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Complete List of Authors:	Lu, Renhua; Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital, Department of Nephrology Gu, Le-yi; Shanghai Jiao Tong University, Department of Nephrology; Shanghai Jiao Tong University, Department of Nephrology Zhang, Weiming; Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital, Department of Nephrology Guo, Yongping; Shanghai Sixth Peoples Hospital, Department of Nephrology Zang, Xiujuan; Shanghai Songjiang District Central Hospital, Department of Nephrology Zhou, Yan; Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital, Department of Radiology Yu, Ling; Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital, Department of Neurology Pan, Shuting; Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital, Clinical Center for Investigation Pang, Huihua; Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital, Department of Nephrology Liu, Shang; Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital, Department of Nephrology Xie, Kewei; Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital, Department of Nephrology Li, Ping; Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital, Department of Nephrology Zeng, Xiaojun; Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital, Department of Nephrology Lu, Yifei; Shanghai University of Traditional Chinese Medicine, Department of pharmacy
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Thiamine and folic acid in the treatment of cognitive impairment in maintenance hemodialysis patients: A prospective, randomized, placebo-controlled, double-blind, multi-center study

Renhua Lu[1], Leyi Gu[1,2*], Weiming Zhang[1], Yongping Guo[3], Xiujuan Zang[4], Yan Zhou[5], Ling Yu[6], Shuting Pan[2], Huihua Pang[1], Shang Liu[1], Kewei Xie[1], Ping Li[1], Xiaojun Zeng[1], Yifei Lu[7]

1 Department of Nephrology, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

2 Clinical Center for Investigation, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

3 Department of Nephrology, East branch of Shanghai Sixth People's Hospital, Shanghai, China

4 Department of Nephrology, Shanghai Songjiang District Central Hospital, Shanghai, China

5 Department of Radiology, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

6 Department of Neurology, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

7 Department of pharmacy, Shanghai University of Traditional Chinese Medicine

*Correspondence: Leyi Gu, Department of Nephrology, Renji Hospital, 160 Pujian Road, Shanghai, China. E-Mail guleyi@aliyun.com

ABSTRACT

Introduction

Cognitive impairment (CI) is the common complications in maintenance hemodialysis (MHD) patients. Recently, the pathogenesis of CI has been discussed and oxidative stress is one of the main mechanisms in these patients. Thiamine and folic acid, which play an important role in relieving the production of reactive oxygen species, reducing homocysteine levels, improving oxidative stress in the nervous system. In pilot study, cognitive function was significantly improved in the group with thiamine and folic supplementation. Based on this result, we hypothesize that thiamine combined with folic acid supplementation may improve cognitive function in patients with MHD.

Methods and analysis In this prospective, randomized, placebo-controlled, double-blind, multi-center study, we enrolled patients undergoing hemodialysis who had the Montreal Cognitive Assessment (MoCA) score lower than 26 to treatment group (thiamine 90mg/day combined with folic acid 30mg/day) or control group (thiamine placebo 90mg/day combined with folic acid placebo 30mg/day). All subjects were followed up for 96 weeks. The primary endpoint is the comparison of ADAS-Cog score between the treatment group and the control group at 96 weeks of follow-up. The secondary endpoints included serum thiamine, folate, homocysteine levels, cranial functional magnetic resonance imaging, and prognosis. The central randomization method will be adopted in this study and the principles of placebo-controlled, double-blind randomized control will be followed. The comparisons among ADAS-Cog scores and other secondary endpoints over time within subjects was conducted by using repeated measure ANOVA, and pairwise t-test with Bonferroni adjustment was performed for multiple comparisons. On the other hand, for comparisons between treatment and control group, simple one-way ANOVA or Wilcoxon rank sum test was used. The chisquare method was used for statistical analysis of the categorical data. Kaplan-Meier survival curve was used for survival analysis. A $p < 0.05$ was considered statistically significant difference. Statistical analyses were conducted with SPSS.

Keywords: End-stage renal disease; Cognitive function; Vitamins B; Double-blind; Multi-center

Ethics and dissemination This trial has been approved by Shanghai Jiao Tong University School of Medicine, Renji Hospital Ethics Committee (KY2019-199). After publication of study results, trial report will be published in peer-reviewed journals and/or in national or international conferences.

Trial registration number ChiCTR2000029297

Strengths and limitations of this study

- This is a prospective, randomized, placebo-controlled, double-blind, multi-center study in maintenance hemodialysis patients addressing the complication of cognitive impairment.
- The trial includes treatment and control group.
- It provides important 96 weeks data on cognitive function, serum thiamine, folate, homocysteine levels, cranial functional magnetic resonance imaging, and mortality.
- Laboratory test and adverse events were closely monitored to ensure the safety of the study during follow-up.

INTRODUCTION

This publication describes the study design and protocol of a clinical trial on thiamine and folic acid in the treatment of cognitive impairment in maintenance hemodialysis patients: A prospective, randomized, placebo-controlled, double-blind, multi-center study (Version 2.0; Date 08 May, 2020).

Background

Cognitive impairment (CI) is one of the common neurological complications in patients with end stage renal disease (ESRD)[1]. Our previous single-center cohort observational study showed that the incidence of CI in patients with maintenance hemodialysis (MHD) was 51.6%[2].

As we know, acute neurological complications such as stroke which is taken seriously concern by nephrologist. However, cognitive impairment may be ignored in patients on MHD by nephrologist. At the same time, because of the dormant clinical symptoms in early cognitive impairment patients, this complication can not be timely diagnosed and treated, significantly reduce the patient's quality of life, and leads to the

poor clinical prognosis. These bring to the country's social and family burden which need to cause the extensive concern of the medical profession.

In our single-center randomized controlled pilot study, 50 MHD patients with cognitive impairment were enrolled, including the treatment group (n=25, Thiamine 90mg/day combined with folic acid 30mg/day) and the control group (n=25, nonintervention). After 2 years of follow-up, cognitive function was significantly improved in the treatment group. The 2-year survival rate was also found to be better in the treatment group than in the control group. In addition, there was no statistically significant difference in safety between two groups.

Based on the results of this pilot study, we hypothesize that thiamine combined with folic acid supplementation may improve cognitive function in patients with MHD and provide a prognostic benefit. This needs to be confirmed by a larger sample size randomized controlled multi-center clinical study.

Rationale

Recently, the relationship between cognitive dysfunction and oxidative stress has been discussed in chronic kidney disease patients, especially dialysis patients[1]. Our previous animal study found that the cognitive function of uremia rats which was assessed by the radial arm water maze test decreased compared with the sham operation group. Histological assessment suggested indicated increased oxidation in the hippocampal neurons and decreased numbers of neurons compared to control mice. This finding is similar to previous studies [3].

