Chinese herbal medicine Tangshen Formula Treatment For Type 2 Diabetic Kidney Disease in Early Stage : study protocol for a randomized controlled trial

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Abstract

Abstract Background: Diabetic kidney disease (DKD) is the main cause of end stage kidney disease (ESKD) and It has become a heavy economic and social burden due to its high prevalence and morbidity. The most effective strategy is that patients with DKD should be diagnosed and treated early. Preliminary studies showed the Chinese herbal Tangshen Formula (TSF) may delay the progression of DKD, reducing micro-and macro-albuminuria and improving renal function. We designed a randomized, double-blind, placebo-controlled trial to evaluate the efficacy of TSF in patients with DKD. Methods/design: This trial is a thirteen-center, randomized, double-blind, placebo-controlled study. 623 participants will be randomized in a 1:1 ratio to an experiment group (TSF plus Losartan) and a control group (placebo plus Losartan). The trial cycle will last 24 weeks. The primary outcome will be the change in Urine microalbumin creatinine ratio □ ACR □ from baseline to week 24. The secondary outcome will be the change in the rate of progression into the clinical proteinuria period after intervention, the rate of urine microalbumin negative conversion, the rate of normal urinary microalbumin, doubling rate of baseline creatinine value and glomerular filtration rate (GFR) between the two groups. Safety in medication will also be evaluated. Discussion: We hypothesize that type 2 diabetic patients in early stage of DKD will benefit from TSF. If successful, this study will provide evidence-based recommendations for clinicians. Trial registration: ClinicalTrials.gov (NCT03009864) Keywords: Diabetes Kidney Disease, Traditional Chinese medicine, Treatment, Randomized controlled trial

Background

Diabetic kidney disease (DKD) is the most common diabetic microvascular complication and the primary cause of end stage kidney disease (ESKD). Pooled data from 54 countries revealed that more than 80% of ESKD were given rise by diabetes, hypertension or a combination both. The prevalence of ESKD was also up to 10 times higher in people with diabetes as those without [1]. Several studies showed that 20% of people with diabetes from the UK [2] and 40% of diabetics in the US will develop chronic kidney disease (CKD), whereas 19% show signs of DKD stage 3 or higher[3]. It is responsible for 40% of patients to experience dialysis after diagnosis of diabetes mellitus in the developed world [4]. Moreover, there also has been a continuous increase in the incidence of ESKD due to the increasing incidence of T2DM, notably in China, with 11.9 % of high incidence of type 2 diabetes [5]. As impairment in renal function progresses, it is bound up with a high risk of mortality, cardiovascular events and hospitalizations [1] and associated with heavy economic burden, estimated average annual healthcare costs ranging from $4573 to $10,322[8]. Some risk factors of DKD have been identified, such as ageing, hypertension and hyperglycemia [6,7]. Current strategies for DKD aimed to delay deterioration of renal function through actively controlling glucose, blood pressure and blood lipid levels, and via restrain of the renin-angiotensin-aldosterone system (RAAS) [6,7,8] such as angiotensin-converting enzyme inhibitors (ACEI), angiotensin II receptor blockers (ARB).

However, these therapies are difficult to achieve in all patients with DKD and stable results in the long term as well as these treatments may not reverse DKD [9,10] and even with treatment of currently

effective therapies, ESKD will finally occur in a proportion of patients, requiring dialysis regimen and kidney transplant. Therefore, the most effective strategy is that diabetic patients kidney disease should be diagnosed and treated early. Type 2 diabetic patients with microalbuminuria is at high risk of progression to overt renal disease, indicating that those patients easily enter the stage of clinical diabetic nephropathy (Stage 4 or 5). In addition, not only of ESKD but also of premature cardiovascular events is predicted by microalbuminuria in diabetes[11-13], whereas effective intervention therapies for microalbuminuria could improve clinical outcomes in DKD.

Although ACEI or ARB showed the evidence of decreasing microalbuminuria in type 2 diabetics with nephropathy [14,15], some adverse events (cough, rhinitis, hyperkalemia, acute kidney injury and angioedema) related ACEI or ARB might cause poor adherence [16-1]. Therefore, it is imperative to seek effectively renoprotective therapies.

Chinese herbal medicine (CHM) has long been widely used in China [20]. Numerous studies have demonstrated the biological activity and therapeutic mechanism of CHM [21-24]. Recent studies show that certain Chinese herbs have renoprotective effects, improving GFR and decreasing proteinuria, especially in patients with microalbuminuria [25-29]. This study is designed to investigate whether TSF may represent a potential remedy for slowing down disease progression in early type 2 diabetic nephropathy. If positive, this work may provide an evidence-based medicine remedy for slowing or preventing the clinical progression of DKD.

