records showed reflux events. At the same time, symptom association probability (SAP) is used to determine the relationship between symptoms and reflux. If SAP > 95%, symptoms will be considered related to reflux.

Diagnostic criteria: 1&3&4 or 2&4&5

Eligibility criteria {10}

Inclusion criteria for subjects

1.Patients meet the diagnostic criteria of NERD in guide and TCM with cold-heat complex syndrome at the same time.

2.18-65 years old.

3.Those who did not receive similar drugs (PPI, H2 receptor antagonist, gastric mucosal protective agent, etc.) within 2 weeks before the trial.

4.Voluntarily participated in this study and signed the informed consent form (ICF).

Exclusion criteria

1.Patients with reflux esophagitis, reflux hypersensitivity (RH) or Barrett esophagus(BE).

2.Patients with a disease history of upper gastrointestinal bleeding or surgery, esophageal stricture, esophageal and gastric tumors and other organic diseases.

3.Patients with serious primary diseases with cardiovascular, cerebrovascular, liver, kidney and hematopoietic system.

4.Taking other drugs or treatments related to NERD (PPI, H2 receptor antagonist, gastric mucosal protective agent, etc.) within 2 weeks.

5.Women who are breastfeeding, during pregnancy or preparing for pregnancy.

6.Patients with anxiety or depression.

7.Patients with a history of mental ill or neurological diseases or language disorders.

8.Allergic constitution or being allergic to the known ingredients of the drug in this study.

9.Currently participating in other clinical trials.

Inclusion criteria for healthy subjects

1.Those who have no typical clinical symptoms of NERD such as acid regurgitation, heartburn and reflux.

2. The score of GERD-Q scale < 8 points.

3. 18-65 years old.

4. Those who voluntarily participate in this study and sign the ICF.

Exclusion criteria for healthy subjects

1. Those who have been diagnosed as esophageal diseases such as NERD, RH and BE.

2. Patients with organic lesions such as upper gastrointestinal bleeding or operation, esophageal stricture, esophageal and gastric tumors.

3. Patients with serious primary diseases such as cardiovascular, cerebrovascular,

liver, kidney and hematopoietic system.

4. Women who are breastfeeding, pregnant or preparing for pregnancy.

5. Patients with anxiety or depression.

6. Those who with a history of mental or neurological diseases or mental or language disorders.

7. Those who have participated in other clinical trials within three months.

Sample size {14}

According to the statistical minimum requirements of the clinical efficacy evaluation of the two groups of samples, it is estimated that the sample size of each group is 30 cases, and the total sample size of the two groups is 60 cases. After the drop rate is calculated 20%, the total number of cases in this study is determined to be 72 cases. And we will additionally recruit 10 healthy controls.

Recruitment {15}

All the cases will be from outpatients of digestive department in Dongfang hospital, Beijing University of Chinese Medicine, who were clinically diagnosed as NERD, and dialectically belonged to the syndrome of intermingled heat and cold.

Allocation {16}

The drugs are allocated according to the random coding sequence of the research center and the number of cases allocated. We will designate a research drug administrator. The drug will be distributed by the research drug administrator according to random and blind code and registered in the "Clinical Research Drug use record form" after the researchers screen the qualified subjects and wrote the research medical

177 records with informed consent.

178 **Randomization and blinding {16.17}**

179 The research drugs will be randomly coded according to the randomized scheme of
180 clinical research in Xiyuan Hospital of the Academy of TCM. (1) the method of random
181 allocation: using SAS software to generate random sequences, randomly grouping, and
182 coding drugs; (2) the distribution scheme of hiding and implementing: the sealed
183 envelope method will be used to hide the random sequences.

184 Blindness will be set for the implementers (doctors) of the study and the subjects
185 (patients). The random code of the drug used in this study will be the unique
186 identification code of the subjects. Each coded drug will be accompanied by an
187 emergency letter for emergency treatment of blindness. In the event of an emergency
188 or when the patient needs to be rescued, it is necessary to know what kind of treatment
189 the patient is receiving. The corresponding emergency letter shall be opened in the
190 presence of at least two researchers, and the emergency letter shall be collected together
191 with the case report form (CRF) after the end of the trial. Blind leakage or emergency
192 letter opening should not exceed 20% before the end of the trial.

