A Real-world Clinical Study on the Treatment of Menopausal Syndrome With Liuwei Dihuang Pills

Study Protocol and Statistical Analysis Plan

2025.01.23

Name of test drug	Liuwei Dihuang Pills
Title	A Real-world Clinical Study on the Treatment of Menopausal Syndrome With Liuwei Dihuang Pills
Observational purpose	To explore the clinical efficacy and application value of Liuwei Dihuang Pills in treating menopause syndrome.
Research technique	Multicenter, large sample, prospective cohort study
Sample capacity	1000 menopausal patients were observed. 800 cases in the exposed group and 200 cases in the non-exposed group.
Study population	1. Age: 45-55 years old, Gender: female; 2. Syndrome of deficiency of kidney yin; 3. Patients with the climacteric syndrome.
Entry information	1. Treatment information: reasons for treatment, treatment departments, etc. 2. General information: gender, age, height, weight, body mass index (Body Mass Index, BMI), lifestyle (such as smoking, drinking, etc.), family history, past history, allergy history, basic diseases and their treatment, etc. 3. Disease related data: western medicine diagnosis, Chinese medicine diagnosis and syndrome differentiation classification, menstrual condition, disease course, medication information (course of treatment, dosage and medication), before and after the symptoms, signs, examination results (such as Kupperman score scale, sex hormones, perimenopausal survival quality scale (MENQOL), TCM syndrome scale, etc.), etc.
Observational indicators	1.General information: gender, age, course of disease, past history, etc 2.Safety measures (required before and after treatment) (1) General physical examination items: heart rate and blood pressure; (2) Laboratory examination items: Blood, Urine, Stool Routine; Fasting Blood Glucose (FPG), Fasting Insulin (FINS),Blood lipids[Total Cholesterol (TC), Triglycerides (TG), High-density Lipoprotein Cholesterol (HDL-C), Low-density Lipoprotein Cholesterol (LDL-C)],Liver function [Alanine Aminotransferase(ALT), Aspartate Aminotransferase(AST)], Renal function [Creatinine(Cr), Blood Urea Nitrogen(BUN)], Thyroid hormone indicators[serum free

	triiodothyronine (FT3), free thyroxine (FT4), thyroid stimulating hormone (TSH)],Electrocardiogram; Gynecological B ultrasound, etc.; (3) Record of adverse events and adverse reactions; 3. Efficacy Indices (Compulsory Pre-treatment and Post-treatment Items): (1) Primary Efficacy Indices: ① Change in Menopausal Symptoms: Modified Kupperman Scale Score; ② Serum Hormone Level Measurement: Serum Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH), Estradiol (E2); (2) Secondary Efficacy Indices: ① Menopausal-Specific Quality of Life Questionnaire; ② Changes in TCM Syndrome Scores; 4.Exploratory Analysis Metabolomics, Modified Proteomics, Micro-RNA, and Other Omics Sample Testing and Analysis.
Therapeutic regimen	In this study, it were divided into exposed and non-exposed groups. Patients in the non-exposed group were conventional treatment, and patients in the exposed group were conventional treatment. It is required to use drugs rationally and dialectically according to the instructions for at least 4 consecutive weeks. Without intervention in the treatment plan, concomitant medication is allowed, and the use of medication and concomitant medication shall be truthfully recorded, including dose, course of treatment, frequency, etc. Type of combined drugs and the corresponding daily dose, daily frequency and course of treatment.During the treatment, both groups had a follow-up assessment for 4 weeks.
Follow-up time	Patients were followed up and cohort analyzed before and 4 weeks after medication, and follow-up for patients with long medication (> 4 weeks) was extended to 3 months. Data were collected by the clinicians.Test patients for liver and kidney function, electrocardiogram, sex hormones and fill in the Kupperman score scale, perimenopausal quality of life scale (MENQOL) and other indicators. The registration observation form is recorded as the original record into the electronic data acquisition system (EDC) for data management. Set up the data verification plan in the study and edit the verification in real

	time.
Scheduling	Completed within 6 months of observation initiation.

1. Research background

1.1 Basis for project approval

Menopause syndrome is a series of symptoms of autonomic dysfunction due to a decline in sex hormones before and after menopause. Often accompanied by menstrual cycle disorders, hot flushes, night sweats, sleep disorders, mood disorders and other neuropsychological symptoms [1]. The core reason lies in the gradual decline of ovarian function, including natural menopause and artificial menopause. Due to the irreversible low-level estrogen state, postmenopausal aging lasts for at least 30 to 40 years, and the postmenopausal state in women occupies the ⅓ for even longer, during which the incidence of complications also significantly increases [2-4]. About 25 million women worldwide enter menopause every year, and 10 million women in China enter menopause every year. The United Nations World Health Organization estimates that by 2030, the population of menopausal women worldwide will reach more than 1.2 billion, and the number of menopausal women in China will exceed 210 million, accounting for about 1/7 of the total population, which needs to attract attention to public health [5].