Thiamine and folic acid belong to the family of water-soluble B vitamins[4]. Thiamine is vitamin B1, which plays an important role in reducing the production of reactive oxygen species in the nervous system and alleviating oxidative stress as the cofactor of transketolase[5, 6]. Folic acid, is also called as vitamin B9, involved in the synthesis of purine and thymine, metabolism of amino acid, as well as hemoglobin production. Folic acid has a direct antioxidant effect, interacts with endothelial nitric

oxide synthase, and affects the bioavailability of nitric oxide cofactors. Moreover, folic acid is essential for the metabolism of homocysteine into methionine, which can reduce homocysteine levels[7].

More important, clinical studies have reported that the application of vitamins B including thiamine and folic acid can reduce the level of oxidative stress index including homocysteine in blood[7,8], thereby reducing oxidative stress and bringing benefits to the treatment of cardiovascular complications in patients with ESRD[9], particularly in those intensive treatment or with a drop in homocysteine levels of more than 20 percent[8]. Nevertheless, recent systematic review and Meta-Analysis indicated that thiamine or folic acid alone can not improves cognitive function healthy older people[10,11].

However, it is not clear whether supplementation with thiamine and folic acid, can improve cognitive function in ESRD patients? Therefor, we designed this prospective, randomized, placebo-controlled, double-blind, multi-center trial to explore whether the intensive combination of thiamine and folic acid can improve cognitive function in MHD patients with CI. The dose of thiamine (90mg/d) is according to the treatment dose of Wernicke's encephalopathy[12] and refeeding syndrome[13]. The dose of folic acid (30mg/d) is refer to the treatment of cardiovascular complications in MHD patients[8].

Objectives

The primary objective of this trial is to verify the hypothesis that thiamine combine with folic acid supplements can improve cognitive function and prognosis in MHD patients with cognitive impairment, and to provide evidence-based for clinical treatment of this complication.

Trial design

This is a prospective, randomized, placebo-controlled, double-blind, multi-center trial. It has two parallel groups with equal allocation including treatment group (Thiamine

90mg/day combined with folic acid 30mg/day) and control group (Thiamine placebo 90mg/day combined with folic acid placebo 30mg/day). The random envelope method was used for center randomization. We used the SPIRIT reporting guidelines[14] in the current study.

METHODS AND ANALYSIS

Study setting

The current trial will be carried out simultaneously in three centers, Renji Hospital, School of Medicine, Shanghai Jiao Tong University (primary center), East branch of Shanghai Sixth People's Hospital (sub-center) and Shanghai Songjiang District Central Hospital (sub-center). In addition, researchers from these three centers have obtained GCP certificates.

In total, nearly 600 MHD patients were follow up at Ren Ji hospital. These three centers have a total of about 1000 MHD patients . According to the results of our previous studies, the incidence rate of cognitive impairment in MHD patients is about 51.6%, so there are enough patients for screening in this study. Patient recruitment will begin imminently and is planned to last 3-6 months.

Eligibility criteria

The target population of this study is patients undergoing maintenance hemodialysis, who should fulfil the following eligibility criteria.

Inclusion criteria

1. Maintenance hemodialysis patients (≥3 months）
2. With cognitive impairment, defined as: MoCA score is less than 26, or MoCA scores is less than 25 if duration of education is less than or equal to 12 years
3. 18-75 years old, male or female

4. Sign the informed consent form

Exclusion criteria

1. Unable to cooperate and complete the study
2. Life expectancy is less than 1 year
3. Participated in other clinical trials within three months
4. Accompanied by severe anemia, infection, tumor, activity bleeding or heart, liver and lung disease
5. Thiamine or folic acid allergies
6. Disorders that may cause cognitive impairment, such as stroke
7. Pregnant or lactating women
8. Subjects judged by the investigator to be unsuitable for inclusion in this study

Intervention

An overview of the trial is provided in figure 1. The researchers screened subjects according to inclusion criteria and exclusion criteria, after which the subject is randomized to the treatment or the control arm by 1:1.

In the treatment arm, the subjects were treated with thiamine (90mg/day) and folic acid (30mg/day).

In the control arm, the subjects were treated with thiamine placebo (90mg/day) and folic acid placebo (30mg/day).

All subjects were followed up every 24 weeks for 96 weeks (table 1). As the trial proceeds, statistical monitoring and concomitant projects may identify need for revisions to the intervention.

Observation item

Demographic data of subjects at baseline were collected including age (year), gender, height(cm), weight (Kg), history of smoking (Smoke every day, no matter how many cigarettes), alcohol abuse (Drink alcohol every day, no matter how much), history of drug abuse (drug dependence, no matter how much), history of education (year, High school is defined as duration of education is more than or equal to 12 years), income (yuan/year, the annual income is more than or less than 30,000 yuan), medical expenses (yuan/year), cognitive impairment family history and work status (Full-time or part-time jobs/retire).

The medical history of the patients, such as the primary cause of MHD, hypertension, diabetes, cardiovascular disease, cerebrovascular disease, cirrhosis, chronic obstructive pulmonary disease, the date of initiate dialysis, Average weekly urine volume (mL/day), as well as folic acid, vitamin B12, active vitamin D and intravenous iron therapy In the last six months.

The following information of haemodialysis treatment was collected at baseline, 24 weeks, 48 weeks, 72 weeks and 96 weeks of follow-up: dialysis duration (h/session), dialysis frequencies (time/week), dialysis modality (HD or HDF, which is the main dialysis modality, for example: HD twice a week and HDF once a week HD means HD), vascular access (fistula or catheter), average weekly ultrafiltration (L/session), hypotension during dialysis treatment in the last week (definition of hypotension: systolic pressure <90 mmHg or diastolic pressure <60 mmHg), use low molecular heparin, weight of pre and post haemodialysis (Kg), blood pressure (BP) of pre and post haemodialysis (mmHg), heart rate of pre and post haemodialysis (BPM).

Laboratory examination data at baseline, 24 weeks, 48 weeks, 72 weeks and 96 weeks of follow-up, such as white blood cell (WBC), haemoglobin (Hb), platelet (Plt), ALT, AST, total protein (TP), total bilirubin (TB), PH, HCO_3^-,K^+, Na^+, Cl^-, albumin (Alb), total cholesterol (TC), Triglyceride (TG), LDL, HDL, Calcium, Phosphorus, iPTH, ferritin, fasting blood glucose (Glu), C-reactive protein (CRP), β_2-microglobulin (β_2-MG), B natriuretic peptides (BNP), thiamine, folic acid and homocysteine were

collected. Transferrin saturation (TSAT) and dialysis adequacy (spKt/V) were calculated.

Cognitive function including MoCA[15], MMSE[16] and ADAS-Cog[17,18] were assessed at baseline, 48 weeks and 96 weeks of follow-up.

Imageological examination including cranial functional magnetic resonance imaging (fMRI) was performed at baseline and 96 weeks of follow-up.

Endpoints

Primary endpoint

The primary endpoint is the comparison of ADAS-Cog score between the treatment group and the control group at 96 weeks of follow-up.