193 **Consent and assent {26a,27}**

194 People who volunteer to join the research group will sign an ICF, which is explained
195 to patients the purpose of the study and has informed the benefits and risks if participate
196 in the study. All the medical records will be kept in Dongfang hospital. It will be allowed
197 to access medical records of patients for researchers, research authorities and ethics
198 committees. The specimens collected in the test will be destroyed after use. Any public

report on the results of this study will not disclose the personal identity of the patient.

Interventions {11}

Intervention description {11a}

TCM group: HWJNR with Omeprazole Enteric-coated Tablets simulator; western medicine group: Omeprazole Enteric-coated Tablets with HWJNR simulator. The course of treatment is set for eight weeks. Medication method and dose: Omeprazole Enteric-coated Tablets/simulator: 20mg, once a day, half an hour before breakfast; HWJNR/simulator: twice a day, one bag at a time, half an hour after breakfast and dinner. Production of formula simulator: the TCM simulator is made by Beijing Kangrentang Pharmaceutical Co., Ltd., according to the preparation process of HWJNR. Both the shape, color, gas and taste will be similar to HWJNR after adding the same kind and equal amount of excipients. Finally, add 5% of the original unit treatment drug to correct color and taste.

Adverse reaction(AE) {11b}

The change or discontinuation of the intervention will be determined by the performance of the participants after taking the drug. The researcher will truthfully reflect the changes of the condition and any AEs during the study period. Our first priority is to protect the safety of the subjects. If the symptoms of gastroesophageal reflux such as reflux and heartburn have not been effectively relieved, Hydrotalcite Chewable Tablets (Daxi) will be used for temporary treatment, and the total dosage of Hydrotalcite Chewable Tablets in the two groups will be counted at the end of the trial.

Compliance{11c}

We will issue CRFs to all subjects and supervise patients to fill CRF every day to test compliance. Compliance with medication% = [actual dose/(specified daily dose × days)] × 100%. All patients will score and record their symptoms every day based on the CRF score content. Messages will be sent through WeChat or by phone to remind the patients of the follow-up visits. Results of physical examinations will be explained ateach visit. All the tests and drug fees during the trial will be covered.

Combined use of drugs {11d}

During the study, drugs with the same efficacy as the study drugs or related to the treatment of NERD will be prohibited, for the purpose of minimizing or avoiding the impact of combined use of drugs on the evaluation of the safety and effectiveness of the trial drugs. If the subjects take drugs to control chronic diseases, such as hypertension, diabetes, etc., they should truthfully and detailedly record the medication in the trial, including drug name (or other treatment name), dosage, frequency and time of use, etc., so that it will be analyzed and reported in summary.

Outcomes {12}

Primary outcomes measurements

GERD-Q scale score: GERD-Q scale is the most recognized and widely used special scale for GERD diagnosis in the world. In addition to diagnosing GERD, it can also evaluate the impact of GERD on quality of life and monitor the effect of treatment with high accuracy. Trained evaluator will record the evaluation at baseline, week 4 and week 8, fill out the GERD-Q questionnaire, and calculate the total score, frequency score and degree score of GERD-Q symptoms. In the study, the score of GERD-Q scale

after 8 weeks treatment will be used as the main outcome index.

Secondary outcomes measurements

(1) Quality of life score: According to the Chinese version of 36-item Short-Form (SF-36) health survey[10], the quality of life will be evaluated from 9 dimensions: physical function, physical function, physical pain, general health, vitality, social function, emotional function and mental health. All the indexes will be recorded at baseline, week 4 and week 8.

(2) Patient Reported Outcomes (PRO) self-rating scale score: There will be a diary card for patients to fill in every day. The researchers will calculate the PRO self-rating scale score according to the patient's diary card at baseline, week 4 and week 8.