The western medicine treatment of menopausal syndrome mainly focuses on psychological therapy and hormone therapy, but hormone supplementation will often lead to diseases of other organs, such as breast cancer, cervical cancer and other [6], which has certain limitations. Treatment of traditional Chinese medicine menopausal syndrome has thousands of years of history, menopausal syndrome in traditional Chinese medicine called "before and after the disease", belongs to the "lily disease" "dirty manic" category [7], the pathogenesis of the disease of the basic pathogenesis for menopause before and after female kidney qi deficiency, decyl day, kidney imbalance of Yin and Yang, is the key to the syndrome before and after the menopause. The imbalance of Yin and Yang in the kidney often involves other viscera, mainly in the

heart, liver and spleen. If the kidney Yin is insufficient, can not help heart fire, the heart fire is high; kidney Yin deficiency, kidney Yin, liver deficiency, liver flexibility, liver Yang hyperactivity; kidney and spleen days, kidney Yang warm, kidney deficiency Yang failure, fire is not warm soil, often lead to spleen kidney Yang deficiency, water dampness, phlegm turbidity, blood, blood stasis, depression, and complex pathological mechanisms and various clinical symptoms [8].

Liuwei Dihuang Pills were first recorded in the "Direct Formula of Children", which is a classic prescription for tonifying kidney, and was listed as the first batch of "Ancient Classic prescriptions" by the State Administration of Traditional Chinese Medicine. The prescription is composed of six traditional Chinese medicines, namely rehmannia, cornwood, peony skin, yam, poria cocos and diarrhea. It has the effect of nourishing Yin and tonifying kidney. It is suitable for kidney Yin loss, dizziness, tinnitus, acid and soft waist and knee, bone steaming and heat, night sweat and sperm and thirst elimination. Compound powder, nourishing Yin and tonifying kidney, tuckahoe removing dampness and invigorating the spleen, Chinese yam nourishing the spleen and kidney, nourishing the liver and kidney, and reducing the heat of the peony peel. It enhances immunity, reduces blood lipid, blood pressure, blood sugar, anti-fatigue, aging, low temperature, and resistance to hypoxia to improve renal function, plant nervous system function, and has a promoting effect on metabolism [9]. It can improve the estrogen level of menopausal women, promote the feedback control of the hypothalamic-pituitary-ovarian axis, improve the endocrine function and the internal environment of the body, and reduce the clinical symptoms [10]. Traditional Chinese medicine treatment of menopausal syndrome from syndrome differentiation has distinct characteristics and advantages. Clinical observation and research have proved that TCM treatment of menopausal syndrome has definite effect and good safety.

Menopause syndrome in addition to menstrual disorders, changeable mood and other main symptoms, some female patients will appear abnormal glucose and lipid metabolism [11,12]. In the perimenopausal stage female estrogen level decreased or fluctuation, resulting in female blood lipid level change [13], estradiol (E2) is an

important lipid metabolism substances, it can promote the decomposition of lipids, excretion, reduce plasma cholesterol, β-lipoprotein levels, make the serum phospholipid, α-lipoprotein content increased[14]. In addition, during the perimenopausal period, endogenous estrogen was decreased significantly, accompanied by weight gain, adipose tissue redistribution and metabolic levels. Studies showed that visceral adipose tissue quality and central obesity were significantly associated with insulin resistance, which led to an increased risk of T2DM in perimenopausal women[15]. To this end, the present study analyzed the clinical effect of the application and the effect of the level of glucose and lipid metabolism. Previous studies have shown that inflammatory response can reflect the degree of insulin resistance, and the heavier the inflammatory response, the more severe the insulin resistance[16]. IL-6, TNF-α and CRP are the commonly used inflammatory markers in clinical practice[17]. Immune function and endocrine dysfunction has obvious correlation between them, and to influence each other, menopausal women body abnormal hormone secretion, can lead to the cell activity is affected by different degree, at the same time if the endocrine balance between ovarian and pituitary disorders, can cause the immune system function, can cause the occurrence of various clinical symptoms of menopausal syndrome. Therefore, the immune function [18] can be observed in patients with menopausal syndrome.

Studies have shown that Liuwei Dihuang Pills combined with western medicine can treat hyperthyroidism kidney Yin deficiency in children, and regulate the corresponding serum free triiodithyronine (FT3), free thyroxine (FT4) and thyroid stimulating hormone (TSH) levels [19]. Sex hormone and thyroid hormone are jointly governed by the thalamic-pituitary axis. At the same time, thyroid hormone can improve the secretion of sex hormone, and sex hormone and thyroid hormone mutually influence[18]. Thyroid hormone plays an important auxiliary role in the regulation of sex hormones. Therefore, the abnormal secretion of thyroid gland makes the clinical symptoms of patients aggravate[20]. In addition, there are few clinical studies on thyroid hormone and glucose and lipid metabolism in perimenopausal women, so further research is needed.