Secondary endpoints

The secondary endpoint are listed below:

1. The comparison of serum thiamine, folate, and homocysteine levels between the treatment and control groups at 96 weeks of follow-up.
2. The comparison of cranial functional magnetic resonance imaging between treatment group and control group at 96 weeks of follow-up.
3. Prognosis comparison between treatment group and control group at 96 weeks of follow-up.

Safety endpoints

Changes in laboratory safety indicators and incidence of adverse events between the treatment and control groups during follow-up.

Withdrawal and drop-out

Shedding criteria

1. The researchers found serious safety problems
2. The effect was so poor that there was no need to continue the trial

Exit criteria

1. Subjects who meet the inclusion criteria and do not complete the study for any reason are considered to be withdrawal subjects, which include subjects' self-withdrawal and subjects' withdrawal determined by the investigator
2. Severe adverse events occurred and the investigator considered it appropriate to withdraw from the study
3. Major Trial Protocol Deviation Subjects are found to not meet the requirements of the trial protocol after randomization
4. Serious other complications occurred during the clinical trial, and the investigators determined that it would not be beneficial to continue the study
5. Unplanned pregnancy
6. Lost follow-up, subjects did not return to the center, and contact with subjects failed

If a subject is lost to follow-up, the investigator should make every effort to contact the subject and encourage the subject to continue to participate in the study as planned. If the subject withdraws their informed consent, the researcher should not evaluate the subject further and should not attempt to collect more data.

For subjects who withdraw from the trial early, the last interview should be arranged within 3 days after the withdrawal (the content of the interview should be determined by the investigator according to the actual situation of the subjects) to complete the efficacy and safety assessment.

Even if the subject is unable to return to the study center for visits, the investigator will complete all available data on the Case Report Form (CRF) and record the reason

for early withdrawal. Whenever possible, the subject must return all drugs. The investigator will take inventory of the drug and complete the Drug Release and Recall Record Form.

Participant timeline

Please refer to figure 1 for details of the visit schedule and participant timeline.

Sample size and recruitment

Based on the previous single-center study, it was assumed that cognitive function (MoCA score) in the treatment group improved from 20 to 26, and set alpha=0.05 and 1-beta=0.9, then at least 100 patients per group are required. In consideration of control shedding or rejection rate within 15% during the test and subsequent analysis requirements, 115 cases were planned to be enrolled in each group. The ratio of the treatment group to the control group was 1:1, with 115 cases in the treatment group and 115 cases in the control group. This study will screen and enroll patients on maintenance hemodialysis in three research centers.

Randomisation

The central randomization method will be adopted in this study and the principles of placebo-controlled, double-blind randomized control will be followed. All eligible subjects will be randomised in a ratio of 1:1 and divided into the treatment and control arms.

Randomization sequence was generated by an independent data manager using SAS (version 9.3) and stored within sealed opaque envelopes. The investigator opened an envelope to obtain a number every time a patient was consented to enter the trial. Based on this number, the patient is assigned the corresponding number of medication (Treatment drugs or placebos).

Blind and unblind

This is a prospective, randomized, placebo-controlled, double-blind, multi-center trial. This study will establish the program of blind setting and blind breaking. There were both blind and non-blind drug administrators in this study. To ensure the

indistinguishable nature of the investigational drug, the blind drug administrator will confirm the indistinguishable nature of the investigational drug and its matching placebo in terms of appearance, taste, etc., before the investigational drug is distributed and unblinded. The non-blind drug administrator creates a master random list of drugs and creates a code break blind authorization number for emergency code break blind. The non-blind drug administrator will securely store the master random list and the code broken blind authorization number list until unblinding. The investigator will develop and maintain a written emergency code blinding procedure to be followed in response to an emergency.

Selected data collection methods

Cognitive function scores including MoCA, MMSE and ADAS-Cog will be performed by board certified neurologists whenever possible. All cognitive function scores will be performed before or during the interval of hemodialysis.

Data statement

The investigator must properly handle all the data obtained during the clinical trial, and truthfully record all adverse events and serious adverse events during the clinical trial, so as to ensure the rights and privacy of the subjects participating in the clinical trial. Personal information of subjects will be collected, shared and maintained to protect confidentiality during and after the study. Only the project investigator will have access to the final trial data set.

A data monitoring committee (DMC) is composed of clinicians and biostatisticians from Clinical Center for Investigation, Renji Hospital, School of Medicine, Shanghai Jiao Tong University who are not involved in this study for the purpose of ensuring the safety of subjects and the quality of study data.

Statistical methods

Data Set Category

1. Full Analysis Set(FAS): the case of using at least once drugs and main efficacy indexes data after the drug administered.

2. Per Protocol Set(PPS): the good compliance case of meeting the main inclusion and exclusion criteria without affecting the main curative effect of prohibiting drugs during the trial.

3. Safety Set(SS): the case of using the investigational drug product at least once with a safety evaluation.

Statistical analysis technique

It will be listed separately that the number of subjects selected and completed the follow-up in the population and centers, identifying three analysis data sets (FAS, PPS, SS) as specified above. The primary analysis only included patients with completed primary outcomes. The comparisons among ADAS-Cog scores and other secondary outcomes over time within subjects was conducted by using repeated measure ANOVA, and pairwise t-test with Bonferroni adjustment was performed for multiple comparisons. On the other hand, for comparisons between treatment and control group, simple one-way ANOVA or Wilcoxon rank sum test was used. The chisquare method was used for statistical analysis of the categorical data. For missing data in secondary efficacy outcomes, they were assumed to be missing at random and multiple imputation was conducted. Kaplan-Meier survival curve was used for survival analysis.

Variables of normal distribution were presented by means with SD while skewed ones were reported as the median and the inter-quartile ranges. Counting data were expressed as constituent ratios or percentages.

A $p < 0.05$ was considered statistically significant difference. Statistical analyses were conducted with SPSS (Version 20.0, SPSS Inc., Chicago, IL, USA).

Harms

Safety endpoints related directly to changes in laboratory safety indicators and incidence of adverse events between the treatment and control groups during follow-up. These endpoints will be listed according to treatment received with a breakdown. Subjects will be followed up in detail, if any complications arise, appropriate treatment will be provided in accordance with current routine medical procedures.

Patient and Public Involvement statement

The trial protocol was developed in part by nephrological and neurological physicians with years of experience in treating MHD patients with CI. At the same time, medical statisticians performed study design and sample size estimation. Patients and the public have not yet been involved directly in the design, or conduct, or reporting, or dissemination plans of this trail protocol.

Ethics and dissemination

This trial has been approved by Shanghai Jiao Tong University School of Medicine, Renji Hospital Ethics Committee (KY2019-199). Other participating sub-centers must also obtain ethics committee approval documents prior to the start of clinical trials. The Good Clinical Practice(GCP)[19] regulations shall be strictly followed during the test implementation.This trial has been registered with the Chinese Clinical Trial Registry (http://www.chictr.org.cn/index.aspx) at 22 Jan. 2020. Trial registration number is ChiCTR2000029297. Amendments to the protocol will be reviewed by Ethics Committees. Informed consent will be obtained before collecting any patient data and patient information. After publication of study results, trial report will be published in peer-reviewed journals and/or in national or international conferences. All researchers involved in the design, discussion and writing of this study protocol, as the authors.