(3) TCM clinical syndrome score: According to the diagnostic criteria of cold-heat complex syndrome， the main and minor symptoms, tongue and pulse will be graded and scored by using a unified table. All the indexes will be recorded at baseline, week 4 and week 8. According to the referred Guidance Principle of Clinical Study on New Drug of Traditional Chinese Medicine[11], all the symptoms will be divided into four grades: none, mild, moderate and severe, with 0, 2, 4 and 6 points in the main symptoms, and 0, 1, 2 and 3 points in the minor symptoms, while the tongue and pulse will be divided into normal and abnormal grades, with 0 and 2 points in the main disease, and 0 and 1 points in the secondary disease.

The clinical efficacy will be evaluated as follows:

a) Clinical recovery: the symptoms disappeared or syndrome score decreased by ≥ 95% from the baseline.

b) Marked efficacy: the syndrome score decreased by ≥70%, but <95% from the baseline.

c) Effective: the syndrome score decreased by ≥ 30%, but < 70% from the baseline.

d) Ineffective: the symptoms and signs were not significantly improved, or even aggravated, and the syndrome score decreased by < 30% from the baseline.

e) Aggravation: the syndrome score after treatment is higher than that before treatment.

TCM syndrome efficacy rate = (Clinical recovery + Marked efficacy + Efficacy) cases /a total number of cases × 100%.

(4) Mechanistic outcome: The samples will be used to study the composition structure and metabolite expression profile of intestinal flora in NERD patients through 16s rRNA sequencing and metabonomic association analysis in TCM group, western medicine group at baseline and week 8, and in normal subjects at baseline. By analyzing the correlation between a variety of differential flora and differential metabolites, we will demonstrate the effects of changes in the structure of intestinal flora on metabolic phenotypes and functional metabolic small molecules. This experiment attempts to screen some key strains through visual tools, further explore their mechanism in the pathogenesis of NERD, identify the advantages of treatment and reveal the potential mechanism of HWJNR. The determination of intestinal flora and metabolites will be performed by Shanghai OE Biotech Co., Ltd (Shanghai, China).

(5) Follow-up recurrence index: The number and percentage of the subjects who with non-medication, maintenance medication, intermittent medication and on-demand

medication in the two groups will be recorded at month 1 and month 3 respectively.

Safety outcomes

Participants will undergo laboratory tests at baseline and week 4, including liver and kidney function (ALT, AST, Scr, BUN), blood routine test, urine routine test, and stool routine test. Other tests include an electrocardiograph examination.

Participant timeline {13}

Table 4 Schedule of enrollment, treatment, and assessment

	Enrollment	Allocation	Treatment period		Follow-up period	
TIMEPOINT		0	4th week	8th week	1st month	3rd month
ENROLLMENT						
Eligibility screen	▲					
Chinese Medicine pattern	▲					
Informed consent		▲				
Randomization		▲				
INTERVENTIONS						
HWJNR with Omeprazole Enteric-coated Tablets simulator			⟷	⟷		
Omeprazole Enteric-coated Tablets with HWJNR simulator						

Assessments						
Examination tests	▲			▲		
Demographics	▲					
Vital signs	▲			▲		
Medical history		▲				
Chinese Medicine symptoms		▲	▲	▲		
GERD-Q		▲	▲	▲		
SF-36		▲	▲	▲		
PRO		▲	▲	▲		
Syndrome score of TCM		▲	▲	▲		
Mechanistic outcome		▲		△*		
Drug recall			▲	▲		
Patient's diary		▲	▲	▲		
Adverse event			▲	▲		
Drug combination		▲	▲	▲		
Recurrence index					▲	▲

*The mechanistic outcome will be analyzed in experimental group (TCM group and western medicine group) at the baseline and week 8, and in normal subjects at baseline.

Biological specimens {26b,33}

Collect human samples including blood, urine and feces. These samples will be used for blood routine test, urine routine test, stool routine test, liver and kidney function test.