The immune system is an indispensable defense system of the human body, and it plays a very important role in menopause. Studies have shown that the immune system of the human body is closely related to the neuroendocrine system. With the arrival of menopause, the neuroendocrine system of women is chaotic and its disease resistance gradually decreases. The ratio of CD4/CD8 in T lymphocyte subsets reflects the immune function of the human body. And the ratio of T cell subsets in the peripheral blood of healthy adults is between 1.5 and 2.0. With the decline of immune function of menopausal women, the composition, efficacy and transmission pathway of T cell subsets are change, mainly manifested as a drop of CD4 and CD8 T cells. As a pleiotropic cytokine produced by a variety of cells, IL-6 mainly participates in the inflammatory response and immune response of the human body through humoral immunity. In the normal immune state, most of them are in the "dormant" state. With the decline of the body's immune function, the serum content of IL-6 increases significantly to enhance the ability of foreign foreign body antigen[21].

1.2 Introduction of the study drugs

Name: Liuwei Dihuang Pills.

Ingredients: cooked rehmannia, wine wood meat, peony skin, yam, poria cocos, diarrhea.

Character: this product is brown to black brown honey Pills; sweet and sour taste.

Indications: nourishing the Yin and tonifying the kidney. For kidney Yin loss, dizziness and tinnitus, waist and knee acid and soft, bone steaming hot flashes, night sweat spermatorrhea.

Usage and dosage: Use drugs according to the instructions.

Specification: No limit.

Storage: seal.

1.3 Overview of human experience

98 patients with menopausal syndrome were randomly divided into treatment group (56 patients) and control group (42 patients). The treatment group took 240 mg each day per day, and the control group took 0.625 mg per day. Both groups were

treated continuously for 3 months. According to the menopause Kupperman symptom score scale, the treatment group hot flashes sweating score decreased significantly (P <0.05), the efficacy was better than that of the control group (P <0.05), and insomnia, emotional excitement and other symptoms also improved [22] to varying degrees. In addition, 120 patients with menopausal syndrome were randomly selected. According to the analysis of Kupperman score results, liuwei Dihuang Pills alone could significantly improve the symptoms of insomnia, anxiety and depression in menopausal patients (P<0.05), but the combined effect with combined estrogen tablets was better [23]. In addition, previous studies have shown that Liuwei Dihuang Pills and its formula combined with metformin have good efficacy in the treatment of diabetes mellitus[24]. Similarly, some studies have shown that Liuwei Dihuang Pills combined with low-dose estrogen progesterone replacement therapy has a certain impact on blood lipid therapy in patients with menopausal syndrome[25].

1.4 Preliminary foundation

This study investigated the effect of Liuwei Dihuang Pills (LWDHP) on menopause syndrome and clarified the underlying mechanism. In this study, using the method of network pharmacology, the KEGG pathway analysis of menopausal syndrome, screening the top 20 pathways, using inflammatory proteomics and menopausal syndrome Menendelian randomization analysis, obtained OR> 1 inflammatory protein, and query the genes regulating inflammatory protein through the NCBI website, screening the target genes of the inflammatory pathway and the inflammatory genes obtained in Mendelian analysis, to construct the corresponding PPI network map. Finally, it is concluded that LWDHP provides a new targeted treatment strategy for menopausal syndrome by regulating inflammatory-related targets and alleviating menopausal syndrome.

1.5 Support topics

This study belongs to the national Key Research and Development Program of Traditional Chinese Medicine modernization research "The establishment of clinical efficacy and safety evaluation of proprietary Chinese patent Medicine based on Systems Biology" (No.2022YFC3502004).

2. Research purpose

To evaluate the efficacy and safety of Liuwei Dihuang Pills in the treatment of menopausal syndrome and its effect on glucose and lipid metabolism.

3. Study design

3.1 Study type

The study type is a multicenter, large sample, prospective cohort study.

3.2 Study subjects

Women aged 45–55 years with menopausal syndrome from multiple centers nationwide were consecutively included in this study.

3.3 Diagnostic criteria

- Climacteric syndrome:

According to the standard [26-28] for the diagnosis of menopausal syndrome stipulated in Obstetrics and Gynecology (9th edition), Guiding Principles for Clinical Research of New Drugs of Traditional Chinese Medicine and Guidelines for the Diagnosis and Treatment of Integrated Chinese and Western Medicine for Menopausal Syndrome (2023 edition), Those who meet the following standards, Can be diagnosed with menopausal syndrome: ① Age 45-55 years old; ② Menstrual disorders for> 3 months or amenorrhea for 3 to 12 months; ③ Accompanied by hot and wet sweating, menstrual disorders, irritability, irritability, anxiety, insomnia, palpitations, chest tightness, headache, dizziness, pain in sexual intercourse, frequent urination, back and limb pain symptoms (hot and wet sweating necessary, Have 4 or more other symptoms); ④ Sex hormone index: serum follicle tin (FSH)> 10 IU / L, Or AMH <1.1μ g/L, It suggests that the ovarian reserve function is reduced; Sealing: FSH> 40 IU / L, And estradiol (E2)<73.4pmol/L (20 pg/mL), The ovarian failure was indicated.