DISCUSSION

Epidemiological study shows the number of dialysis patients is expected to rise to 2.162 million by 2030 in Asia[20]. Meanwhile, cognitive impairment is a common complications in ESRD patients[1]. In pilot study, we enrolled 50 maintenance hemodialysis patients with cognitive impairment, including the treatment group (n=25) who received thiamine 90mg/day combined with folic acid 30mg/day and the control group (n=25) who was the blank control group. After 2 years of follow-up, cognitive function which was evaluated by MOCA score was significantly improved in the

treatment group. In addition, there was no statistically significant difference in safety between the two groups.

Based on the results of this pilot study, we designed this prospective, randomized, placebo-controlled, double-blind, multi-center trial. In this study, MHD patients with CI from several large-scale hemodialysis centers in Shanghai were enrolled in a prospective, randomized, controlled, double-blind study. According to the sample size of the pilot study, 230 subjects were expected to be enrolled. The subjects were randomly divided into treatment group (n=115, thiamine 90mg/day combined with folic acid 30mg/day) and control group (n=115, thiamine placebo 90mg/day combined with folic acid placebo 30mg/day) and followed up for 96 weeks. Cognitive function scores, serum levels of thiamine, folate and homocysteine, functional magnetic resonance imaging of the brain, and safety measures were monitored regularly.

This study hopes to verify added thiamine and folic acid reduces homocysteine levels, so as to relieve the oxidative stress and improve cognitive function in patients with MHD. To explore the benefits of supplementation with thiamine and folic acid in the prognosis of MHD patients with CI. To evaluate the safety of thiamine and folic acid in MHD patients. This study may provides evidence-based evidence for the clinical treatment of this complication and ultimately lays a foundation for the organization and implementation of higher level clinical research in this field.

This exploratory study would be conducted to evaluate the efficacy of thiamine and folic acid supplement in the treatment of MHD patients complicated with CI, so as to help clinically find an effective treatment for this complication and reach the leading domestic and international level. It is helpful to promote the cross-collaboration and common development of multi-centers and multi-disciplines, improve the ability and level of the treatment of nervous system complications in MHD patients, and lay a foundation for the organization and implementation of higher level clinical research in this field.

Through this study, a breakthrough can be achieved in the treatment of MHD patients complicated with CI, blocking the disease progression of MHD patients, reducing the hospitalization rate, medical costs and mortality of MHD patients caused by CI, improving the quality of life of MHD patients, thus improving the prognosis, and having a good clinical application prospect.

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Author Contributors

Renhua Lu and Leyi Gu were responsible for the design of the entire study, including the drafting, revision and submission of clinical trial protocols. Weiming Zhang, Yongping Guo, Xiujuan Zang, Huihua Pang, Shang Liu, Kewei Xie, Ping Li and Xiaojun Zeng are responsible for the discussion and revision of clinical trial protocol. Yan Zhou is responsible for designing the imaging tests. Ling Yu takes charge of the design of cognitive function tests. Shuting Pan is responsible for the design of statistical methods. Yifei Lu is in charge of the pharmacological mechanisms and dosage selection of experimental drugs. All authors have read and approved the final manuscript.

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Competing interests None declared.

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Table 1 The visit schedule of this study

	screen	Randomization	Follow-up period			
Visiting period (weeks)		T0	T1	T2	T3	T4
	-4	0	24±4	48±4	72±4	96±4
Informed consent	×					
Inclusion and Exclusion Criteria	×					
Demographic data	×					
Medical history	×					
Information of haemodialysis	×		×	×	×	×
Laboratory examination	×		×	×	×	×
Cognitive function	×			×		×
Imageological examination	×					×
Drug Administration		×	×	×	×	×
Adverse Event	×	×	×	×	×	×

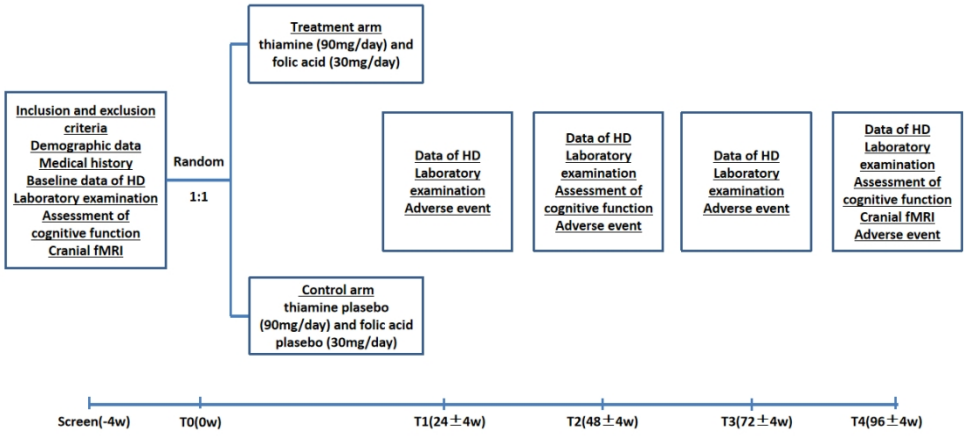

Figure 1 An overview of this trial

338x190mm (96 x 96 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

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Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	12
Protocol version	#3	Date and version identifier	2

Funding	#4	Sources and types of financial, material, and other support	14,15
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	14
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	n/a, no sponsor
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	n/a, no sponsor and funder
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	n/a, no committees
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3,4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4,5
Objectives	#7	Specific objectives or hypotheses	4,5

Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	5,6
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	8,9
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	6,7
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8,9
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood	7,8

		pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9,10
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9,10
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implementation		interventions	
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	10
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	6,7
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	8,9
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	10
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in	11

		the protocol	
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	11
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	10
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	8,9
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11,12
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n/a, no auditing
Ethics and dissemination			

Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	12
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	12
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	12
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a, not applicable
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	10
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	10
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	11
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication	12

		restrictions	
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	12
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	12
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	n/a, not applicable
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a, not applicable

BMJ Open

Protocol for thiamine and folic acid in the treatment of cognitive impairment in maintenance hemodialysis patients: A prospective, randomized, placebo-controlled, double-blind, multi-center study