The feces will additionally be used to detect the relationship between fecal flora and metabolites. All specimens will be destroyed after examinations.

Data collection and management {18,19}

Recording requirements of the CRF

(1) All records will be collected by trained and qualified investigators and screened by a qualified investigator. Researchers must fill in the research forms in a timely, accurate, complete and standardized manner in accordance with the relevant regulations and instructions. The main researchers will be responsible for the authenticity of the research data.

(2) The laboratory test report must be pasted on the "Research Medical record". The laboratory test data or description recorded in the CRF should be checked correctly with the original test report in the Research Medical record. Once the CRF is complete, the original record will not be changed, even if any modifications are made. The completed CRFs will be reviewed by a clinical inspector.

(3) The data of laboratory examination items outside the normal range should be confirmed by the researchers before and after the study, and those with clinical significance should be reexamined, and those with clinical significance found during or at the end of the study should be recorded and followed up according to adverse events.

(4) all data will be kept by a special person.

Selection of dataset

(1) Full Analysis Set (FAS): FAS refers to the intention-to-treat (ITT) analysis of all patients who have been randomized into groups and assigned random numbers (called

willing treatment groups). Errors that do not meet the inclusion criteria or exclusion criteria are excluded and are not included in ITT analysis.

(2) In line with Per-Protocol set (PPS): PPS refers to the statistical analysis of all cases that in accordance with the clinical observation plan, with good compliance (Compliance with medication ranging from 80% to 120% will be eligible for the protocol analysis set), not take prohibited drugs during the observation period, and completed the CRF requirements.

(3) Safety Set (SS): Safety data should include all subjects who have received at least one treatment and at least one safety assessment after randomization.

Statistical analysis {20}

All the analysis results will be generated by SPSS 25.0 software. Unilateral test will be used in all statistical tests, and the significance level $P \leq 0.05$. The quantitative index will be described by the basic statistical description method of numerical variable index, and the arithmetic mean, standard deviation, median, minimum and maximum will be calculated according to the data distribution. Counting data to calculate the number and percentage of cases under the corresponding classification.

Data monitoring {5d, 21, 23}

The data Monitoring Committee is not required because (1)the primary and secondary outcomes are only self-reported symptoms of the patient, and (2) the known risk is small: Omeprazole Enteric-coated Tablets are a first-line and a mature treatment for NERD patients; TCM is a conventional treatment in China, and HWJNR did not show potential toxicity in the previous pilot clinical observation of our research group.

So there will be no interim analyses, stopping rules, and auditing for the trial.

Adverse event reporting and harms {22,30}

An adverse event (AE) refers to any adverse medical event that occurs to the patient or clinical investigation object during the period of management of drugs, but it does not necessarily have a causal relationship with this treatment. According to the standards developed by the Adverse Drug reaction Supervision Center of the Ministry of Health, we will classify them according to the five-level classification of "affirmative, probable, probable, suspicious and impossible". The former four will be taken together to study the AEs of drugs, and the incidence of AEs will be calculated accordingly. (Table 5)

Table 5 Adverse Drug reaction Supervision Center of the Ministry of Health. Evaluation criteria for the relationship between AE and Research Drug use

Judgment index	Judgment result				
	Definitely	probably	probably	suspiciously	impossible
1. Whether there is a reasonable relationship between the time of starting medication and the time of suspicious occurrence.	+	+	+	+	+
2. Whether the	+	+	+	-	-

suspected ADR (ADR) conforms to the known ADR type of the drug					
3. Whether the suspected ADR can be explained by the patient's pathological condition, combined medication, therapy or previous therapy.	-	-	±	±	+
4. It is doubtful whether the withdrawal or reduced dose of ADR alleviates or disappears	+	+	±	±	-
5. Does the same reaction occur again after re-exposure to suspicious drugs	+	?	?	?	-

Description: + for positive-for negative ±for positive or negative? Indicates that the situation is

unknown.