Menopause stage criteria: (2011 reproductive aging stage + 10 system menstrual cycle length as the standard).

1. The length of the menstrual cycle varies (i. e., menstrual disorder), and the 10 menstrual cycles have 2 or more changes in the adjacent menstrual cycle for 7 days, that is, the early transition of menopause.

2. The menstrual cycle is 60 days, and FSH> 25 IU / L, or late menopausal transition.

3. One year after menstruation, that is, early menopause

These three periods can be called menopause

- TCM diagnosis of menopausal syndrome:

It meets the clinical diagnostic standard [27,29] of before and after menopause in Gynecology of Traditional Chinese Medicine and Guiding Principles of Clinical Research of New Chinese Medicine, and is kidney Yin deficiency. Main syndrome: temporary hot flashes, accompanied by sweating; secondary syndrome: dizziness, tinnitus, irritability, dry vagina, dry mouth and constipation; tongue pulse: red tongue, dry moss, fine pulse count.

One or more of the above items can be diagnosed.

3.4 Inclusion criteria

(1) Age: 45-55 years old (including both ends), female;

(2) Meet the western medicine diagnostic criteria for menopausal syndrome;

(3) Meet the TCM syndrome differentiation standard of kidney and Yin deficiency syndrome;

(4) Informed consent, and the voluntary signing of the informed consent form.

3.5 Exclusion criteria

(1) Those allergic to the ingredients and excipients;

(2) Patients with other endocrine diseases;

(3) The presence of the reproductive system organic lesions;

(4) Patients with bilateral oophorectomy, hysterectomy, and ovarian dysfunction;

(5) Patients with endometrial hyperplasia, unexplained vaginal bleeding;

(6) Patients with malignant tumors, hematopoietic system and immune system diseases;

(7) Serious diseases of cardiovascular and cerebrovascular diseases, liver and kidney organs (serum transaminase level is more than 1.5 times higher than the upper limit of normal value, and serum creatinine is higher than the upper limit of normal value);

(8) Psychiatric illness and alcohol or drug dependence;

(9) with mental illness or communication disorder;

(10) According to the judgment of the investigator, other lesions that reduce the possibility of enrollment or complicate the enrollment are not suitable to participate in this clinical study;

3.6 Test discontinuation criteria

(1) With serious adverse events or serious adverse reactions;

(2) Request withdrawal from the investigator;

(3) Patients with serious complications or other serious diseases who occurred during the study.

3.7 Discontinue the handling of the cases

Record the reason for the discontinuation, investigate the treatment history, record the time of the last treatment and the evaluation index at the discontinuation. Analyze the possible impact of discontinued cases on the study conclusions, complete and complete the observation form.

3.8 Treatment protocol

In this study, it were divided into exposed and non-exposed groups. Patients in the non-exposed group were conventional treatment, and patients in the exposed group were conventional treatment + Liuwei Dihuang Pills. It is required to use drugs rationally and dialectically according to the instructions for at least 4 consecutive weeks. Without any intervention in the treatment plan, concomitant medication is allowed, and the use of medication and concomitant medication shall be truthfully recorded, including dose, course of treatment, frequency, etc. Type of combined drugs and the corresponding daily dose, daily frequency and course of treatment. During the treatment, both groups underwent a follow-up assessment for 4 weeks. Study termination follow-up conditions include patient death, loss to follow-up, or reaching the study endpoint.

3.9 General Information:

(1) Treatment information: reasons for treatment, treatment departments, etc.

(2) Basic information: gender, age, height, weight, occupation; family history, past medical history, allergy history, menstrual history, basic diseases and their treatment, etc.

(3) Disease-related data: diagnostic information, including western medicine

diagnosis, TCM diagnosis and syndrome differentiation; onset and duration of onset, diagnostic examination results, description and symptoms and signs before medication, etc. Medication information, including course of treatment, dose, combined medication (description of frequency of hormone combination, frequency of nourishing Yin and kidney drugs, frequency of other combined drugs);

3.10 Outcome indicators:

Comparative analysis of clinical characteristics of patients before and after medication, including:

（1） Efficacy indicators:

1) scale:

- Western medicine scale: Kupperman score scale [33], perimenopausal quality of life scale [34];
- Chinese medicine scale: Chinese medicine syndrome score table.

2) Inspection indicators:

- Sex hormones: serum folliculogectin (FSH), luteinizing hormone (LH), estradiol (E2);
- Glucose: fasting blood glucose (FPG), fasting insulin (FINS),Glycated Albumin (GA);
- Blood lipids: total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C);
- Thyroid hormone indicators: serum free triiodothyronine (FT3), free thyroxine (FT4), thyroid stimulating hormone (TSH).