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Complete List of Authors:	Lu, Renhua; Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital, Department of Nephrology Gu, Le-yi; Shanghai Jiao Tong University, Department of Nephrology; Shanghai Jiao Tong University, Department of Nephrology Zhang, Weiming; Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital, Department of Nephrology Guo, Yongping; Shanghai Sixth Peoples Hospital, Department of Nephrology Zang, Xiujuan; Shanghai Songjiang District Central Hospital, Department of Nephrology Zhou, Yan; Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital, Department of Radiology Yu, Ling; Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital, Department of Neurology Pan, Shuting; Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital, Clinical Center for Investigation Pang, Huihua; Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital, Department of Nephrology Liu, Shang; Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital, Department of Nephrology Xie, Kewei; Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital, Department of Nephrology Li, Ping; Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital, Department of Nephrology Zeng, Xiaojun; Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital, Department of Nephrology Lu, Yifei; Shanghai University of Traditional Chinese Medicine, Department of pharmacy
Primary Subject Heading:	Urology
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Protocol for thiamine and folic acid in the treatment of cognitive impairment in maintenance hemodialysis patients: A prospective, randomized, placebo-controlled, double-blind, multi-center study

Renhua Lu[1], Leyi Gu[1,2*], Weiming Zhang[1], Yongping Guo[3], Xiujuan Zang[4], Yan Zhou[5], Ling Yu[6], Shuting Pan[2], Huihua Pang[1], Shang Liu[1], Kewei Xie[1], Ping Li[1], Xiaojun Zeng[1], Yifei Lu[7]

1 Department of Nephrology, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

2 Clinical Center for Investigation, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

3 Department of Nephrology, East branch of Shanghai Sixth People's Hospital, Shanghai, China

4 Department of Nephrology, Shanghai Songjiang District Central Hospital, Shanghai, China

5 Department of Radiology, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

6 Department of Neurology, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

7 Department of pharmacy, Shanghai University of Traditional Chinese Medicine

*Correspondence: Leyi Gu, Department of Nephrology, Renji Hospital, 160 Pujian Road, Shanghai, China. E-Mail guleyi@aliyun.com

ABSTRACT

Introduction

Cognitive impairment (CI) is the common complications in maintenance hemodialysis (MHD) patients. Recently, the pathogenesis of CI has been discussed and oxidative stress is one of the main mechanisms in these patients. Thiamine and folic acid, which play an important role in relieving the production of reactive oxygen species, reducing homocysteine levels, improving oxidative stress in the nervous system. In pilot study, cognitive function was significantly improved in the group with

thiamine and folic supplementation. Based on this result, we hypothesize that thiamine combined with folic acid supplementation may improve cognitive function in patients with MHD.

Methods and analysis In this prospective, randomized, placebo-controlled, double-blind, multi-center study, we will enroll patients undergoing hemodialysis who has the Montreal Cognitive Assessment (MoCA) score lower than 26 to treatment group (thiamine 90mg/day combined with folic acid 30mg/day) or control group (thiamine placebo 90mg/day combined with folic acid placebo 30mg/day). All subjects will be followed up for 96 weeks. The primary endpoint is the comparison of Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog) score between treatment group and control group at 96 weeks of follow-up. The secondary endpoints include serum thiamine, folate, homocysteine levels, cranial functional magnetic resonance imaging, and survival. The central randomization method will be adopted and the principles of placebo-controlled, double-blind randomized control will be followed. The comparisons among ADAS-Cog scores and other secondary endpoints over time within subjects is conducted by using repeated measure ANOVA or generalized estimating equations (GEE). Pairwise t-test with Bonferroni adjustment is performed for multiple comparisons. On the other hand, for comparisons between treatment and control group, simple one-way ANOVA, GEE or Wilcoxon rank sum test is used. The chisquare method is used for statistical analysis of the categorical data. Kaplan-Meier survival curve is used for survival analysis. A $p<0.05$ is considered statistically significant difference.

Keywords: End-stage renal disease; Cognitive function; Vitamins B; Double-blind; Multi-center

Ethics and dissemination This trial has been approved by Shanghai Jiao Tong University School of Medicine, Renji Hospital Ethics Committee (KY2019-199). After publication of study results, trial report will be published in peer-reviewed journals and/or in national or international conferences.

Trial registration number ChiCTR2000029297

Strengths and limitations of this study

- This is a prospective, randomized, placebo-controlled, double-blind, multi-center study in maintenance hemodialysis patients addressing the complication of cognitive impairment.
- The trial includes treatment and control group.
- It provides important 96 weeks data on cognitive function, serum thiamine, folate, homocysteine levels, cranial functional magnetic resonance imaging, and mortality.

- Laboratory test and adverse events are closely monitored to ensure the safety of the study during follow-up.
- Although unified training is conducted, cognitive score measurement by different researchers may differ in the evaluation of cognitive function of subjects in a multi-center study.

INTRODUCTION

This publication describes the study design and protocol of a clinical trial on thiamine and folic acid in the treatment of cognitive impairment in maintenance hemodialysis patients: A prospective, randomized, placebo-controlled, double-blind, multi-center study (Version 2.0; Date 08 May, 2020).

Background

Cognitive impairment (CI) is one of the common neurological complications in patients with end stage renal disease (ESRD)[1]. Our previous single-center cohort observational study showed that the incidence of CI in patients with maintenance hemodialysis (MHD) was 51.6%[2].

As we know, acute neurological complications such as stroke which is taken seriously concern by nephrologist. However, cognitive impairment may be ignored in patients on MHD by nephrologist. At the same time, because of the dormant clinical symptoms in early cognitive impairment patients, this complication can not be timely diagnosed and treated, significantly reduce the patient's quality of life, and leads to the poor clinical prognosis. These bring to the country's social and family burden which need to cause the extensive concern of the medical profession.

In our single-center randomized controlled pilot study, 50 MHD patients with cognitive impairment were enrolled, including the treatment group (n=25, Thiamine 90mg/day combined with folic acid 30mg/day) and the control group (n=25, nonintervention). After 2 years of follow-up, cognitive function was significantly improved in the treatment group. The 2-year survival rate was also found to be better in the treatment group than in the control group. In addition, there was no statistically significant difference in safety between two groups.

Based on the results of this pilot study, we hypothesize that thiamine combined with folic acid supplementation may improve cognitive function in patients with MHD and provide a prognostic benefit. This needs to be confirmed by a larger sample size randomized controlled multi-center clinical study.

Rationale

Recently, the relationship between cognitive dysfunction and oxidative stress has been discussed in chronic kidney disease patients, especially dialysis patients[1]. Our previous animal study found that the cognitive function of uremia rats which was assessed by the radial arm water maze test decreased compared with the sham operation group. Histological assessment suggested indicated increased oxidation in the hippocampal neurons and decreased numbers of neurons compared to control mice. This finding is similar to previous studies [3].

Thiamine and folic acid belong to the family of water-soluble B vitamins[4]. Thiamine is vitamin B1, which plays an important role in reducing the production of reactive oxygen species in the nervous system and alleviating oxidative stress as the cofactor of transketolase[5, 6]. Folic acid, is also called as vitamin B9, involved in the synthesis of purine and thymine, metabolism of amino acid, as well as hemoglobin production. Folic acid has a direct antioxidant effect, interacts with endothelial nitric oxide synthase, and affects the bioavailability of nitric oxide cofactors. Moreover, folic acid is essential for the metabolism of homocysteine into methionine, which can reduce homocysteine levels[7].