Before the trial, no AE has been reported in the clinical application of HWJNR. The possible AEs of Omeprazole Enteric-coated Tablets include diarrhea, headache, nausea, abdominal pain, flatulence and constipation, and occasional increase of serum aminotransferase (ALT, AST), rash, dizziness and so on. These AEs are usually mild and can disappear automatically, which have nothing to do with the dose. Since the Omeprazole Enteric-coated Tablets used in this study belong to the routine treatment of NERD, these AEs may also occur even if the subjects do not participate in this clinical study. In the course of the study, we will closely monitor AE and take measures to prevent them. Once the damage related to the study occurs, the research assistant will record the detailed information including symptoms, signs, severity, start date, duration, lab results, intervention, and the results of adverse events at every visit. And the subjects will have the right to receive corresponding compensation.

Serious adverse reaction (SAE) refers to any adverse medical accident that occurs at any dose, including death, life-threatening conditions, the need for hospitalization or prolongation of existing hospitalization, persistent or significant disability/incapacity, or congenital anomaly/birth defect. Once severe adverse events (SAEs) occur, the event must be reported to the principal investigator, the Ethics Committee and the Beijing Food and Drug Administration within 24 hours. The principal investigator has the power to terminate the trial if necessary.

Discussion

NERD is characterized by the presence of typical gastroesophageal reflux disease

(GERD) symptoms associated with pathological acid reflux, including heartburn and regurgitation, but the absence of esophageal erosion[12][13]. NERD presents in approximately 70% of patients with GERD， which has a great impact on life quality [3]. The factors that affect NERD have not yet been clarified[14]. There are several hypotheses about the etiology of NERD, such as esophageal visceral hypersensitivity, sustained esophageal contractions, and abnormal tissue resistance, etc[15]. Recently, studies focus on abnormal microbial-brain-gut interaction and low-level inflammation of GERD mediated by hypoxia-inducible factor[16][17].

The gastrointestinal tract (GIT) is found to be the largest microecosystem in the human body. Although the intestinal tract is not directly connected to the esophagus anatomically, the intestinal flora is directly or indirectly related to GERD[18] . The function of GIT is co-dominated by the central nervous system (CNS), the autonomic nervous system, and the enteric nervous system (ENS). There is a two-way information exchange system of nerve conduction and endocrine hormone regulation between GIT and brain, which is called " gut-brain axis (GBA). Intestinal flora can participate in GBA through immunity, neuroendocrine, vagus nerve and short-chain fatty acids. It is directly or indirectly involved in the regulation of gastrointestinal motility, sensation and secretion, which is beneficial to the maintenance of gastrointestinal homeostasis[19][20][21]. Mast cells (MC) are important effectors of GBA that translate the stress signals into the release of a wide range of neurotransmitters and proinflammatory cytokines[22], which may profoundly affect the gastrointestinal physiology. And the "microbial brain-gut axis" plays a main role in the mechanism of GERD. The study of

chronic metabolic diseases through the study of intestinal flora has become one of the hotspots of microecological research. However, at present, most of the studies on GERD, especially NERD and intestinal microorganisms are still in the exploratory stage and need to be further studied.

Intestinal microbiome involved the host metabolic processes including production, transportation and metabolism of polysaccharides, lipids, amino acids, peptides, nucleotides, vitamins, bile acids and xenobiotics. Carbohydrates and dietary fiber in food residues are fermented by gut microbiota to produce short chain fatty acids(SCFAs), such as propionate, acetate, and butyrate, which play multifunctional roles on the gut microbiota and intestinal epithelial cells[23]. Kimura et al[24]. proved for the first time that the intake of dietary fiber increased the minimum resting pressure of LES and reduced the amount of acid, weak acid and total reflux of NERD from the perspective of SCFA, which indirectly proved that intestinal metabolites have a certain influence on NERD. Kennedy et al. [25]believe that butyrate may regulate visceral hypersensitivity due to the following reasons: (1) the decrease in visceral perception due to butyrate treatment could be due to direct modulation of 5-hydroxytryptamine (5-HT or serotonin) release, which can increase the compliance of the hollow viscera leading to decrease in perception, (2) activation of transient receptor potential vanilloid 1 (TRPV1) receptors in the colonic mucosa by butyrate which in turn may indirectly lead to 5-HT release in the gut to alter the perception, (3) overstimulation (i.e., high concentration of butyrate) or repetitive stimulation (i.e., multiple application) of TRPV1 receptors can cause rapid deactivation of the channel due to excessive influx of