(2) Safety indicators:

① Safety indicators adverse events / reactions clinical manifestations (symptoms and signs);

② Vital signs: blood pressure, respiration, heart rate and body temperature;

③ Blood, urine routine, stool occult blood;

④Liver and kidney function: glutamate aminotransferase, glutamate transaminase, creatinine, urea nitrogen;

And a ⑤ 12-lead ECG.

(3) Exploratory indicators:

Biosample collection, biomarker detection and analysis.Thirty blood samples were collected from patients in each group before and after treatment for pre-treatment and post-treatment testing. Metabolomics, modified proteomics, Micro-RNA, and other omics sample testing and analysis were performed to assess treatment efficacy.

3.11 Case Screening and Sample Size

In this study, 1,000 patients were collected for the menopausal syndrome. There were 800 patients in the exposed group and 200 patients in the non-exposed group (including 100 patients with blood collection; 900 patients without blood collection).

3.12 Relevant information collected for each follow-up time point

Patients were followed up and cohort analyzed before and 4 weeks after medication, and follow-up for patients with long medication (> 4 weeks) was extended to 3 months. Data were collected by the clinicians. The liver and kidney function, electrocardiogram, sex hormones, glucose and lipid metabolism were tested and the Kupperman score scale, perimenopausal period quality of life scale and other indicators were filled in. The registration observation form is recorded as the original record into the electronic data acquisition system (EDC) for data management. Set up the data verification plan in the study and edit the verification in real time.

- Specific follow-up steps

1) Visit 1 (V1) —— Screening Period / Enrollment

Before enrollment

(1) Sign a written informed consent form;

(2) Obof demographic data;

(3) Medical history, treatment history, surgery history, personal history, allergy history, menstrual history;

(4) Measurement of vital signs;

(5) Complete the Kupperman score scale and perimenopausal quality of survival scale (MENQOL);

(6) TCM syndrome differentiation;

(7) Fill in the TCM certificate score;

(8) Investigate the situation of menopause;

(9) Laboratory examination:

- Routine examination: blood routine, urine routine, liver and kidney function;
- Sex hormones: serum folliculogectin (FSH), luteinizing hormone (LH), estradiol (E2);
- Blood glucose: fasting blood glucose (FPG), fasting insulin (FINS),Glycated Albumin (GA);
- Blood lipids: total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C);
- Thyroid hormone indicators: serum free triiodothyronine (FT3), free thyroxine (FT4), thyroid stimulating hormone (TSH).
- RNA test: serum;

(10) A 12-lead ECG;

(11) Abdominal ultrasound, pelvic cavity (gynecological) ultrasound, breast ultrasound, thyroid ultrasound;

(12) Check the inclusion / exclusion criteria;

(13) Issue the patient's electronic questionnaire (questionnaire star).

2) Visit 2 (V2)——Treatment Period

The first 15 ± 2 days after enrollment.

(1) Fill in the symptom Kupperman score scale and perimenopausal quality of life scale (MENQOL);

(2) Fill in the TCM certificate score form;

(3) Investigate the situation of menopausal syndrome;

(4) Record the concomitant medication;

(5) Record the adverse events;

(6) Recovery the subject's medication diary card, used drug packaging and unused drug packaging

Drug, and record;

(7) Recovery and distribution of patient questionnaires;

(8) Issuance of medication and subject medication diary cards.

3) Visit 3— medication completion / follow-up period

Day 30 of medication after enrollment.

(1) Measure the vital signs;

(2) Recovery the patient questionnaire;

(3) Complete the Kupperman score scale and perimenopausal quality of survival

scale (MENQOL);

(4) Fill in the TCM certificate score form;

(5) To investigate the situation of menopausal syndrome;

(6) Record the concomitant medication;

(7) Record the adverse events;

(8) Laboratory test of blood routine, urine routine, liver and kidney function;

- Routine examination: blood routine, urine routine, liver and kidney function;
- Sex hormones: serum folliculogectin (FSH), luteinizing hormone (LH), estradiol (E2);
- Glucose: fasting blood glucose (FPG), fasting insulin (FINS);
- Blood lipids: total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C);
- Thyroid hormone indicators: serum free triiodothyronine (FT3), free thyroxine (FT4), thyroid stimulating hormone (TSH);
- RNA test: serum;

(9) Abdominal ultrasound, pelvic cavity (gynecological) ultrasound, breast ultrasound, thyroid ultrasound;

(10) Fill in the study completion summary form.

- Unscheduled visit / early exit visit

If the subject has an AE during the study or requires additional visit / examinations, record in the unscheduled visit; if the subject withdraws from the trial early, the investigator should conduct safety assessment and effectiveness assessment whenever possible, record in the early exit visit and complete a summary of trial completion.