More important, clinical studies have reported that the application of vitamins B including thiamine and folic acid can reduce the level of oxidative stress index including homocysteine in blood[7,8], thereby reducing oxidative stress and bringing benefits to the treatment of cardiovascular complications in patients with ESRD[9], particularly in those intensive treatment or with a drop in homocysteine levels of more than 20 percent[8]. Nevertheless, recent systematic review and Meta-Analysis indicated that thiamine or folic acid alone can not improves cognitive function healthy older people[10,11].

However, it is not clear whether supplementation with thiamine and folic acid, can

improve cognitive function in ESRD patients? Therefor, we designed this prospective, randomized, placebo-controlled, double-blind, multi-center trial to explore whether the intensive combination of thiamine and folic acid can improve cognitive function in MHD patients with CI. The dose of thiamine (90mg/d) is according to the treatment dose of Wernicke's encephalopathy[12] and refeeding syndrome[13]. The dose of folic acid (30mg/d) is refer to the treatment of cardiovascular complications in MHD patients[8].

Objectives

The primary objective of this trial is to verify the hypothesis that thiamine combine with folic acid supplements can improve cognitive function and prognosis in MHD patients with cognitive impairment, and to provide evidence-based for clinical treatment of this complication.

Trial design

This is a prospective, randomized, placebo-controlled, double-blind, multi-center trial. It has two parallel groups with equal allocation including treatment group (Thiamine 90mg/day combined with folic acid 30mg/day) and control group (Thiamine placebo 90mg/day combined with folic acid placebo 30mg/day). The random envelope method was used for center randomization. We used the SPIRIT reporting guidelines[14] in the current study.

METHODS AND ANALYSIS

Study setting

The current trial will be carried out simultaneously in three centers, Renji Hospital, School of Medicine, Shanghai Jiao Tong University (primary center), East branch of Shanghai Sixth People's Hospital (sub-center) and Shanghai Songjiang District Central Hospital (sub-center). In addition, researchers from these three centers have obtained GCP certificates.

In total, nearly 600 MHD patients were follow up at Ren Ji hospital. These three centers have a total of about 1000 MHD patients. According to the results of our previous studies, the incidence rate of cognitive impairment in MHD patients is about

51.6%, so there are enough patients for screening in this study. Patient recruitment will begin imminently and is planned to last 3-6 months.

Eligibility criteria

The target population of this study is patients undergoing maintenance hemodialysis, who should fulfil the following eligibility criteria.

Inclusion criteria

1. Maintenance hemodialysis patients (≥3 months）
2. With cognitive impairment, defined as: MoCA score is less than 26, or MoCA scores is less than 25 if duration of education is less than or equal to 12 years
3. 18-75 years old, male or female
4. Sign the informed consent form

Exclusion criteria

1. Unable to cooperate and complete the study
2. Life expectancy is less than 1 year
3. Participated in other clinical trials within three months
4. Accompanied by severe anemia, infection, tumor, activity bleeding or heart, liver and lung disease
5. Thiamine or folic acid allergies
6. Disorders that may cause cognitive impairment, such as stroke
7. Pregnant or lactating women
8. Subjects judged by the investigator to be unsuitable for inclusion in this study

Intervention

An overview of the trial is provided in figure 1. The researchers screen subjects according to inclusion criteria and exclusion criteria, after which the subject is randomized to the treatment or the control arm by 1:1.

In the treatment arm, the subjects are treated with thiamine tablets (10mg/tablet, three tablets three times a day, 90mg/day) and folic acid tablets (5mg/tablet, two tablets three times a day, 30mg/day).

In the control arm, the subjects are treated with thiamine placebo tablets (10mg/tablet, three tablets three times a day, 90mg/day) and folic acid placebo tablets

(5mg/tablet, two tablets three times a day, 30mg/day).

All subjects will be followed up every 24 weeks for 96 weeks (table 1). As the trial proceeds, statistical monitoring and concomitant projects may identify need for revisions to the intervention.

Observation item

Demographic data of subjects at baseline will be collected including age (year), gender, height(cm), weight (Kg), history of smoking (Smoke every day, no matter how many cigarettes), alcohol abuse (Drink alcohol every day, no matter how much), history of drug abuse (drug dependence, no matter how much), history of education (year, High school is defined as duration of education is more than or equal to 12 years), income (yuan/year, the annual income is more than or less than 30,000 yuan), medical expenses (yuan/year), cognitive impairment family history and work status (Full-time or part-time jobs/retire).

The medical history of the patients, such as the primary cause of MHD, hypertension, diabetes, cardiovascular disease, cerebrovascular disease, cirrhosis, chronic obstructive pulmonary disease, the date of initiate dialysis, average weekly urine volume (ml/day), as well as folic acid, vitamin B12, active vitamin D and intravenous iron therapy in the last six months will be collected.

The following information of hemodialysis treatment will be collected at baseline, 24 weeks, 48 weeks, 72 weeks and 96 weeks of follow-up: dialysis duration (h/session), dialysis frequencies (time/week), dialysis modality (HD or HDF, which is the main dialysis modality, for example: HD twice a week and HDF once a week HD means HD), vascular access (fistula or catheter), average weekly ultrafiltration (L/session), hypotension during dialysis treatment in the last week (definition of hypotension: systolic pressure <90 mmHg or diastolic pressure <60 mmHg), use low molecular heparin, weight of pre and post haemodialysis (Kg), blood pressure (BP) of pre and post haemodialysis (mmHg), heart rate of pre and post haemodialysis (BPM).

Laboratory examination data at baseline, 24 weeks, 48 weeks, 72 weeks and 96 weeks of follow-up, such as white blood cell (WBC), haemoglobin (Hb), platelet (Plt), ALT,

AST, total protein (TP), total bilirubin (TB), PH, HCO_3^-,K^+, Na^+, Cl^-, albumin (Alb), total cholesterol (TC), Triglyceride (TG), LDL, HDL, Calcium, Phosphorus, iPTH, ferritin, fasting blood glucose (Glu), C-reactive protein (CRP), β_2-microglobulin (β_2-MG), B natriuretic peptides (BNP), thiamine, folic acid and homocysteine will be collected. Transferrin saturation (TSAT) and dialysis adequacy (spKt/V) will be calculated.

Cognitive function including MoCA[15], MMSE[16] and ADAS-Cog[17,18] will be assessed at baseline, 48 weeks and 96 weeks of follow-up.

Imageological examination including cranial functional magnetic resonance imaging (fMRI) will be performed at baseline and 96 weeks of follow-up.

Endpoints

Primary endpoint

The primary endpoint is the comparison of ADAS-Cog score between the treatment group and the control group at 96 weeks of follow-up.