Ca++, (4) butyrate can weaken visceral sensation by inhibiting histone deacetylase (HDAC).

In a word, we hope that this trial will provide evidence for the efficacy and safety of HWJNR with cold-heat complex syndrome. We also expect to have a deeper understanding of the dominant links of treatment, reveal the potential mechanism of HWJNR, and study the composition structure and metabolite expression profile of intestinal flora in patients with NERD.

Trial status

This updated version of the trial protocol (version 2.0, 11 November 2020) has been reviewed and approved by the Ethics Review Committee of Dongfang on 7 December 2020. All relevant researchers have completed the standardization training concerning this trial in December 2020. The first patient was recruited in the trial on 15 January 2021. By the time our manuscript was submitted, we had recruited 10 volunteers. Recruitment is scheduled to be completed in December 2022.

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Abbreviations

437	AE	Adverse reaction
438	BE	Barrett esophagus
439	CNS	Central Nervous System
440	CRF	Case Report Form
441	ENS	Enteric Nervous System
442	FAS	Full Analysis Set
443	GBA	Gut-Brain Axis
444	GERD	Gastroesophageal Reflux Disease
445	GERD-Q	Gastroesophageal Reflux Disease health related quality of life questionnaire
446	GIT	Gastrointestinal Tract
447	HDAC	Histone deacetylase
448	HWJNR	Hewei Jiangni recipe
449	ICF	Informed Consent Form
450	ITT	Intention-To-Treat
451	MC	Mast cell
452	NERD	Non-erosive gastroesophageal reflux
453	PPI	Proton pump inhibitor
454	PPS	Per-Protocol set
455	PRO	Patient Reported Outcomes
456	RDQ	Reflux Diagnostic Questionnaire
457	RH	Reflux hypersensitivity
458	SPIRIT	Standard Protocol Items: Recommendations for Interventional Trials

SAE	Serious adverse reaction
SAP	Symptom Association Probability
SCFA	Short chain fatty acids
SS	Safety Set
TCM	Traditional Chinese Medicine
TRPV	Transient receptor potential vanilloid
5-HT	5-hydroxytryptamine

Authors' contributions {5c, 31b}

The manuscript was jointly drafted by ZXS and CY. TX: key revision; LXH, LJX, XCE: data collection and design coordination; SL, SXJ, ZXC: participate in the design. All the authors read and approved the final manuscript.

Funding {4}

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Availability of data and materials {29}

Not applicable, there has not been available data and materials.

Ethics and dissemination {24,25,31a,31c}

This study has been approved by the Ethics Committee of Dongfang Hospital, Beijing University of Chinese Medicine (reference number JDF-IRB-2020033602). The revision of the agreement, the report of adverse events and the annual review will be supervised by the Ethics Committee of Oriental Hospital of Beijing University of

Traditional Chinese Medicine. ICFs will be obtained from all subjects.

If there is a change in the principal researcher or any change to the approval document in the course of the study, an application for amendment review will be submitted to the Ethics Committee. in the event of any circumstances that may significantly affect the conduct of the study or increase the risk of the subjects, a written application will be submitted to the Ethics Committee in a timely manner.

Findings from this study will be published in a peer-reviewed journal.

Consent for publication {32}

Consent to published de-identified information is included in the written informed consent process. However, no data is being published at this time.

Competing interests {28}

There are no competing interests in this work.