4. Analysis method

4.1 Hierarchical analysis

(1) Different medication duration and daily dosage (high and low dose), presence of age, menstruation, course of disease and whether the concomitant medication and disease as stratification factors;

(2) Explore the correlation between hormone indicators of menopausal syndrome and blood glucose and lipid indicators;

(3) After taking Liuwei Dihuang Pills, blood lipid index in patients with

abnormal glucose metabolism; blood glucose index in patients with menopausal syndrome with abnormal dyslipidemia; and blood glucose in patients with menopausal syndrome with abnormal glucose and lipid metabolism.

4.2 Analysis of the influencing factors of disease recovery

According to the results of the stratified analysis and combined with the medical opinions, the independent variables were determined, and the effect index was taken as the dependent variable, and the Logistic stepwise regression model was established to analyze the influencing factors of disease recovery.

4.3 Tendency matching analysis

Considering that the baseline imbalance between the indicated populations may affect the analysis results, so the propensity matching method was adopted for matching, and factors such as whether to use drugs were taken as dependent variables, and clinical characteristics were used as independent variables to estimate the tendency score.

6. Statistical methods

The general demographic characteristics of the study subjects at baseline were first descriptive and group described. For continuous variables (such as age) with normal distribution and mean standard deviation, the differences between groups were compared by t-test or analysis of variance (analysisofvariance, ANOVA); without normal distribution, the median (first quartile, third quartile) was used, and the differences between groups were compared by Wilcoxon rank sum test or Kruskal-Wallis rank sum test. Categorical variables were expressed using frequency and percentage (n,%), and differences between groups were compared using the Pearson x2 test. Linear regression model was used for continuity variables and Mantel-Haenszel x2 test for multi-group variables.

7. Quality control

Standardized training. Information collection should be carried out when the environment is quiet and clean, the air is fresh, natural light or artificial lighting, and the patients are emotionally stable and relaxed. After signing the informed consent,

the basic information should be collected. Follow the principle of objectivity and standard to collect data and fill in scales to ensure the authenticity and reliability of information. Be familiar with the specific meanings of all scales and representative items in this study, so as to reduce the impact of subjective factors on the trial. After the collection, the information should be checked again to avoid missing filling and misfilling. The physician with the title of chief physician should judge the information again to ensure the data quality.

8. Ethics issues

This study adhered with the Declaration of Helsinki and protected the treatment information of all subjects. This study is a specific study based on the database of the registration study of menopausal syndrome cases, through the national clinical research ethics approval.

9. Participants gave their informed consent

The investigator and the patient signed the informed consent form and gave copies to the patient.

10. Medical treatment and protection of the subjects

In the study, patient privacy as well as personal information will be strictly protected.

11. Definition and collection method of relevant indicator variables

11.1according to the modified Kupperman scoring scale [33]

Scoring method: symptom degree * symptom index

Symptom index: hot flashes and sweating 4 points, abnormal sensation (cold, hot, numbness, pain, etc.), insomnia, irritability, sex pain, urinary tract symptoms 2 points, depression, dizziness, fatigue, joint muscles, limb pain, headache, etc. 1 points. Degree of symptoms: asymptomatic 0 points, occasional symptoms 1 point, symptoms last 2 points, affecting life 3 points. Evaluation of the severity of the disease: mild total score was 15-20, moderate total score was 21-35, and severe total

score was greater than 35.

Modified Kupperman rating scale

Modified Kupperman rating scale						
surname and personal name: age: contact number:						
symptom	base score	0 Points	1 Points	Two points	Three points	grade
Hot and sweaty	four	not have	<3 Times / day	3-9 times / day	Vacation 10 times / sky	
abnormal sensation	two	not have	With the weather close	Usually there is cold Hot pain numbness feel	Hot and cold pain lose	
lose sleep	two	not have	once in a while	Often, Ann Sleep pills are effective	Affect the work life	
mood swing	two	not have	once in a while	Often, no Self-consciousness	Know, no Can self-control	
Depression, suspicion heart	one	not have	once in a while	Often, can automatic control	Lost life confidence	
circumgyration	one	not have	once in a while	Often, no Influence life	Influence daily life	
Life is tired	one	not have	once in a while	On the fourth floor sleepy difficult	daily life Restricted	
Osteoarthralgia	one	not have	once in a while	Often, no Influence function	dysfunction	
headache	one	not	once in a	Often, can	Need to take	

		have	while	bear	medicine	
cardiopalmus	one	not have	once in a while	Often, no influence	Need treatment	
formication	one	not have	once in a while	Often, can bear	Need treatment	
sexual life	two	not have	Under the normal sex desire fall	Sexual life is difficult difficult	sexual anesthesia	
Urinary infection	two	not have	once in a while	> 3 times / year, can from heal	> 3 times / year, required medicine	

11.2 Perimenopausal Quality of survival scale (MENQOL) [34]

Perimenopausal Quality of Survival Scale (MENQOL)