Secondary endpoints

The secondary endpoint are listed below:

1. The comparison of serum thiamine, folate, and homocysteine levels between the treatment and control groups at 96 weeks of follow-up.
2. The comparison of cranial functional magnetic resonance imaging between treatment group and control group at 96 weeks of follow-up.
3. Survival comparison between treatment group and control group at 96 weeks of follow-up.

Safety endpoints

Changes in laboratory safety indicators and incidence of adverse events between the treatment and control groups during follow-up.

Withdrawal and drop-out

Shedding criteria

1. The researchers find serious safety problems
2. The effect is so poor that there was no need to continue the trial

Exit criteria

1. Subjects who meet the inclusion criteria and do not complete the study for any reason are considered to be withdrawal subjects, which include subjects' self-withdrawal and subjects' withdrawal determined by the investigator
2. Severe adverse events occurred and the investigator considered it appropriate to withdraw from the study
3. Major Trial Protocol Deviation Subjects are found to not meet the requirements of the trial protocol after randomization
4. Serious other complications occurred during the clinical trial, and the investigators determined that it would not be beneficial to continue the study
5. Unplanned pregnancy
6. Lost follow-up, subjects do not return to the center, and contact with subjects failed

If a subject is lost to follow-up, the investigator should make every effort to contact the subject and encourage the subject to continue to participate in the study as planned. If the subject withdraws their informed consent, the researcher should not evaluate the subject further and should not attempt to collect more data.

For subjects who withdraw from the trial early, the last interview should be arranged within 3 days after the withdrawal (the content of the interview should be determined by the investigator according to the actual situation of the subjects) to complete the efficacy and safety assessment.

Even if the subject is unable to return to the study center for visits, the investigator will complete all available data on the Case Report Form (CRF) and record the reason for early withdrawal. Whenever possible, the subject must return all drugs. The investigator will take inventory of the drug and complete the Drug Release and Recall Record Form.

Participant timeline

Please refer to figure 1 for details of the visit schedule and participant timeline.

Sample size and recruitment

Based on the previous single-center study, it was assumed that cognitive function (MoCA score) in the treatment group improved from 20 to 26, and set alpha=0.05 and 1-beta=0.9, then at least 100 patients per group are required. In consideration of

control shedding or rejection rate within 15% during the test and subsequent analysis requirements, 115 cases were planned to be enrolled in each group. The ratio of the treatment group to the control group was 1:1, with 115 cases in the treatment group and 115 cases in the control group. This study will screen and enroll patients on maintenance hemodialysis in three research centers.

Randomisation

The central randomization method will be adopted in this study and the principles of placebo-controlled, double-blind randomized control will be followed. All eligible subjects will be randomised in a ratio of 1:1 and divided into the treatment and control arms.

Randomization sequence will be generated by an independent data manager using SAS (version 9.3) and stored within sealed opaque envelopes. The investigator open an envelope to obtain a number every time a patient is consented to enter the trial. Based on this number, the patient is assigned the corresponding number of medication (Treatment drugs or placebos).

Blind and unblind

This is a prospective, randomized, placebo-controlled, double-blind, multi-center trial. This study will establish the program of blind setting and blind breaking. There are both blind and non-blind drug administrators in this study. To ensure the indistinguishable nature of the investigational drug, the blind drug administrator will confirm the indistinguishable nature of the investigational drug and its matching placebo in terms of appearance, taste, etc., before the investigational drug is distributed and unblinded. The non-blind drug administrator creates a master random list of drugs and creates a code break blind authorization number for emergency code break blind. The non-blind drug administrator will securely store the master random list and the code broken blind authorization number list until unblinding. The investigator will develop and maintain a written emergency code blinding procedure to be followed in response to an emergency.

Selected data collection methods

Cognitive function scores including MoCA, MMSE and ADAS-Cog will be performed by board certified neurologists whenever possible. All cognitive function

scores will be performed before or during the interval of hemodialysis.

Data statement

The investigator must properly handle all the data obtained during the clinical trial, and truthfully record all adverse events and serious adverse events during the clinical trial, so as to ensure the rights and privacy of the subjects participating in the clinical trial. Personal information of subjects will be collected, shared and maintained to protect confidentiality during and after the study. Only the project investigator will have access to the final trial data set.

A data monitoring committee (DMC) is composed of clinicians and biostatisticians from Clinical Center for Investigation, Renji Hospital, School of Medicine, Shanghai Jiao Tong University who are not involved in this study for the purpose of ensuring the safety of subjects and the quality of study data.

Statistical methods

Data Set Category

1. Full Analysis Set(FAS): the case of using at least once drugs and main efficacy indexes data after the drug administered.
2. Per Protocol Set(PPS): the good compliance case of meeting the main inclusion and exclusion criteria without affecting the main curative effect of prohibiting drugs during the trial.
3. Safety Set(SS): the case of using the investigational drug product at least once with a safety evaluation.

Statistical analysis technique

It will be listed separately that the number of subjects selected and completed the follow-up in the population and centers, identifying three analysis data sets (FAS, PPS, SS) as specified above. The primary analysis only include patients with completed primary outcomes. The comparisons among ADAS-Cog scores and other secondary outcomes over time within subjects is conducted by using repeated measure ANOVA or generalized estimating equations (GEE), and pairwise t-test with Bonferroni adjustment is performed for multiple comparisons. On the other hand, for comparisons between treatment and control group, simple one-way ANOVA, GEE or Wilcoxon rank sum test is used. The chisquare method is used for statistical analysis of the

categorical data. For missing data in secondary efficacy outcomes, they are assumed to be missing at random and multiple imputation was conducted. Kaplan-Meier survival curve is used for survival analysis.

Variables of normal distribution are presented by means with SD while skewed ones are reported as the median and the inter-quartile ranges. Counting data are expressed as constituent ratios or percentages.

A $p<0.05$ is considered statistically significant difference. Statistical analyses are conducted with SPSS (Version 20.0, SPSS Inc., Chicago, IL, USA).

Harms

Safety endpoints relate directly to changes in laboratory safety indicators and incidence of adverse events between the treatment and control groups during follow-up. These endpoints will be listed according to treatment received with a breakdown. Subjects will be followed up in detail, if any complications arise, appropriate treatment will be provided in accordance with current routine medical procedures.

Patient and Public Involvement statement

The trial protocol was developed in part by nephrological and neurological physicians with years of experience in treating MHD patients with CI. At the same time, medical statisticians performed study design and sample size estimation. Patients and the public have not yet been involved directly in the design, or conduct, or reporting, or dissemination plans of this trail protocol.