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Figures

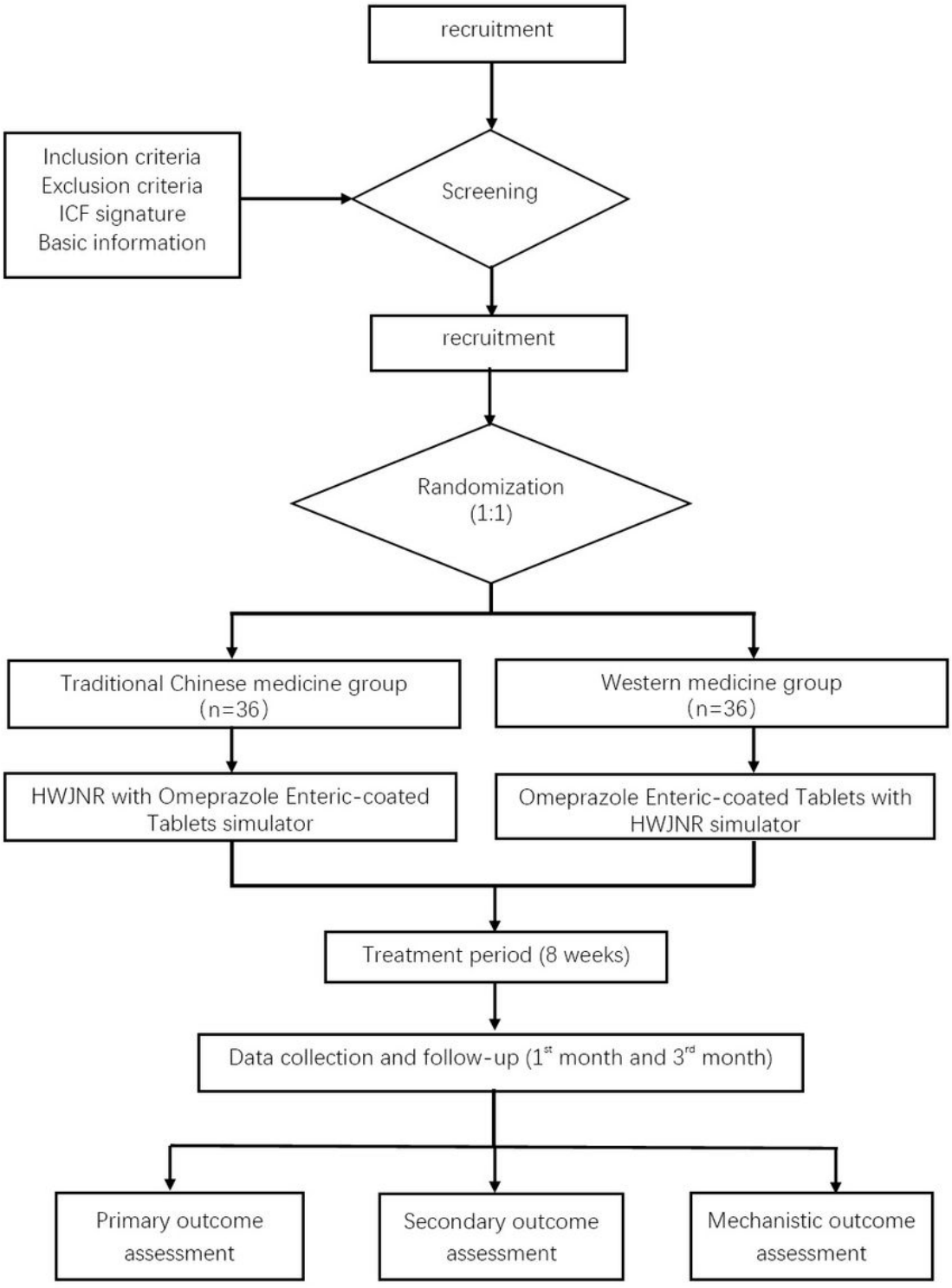

Figure 1

The process of the study design

Supplementary Files

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