Perimenopausal Quality of Survival Scale (MENQOL)							
During the last month, did you have the symptoms in the following questionnaire? Please choose one of the levels, "0", means no impact at all, "6" means extreme impact, the closer to 0, the less affected, the closer to 6, the more affected. Please select a best situation for you in 0-6, and outline "(".							
symptom	The degree of symptoms						
	0 Points	1 Points	Two points	Three points	4 Points	5 Points	Six points
1. Heat (burst of heat)	☐	□	□	□	□	□	□
2. Night sweats (sweating after falling asleep at night)	□	□	□	□	□	□	□
3. Sweat comes out during the day	□	□	□	□	□	□	□
Not satisfied with their own life	□	□	□	□	□	□	□
5. Feel anxious or nervous	□	□	□	□	□	□	□
Memory loss	□	□	□	□	□	□	□
Do things are not as handy as before	□	□	□	□	□	□	□
Feel depressed, depressed or depressed	□	□	□	□	□	□	□
Lack of patience with others	□	□	□	□	□	□	□
Always want to stay there alone	□	□	□	□	□	□	□
11, gastrointestinal flatulence or distension pain	□	□	□	□	□	□	□
12, muscle and joint pain	□	□	□	□	□	□	□
Feel tired or exhausted	□	□	□	□	□	□	□
Sleep problems	□	□	□	□	□	□	□
15. Cervical pain or headache	□	□	□	□	□	□	□
Physical strength decline	□	□	□	□	□	□	□

Poor appearance and spirit	□	□	□	□	□	□	□
Feel a lack of energy	□	□	□	□	□	□	□
Tired and dry	□	□	□	□	□	□	□
20, and the weight gain	□	□	□	□	□	□	□
Face hair increased	□	□	□	□	□	□	□
Appearance, skin quality changes	□	□	□	□	□	□	□
23. The joints feel swollen and uncomfortable	□	□	□	□	□	□	□
Low back pain	□	□	□	□	□	□	□
Urinary frequency	□	□	□	□	□	□	□
26. Urinated control when laughing or coughing	□	□	□	□	□	□	□
Sexual desire changes	□	□	□	□	□	□	□
Dry vagina during sex	□	□	□	□	□	□	□
29. Avoidative sexual behavior	□	□	□	□	□	□	□

11.3 The scoring standard of TCM syndrome is formulated with reference to the relevant contents in the Guiding Principles for Clinical Research of New Chinese Medicine and the Standard for Diagnosis and Efficacy of TCM Certificate (the fourth edition).

Traditional Chinese medicine syndrome score table

Symptoms / Score	No (0)	Light (1)	Medium (2)	Heavy (3)
Heat sweat out	not have	Occasionally the head is hot sweat	Hot flashes in the back and chest	Body and hot flashes, sweat out of the water
lose sleep	not have	4-5 Hours	2-3 Hours	pernoctation
Menstrual disorders or amenorrhea	not have	Accidental	often	repeatedly
dysphoria in chestpalms-soles	not have	Accidental	often	repeatedly
soreness and weakness of waist and knees	not have	Sometimes it hurts	Change position is required	Persistent pain, medication
Dry mouth and dry throat	not have	light	Drinking water to relieve	Drink and don't understand
dizzy	not have	Accidental	often	repeatedly
tinnitus	not have	Accidental	often	repeatedly
Panpulsive palpitations	not have	mild	moderate	severe
Urine little color yellow	not have	once in a while	often	repeatedly
Both eyes dry	not have	once in a while	often	repeatedly

The skin is dry and itchy	not have	Accidental	often	repeatedly
The pussy is dry	not have	mild	moderate	severe
constipation	not have	once in a while	often	repeatedly

一、Blood samples were collected for the SOP

1 Blood collection package

1.1 Blood vessel collection

（1）2 common blood vessels (hospital equipped, red trunk tube, without anticoagulant) Use 1 dose before medication and 1 dose after medication;

（2）14 EP tubes (issued by the general research group) 7 before and 7 after medication;

1.2 4 disposable straws (equipped by the general research group);

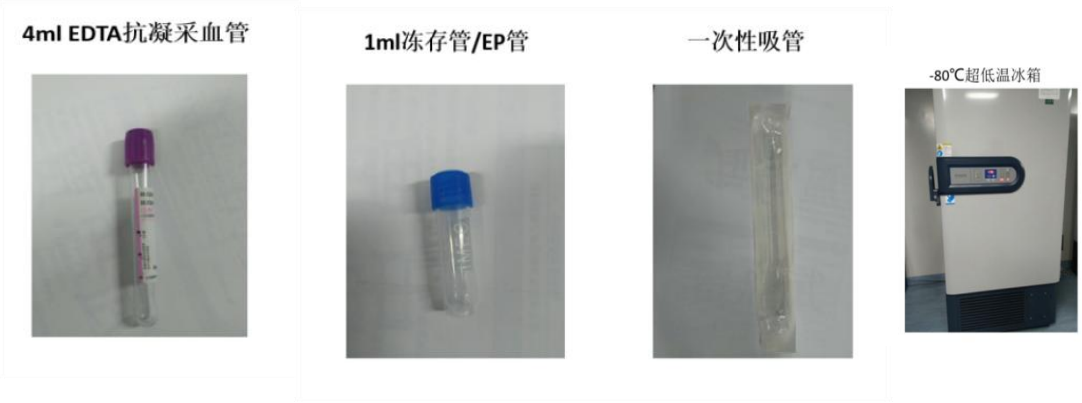
1.3 Frozen storage box (equipped by the general research group);