Ethics and dissemination

This trial has been approved by Shanghai Jiao Tong University School of Medicine, Renji Hospital Ethics Committee (KY2019-199). Other participating sub-centers must also obtain ethics committee approval documents prior to the start of clinical trials. The Good Clinical Practice(GCP)[19] regulations shall be strictly followed during the test implementation.This trial has been registered with the Chinese Clinical Trial Registry (http://www.chictr.org.cn/index.aspx) at 22 Jan. 2020. Trial registration number is ChiCTR2000029297. Amendments to the protocol will be reviewed by Ethics Committees. Informed consent will be obtained before collecting any patient data and patient information. After publication of study results, trial report will be

published in peer-reviewed journals and/or in national or international conferences. All researchers involved in the design, discussion and writing of this study protocol, as the authors.

DISCUSSION

Epidemiological study shows the number of dialysis patients is expected to rise to 2.162 million by 2030 in Asia[20]. Meanwhile, cognitive impairment is a common complications in ESRD patients[1]. In pilot study, we enrolled 50 maintenance hemodialysis patients with cognitive impairment, including the treatment group (n=25) who received thiamine 90mg/day combined with folic acid 30mg/day and the control group (n=25) who was the blank control group. After 2 years of follow-up, cognitive function which was evaluated by MOCA score was significantly improved in the treatment group. In addition, there was no statistically significant difference in safety between the two groups.

Based on the results of this pilot study, we designed this prospective, randomized, placebo-controlled, double-blind, multi-center trial. In this study, MHD patients with CI from several large-scale hemodialysis centers in Shanghai will be enrolled in a prospective, randomized, controlled, double-blind study. According to the sample size of the pilot study, 230 subjects will be expected to be enrolled. The subjects will be randomly divided into treatment group (n=115, thiamine 90mg/day combined with folic acid 30mg/day) and control group (n=115, thiamine placebo 90mg/day combined with folic acid placebo 30mg/day) and followed up for 96 weeks. Cognitive function scores, serum levels of thiamine, folate and homocysteine, functional magnetic resonance imaging of the brain, and safety measures will be monitored regularly.

This study hopes to verify added thiamine and folic acid reduces homocysteine levels, so as to relieve the oxidative stress and improve cognitive function in patients with MHD. To explore the benefits of supplementation with thiamine and folic acid in the prognosis of MHD patients with CI. To evaluate the safety of thiamine and folic acid in MHD patients. This study may provides evidence-based evidence for the clinical

treatment of this complication and ultimately lays a foundation for the organization and implementation of higher level clinical research in this field.

This exploratory study would be conducted to evaluate the efficacy of thiamine and folic acid supplement in the treatment of MHD patients complicated with CI, so as to help clinically find an effective treatment for this complication and reach the leading domestic and international level. It is helpful to promote the cross-collaboration and common development of multi-centers and multi-disciplines, improve the ability and level of the treatment of nervous system complications in MHD patients, and lay a foundation for the organization and implementation of higher level clinical research in this field.

Through this study, a breakthrough can be achieved in the treatment of MHD patients complicated with CI, blocking the disease progression of MHD patients, reducing the hospitalization rate, medical costs and mortality of MHD patients caused by CI, improving the quality of life of MHD patients, thus improving the prognosis, and having a good clinical application prospect.

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Author Contributors

Renhua Lu and Leyi Gu were responsible for the design of the entire study, including the drafting, revision and submission of clinical trial protocols. Weiming Zhang, Yongping Guo, Xiujuan Zang, Huihua Pang, Shang Liu, Kewei Xie, Ping Li and Xiaojun Zeng are responsible for the discussion and revision of clinical trial protocol. Yan Zhou is responsible for designing the imaging tests. Ling Yu takes charge of the design of cognitive function tests. Shuting Pan is responsible for the design of statistical methods. Yifei Lu is in charge of the pharmacological mechanisms and dosage selection of experimental drugs. All authors have read and approved the final manuscript.

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Competing interests None declared.

Word count 4852.

Figure 1 An overview of this trial

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Table 1 The visit schedule of this study

	screen	Randomization	Follow-up period			
Visiting period (weeks)	-4	T0 0	T1 24±4	T2 48±4	T3 72±4	T4 96±4
Informed consent	×					
Inclusion and Exclusion Criteria	×					
Demographic data	×					
Medical history	×					
Information of haemodialysis	×		×	×	×	×
Laboratory examination	×		×	×	×	×
Cognitive function	×			×		×
Imageological examination	×					×
Drug Administration		×	×	×	×	×
Adverse Event	×	×	×	×	×	×

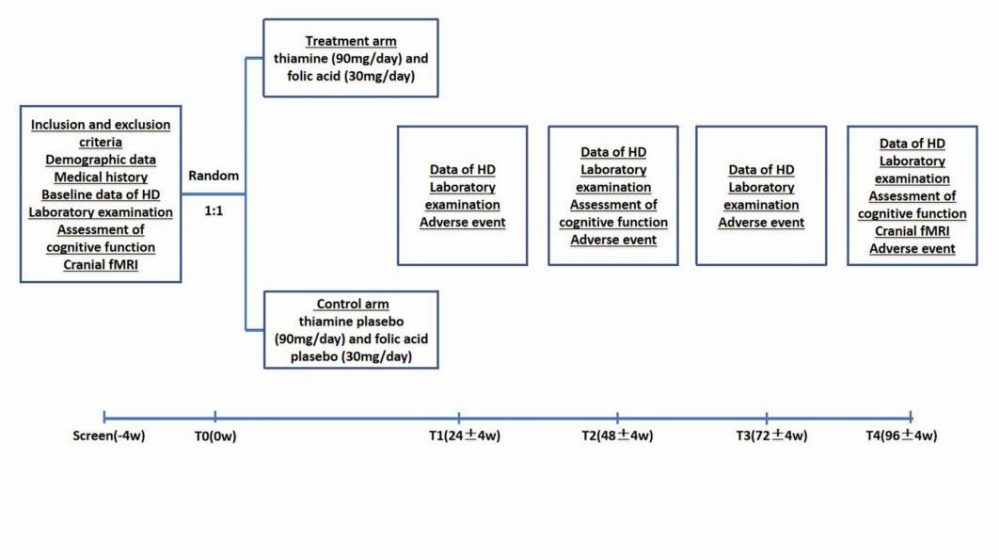

Figure 1 An overview of this trial

90x90mm (300 x 300 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	12
Protocol version	#3	Date and version identifier	2

Funding	#4	Sources and types of financial, material, and other support	14,15
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	14
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	n/a, no sponsor
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	n/a, no sponsor and funder
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	n/a, no committees
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3,4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4,5
Objectives	#7	Specific objectives or hypotheses	4,5

Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	5,6
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	8,9
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	6,7
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8,9
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood	7,8

		pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9,10
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9,10
Allocation:	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to	9,10

implementation		interventions	
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	10
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	6,7
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	8,9
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	10
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in	11

		the protocol	
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	11
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	10
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	8,9
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11,12
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n/a, no auditing
Ethics and dissemination			

Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	12
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	12
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	12
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a, not applicable
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	10
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	10
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	11
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication	12

		restrictions	
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	12
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	12
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	n/a, not applicable
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a, not applicable