10 translucent frozen storage boxes (frozen storage EP tube, 81 holes);

1.4 Blood sample Collection and FreeStorage Registration Card

1.5 marker for marking patient number on each wall (avoid label);

1.6 Low-temperature centrifuge (4℃, 4000rpm), refrigerated refrigerator (4℃), -80℃ ultra-cold refrigerator.



2 Blood sampling process

2.1 Preparation for patient blood collection

5ml of median elbow venous blood samples were collected at 2-4 days of menstruation and fasting for over 8 hours; for rare menstruation and amenorrhea for> 2 months, urine pregnancy test was negative and color ultrasound examination: no bilateral ovarian follicles> 10mm, endometrial thickness of <5mm, 5 mL of median elbow venous blood samples were collected during the fasting state for over 8 hours; 5ml of median elbow venous blood samples were collected during fasting for over 8

hours.

2.2 Special personnel shall be responsible for preparing for blood collection

The department shall designate a special person to guide the subject to the subject to the special blood collection department, and the special person shall be responsible for blood collection. The blood collection person shall check whether the blood collection consumables labels are consistent with the patient, and sign in the column of "Blood Collection and Frozen Storage Registration Card", "Blood Collection", "whether", "Blood collection time" and "Blood collection amount". If there is any abnormality in blood sample collection, it should be explained in the remarks column (such as blood collection delay, hemolysis, etc.).

2.3 Collect venous blood

(1) 4ml of each common blood collection vessel (special label with patient number);

Note: gentle gently to avoid physical hemolysis; blood collection should be sufficient. After the blood sample is collected, the designated person shall process and preserve the blood sample in time.

3 Blood samples

3.1Ordinary blood vessels

Blood sampling was used with 1 branch before medication.

3.2 EP vessels

After blood collection, the designated specimens in 4℃ refrigerator for 1 hour, serum fully precipitated, low temperature (4℃) for 10 minutes (4000rpm), serum into EP tube, packaging specifications: 200 micro liters / tube * 5 tubes, 0.5ml / tube * 2 tubes, a total of 14 tubes, paste a special label with patient number, before and after medication.

3.3 RNA Blood Collection Tube

Attach a label with the patient’s ID. Collect 2.5 mL of blood, then invert the tube gently several times to mix the additive with blood.

The above operation requires special personnel to cooperate with the blood

collector, so as not to expose the blood sample for too long at room temperature.

4 Blood samples frozen storage

All samples shall be stored in the corresponding frozen storage box after completion and shall be stored in the-80℃ refrigerator immediately. If it cannot be placed in the-80℃ refrigerator immediately, it should be temporarily stored in the 4℃ refrigerator for no more than 30 minutes, and then placed in the-80℃ refrigerator before and after medication.

5 Precautions

5.1 Do not shake or tilt the blood vessel after centrifugation.

5.2 Always tighten the EP pipe cap to prevent leakage.

5.3 Strictly prevent repeated freezing and thawing.

5.4 The distributor shall sign in the column of "distributor" and "Assembly" of Blood sample Collection and Frozen Storage Card.

5.5 Frozen holder sign in the column of "Frozen holder", "Frozen holder", "Frozen time", "refrigerator No." and "Frozen layer".

6 Transportation of the blood samples

All kinds of frozen samples are regularly transferred (every 3 months) to the cold chain transportation company for transfer and recovery to the biological sample bank of the Institute of Clinical Traditional Chinese Medicine of China Academy of Chinese Medical Sciences, and the unified testing unit is the Medical Experimental Center of China Academy of Chinese Medical Sciences. The cold chain transportation company is Beijing Yingyu Logistics Co., LTD. Before transportation, the Institute of Clinical Basic Medicine of Traditional Chinese Medicine of China Academy of Chinese Medicine has a special person to communicate with the contact person of the clinical hospital about the time and handover procedures.

6.1 Before transportation

The head of biological samples of the general research group shall regularly coordinate with the project leader of the cold chain transportation company, the head of biological samples of the sub-center and the head of the biological sample bank to confirm the number of biological samples to be transferred, the number of incubators

required for transportation and the transportation time. Confirm the dry ice quantity and the thermometer quality with the cold chain transportation project leader. Ensure the sample safety during transportation.

6.2 After transportation

After the transportation, ask from the project leader of the cold chain transportation company for the temperature record table;

The person in charge of the biological samples of the sub-center shall report the biological samples of the sub-center regularly. Fill in the Blood sample Delivery Form and Statistical Form and transport them with the box. And send the electronic version to the research group email: wlxing@126.com.

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