Methods/design

Study design

This protocol will be designed as a randomized, placebo-controlled and multicenter trial. Participants, investigators and the statisticians will be blinded. 632 subjects will be recruited at the following 13 tertiary A hospitals in mainland China：Guang' anmen Hospital of the China Academy of Chinese Medical Sciences, The First Affiliated Hospital of Anhui University of Traditional Chinese Medicine, Beijing Hospital of Traditional Chinese Medicine, Hubei Hospital of Traditional Chinese Medicine, Zouping County Hospital of Traditional Chinese Medicine, Zibo Wanjie Hospital and Shijiazhuang Hospital of Traditional Chinese Medicine, Zhengzhou city Hospital of Traditional medicine, Xingtai city Hospital of Traditional medicine, Shexian country Hospital of Traditional medicine, Baoding city of Hosptial of Traditional medicine, Yantai bai shi Hospital of Traditional medicine, Jilin province of Hosptial of Traditional medicine. The trial will be implemented base on the principles of good clinical practice and reported according to the CONSORT statement [30,31]. The trial flow is illustrated in **Fig. 1**. The Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) [32] Checklist will be showed in **Additional file 1**. This study has been registered in ClinicalTrials.gov (NCT03009864).

Participants

Diagnostic criteria

The diagnostic criteria of this trial will be set based on the Chinese Diabetes Society guidelines [6] and American Diabetes Association guidelines [7],National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) guidelines [33], and the Kidney Disease Improving Global Outcomes (KDIGO) 2012 Clinical Practice Guideline [34], For a diagnosis of early stage of DKD, patients with DM will have to have the following conditions:

1. Diagnosed as type 2 diabetes.
2. Type 2 diabetic patients with microalbuminuria and Urinary microalbumin excretion rate (UAER)
3. For microalbuminuria, two repeated tests performed within six months will need to produce abnormal results;
4. UAER of 30-300mg/24h or urinary albumin-to-creatinine ratio (ACR) of 30-300 mg/g (mg/mmol)
5. Have clinical manifestations of kidney diseases, such as oedema, anaemia, renal dysfunction, etc.

Inclusion criteria

1. The patient was diagnosed with DKD with microalbuminuria.
2. Age in 30-70;
3. Sign informed consent;

Exclusion criteria

1. Proteinuria caused by non-diabetic kidney in the patient, such as gout, primary hypertension, tumors, and proteinuria caused by chronic kidney disease.
2. Cardiovascular, liver, kidney, hematopoietic system, or other primary severe disease; serum transaminase more than double the standard value; serum creatinine (SCr) higher than the upper limit of normal; and psychiatric patients.
3. Pregnancy, preparation for pregnancy, or lactation, or any history of drug allergy.
4. The patient developed renal failure (anaemia and uremia).
5. Participation in another clinical trial or use of any other drug within the previous month.
6. Recent use of ACEI or ARB in the past one month except Losartan
7. Any excessive consumption of alcohol or any consumption of psychoactive substances, drug

abuse, or drug dependence during the past five years.

8. According to the researcher's judgment, some other diseases or conditions reduce the possibility of enrollment or complicate the enrollment, such as frequent changes of jobs and unstable living environment, which may easily lead to loss of visits.

Randomization and Concealment

Specific randomization sequence will be computer-generated by a computerized random number generator from an independent clinical research organization (CRO) of Institute of Clinical Medicine of Chinese Academy of Chinese Medical Sciences which is not involved in the study. All eligible patients will be randomized to the experimental group or the control group at a 1:1 ratio. An independent non-investigator will protect concealment list. The medical information will be confidential and not be available to any investigator for the duration of the study. If a medical emergency occurs, the individual's randomization code and group allocation can be identified.

Blinding

This study will be designed as a double-blind. Not only were subjects and investigators blinded in these trials, but drug administration, statisticians and curative evaluators were also masked. Treatment allocation will be uncovered after the completion of the study. In addition, TSF and placebo cannot be distinguished in the taste, smell, and appearance. After production, study drugs will be packaged and transferred to numbered bottles by designated pharmacists in accordance with the randomized list. No one can tell the difference except one who is in charge of concealment.

Intervention

All subjects in two groups will be received the conventional treatment, including oral Losartan (50mg/once a day), diet, exercise, and oral medicine, to ensure access to steady levels of blood glucose, blood lipids, and blood pressure and based on the recommendations of Treatment of Chinese Diabetes Society guidelines [6],and American Diabetes Association guidelines[7]. Subjects will be randomly assigned to receive placebo (6 g/bag twice per day) or Tangshan Formula (6 g/bag two times per day) by Specified sequence number from the central randomization system. The treatment will last for 24 consecutive weeks.

Outcomes

Primary outcomes

Urinary microalbumin (MAlb) is a critical indicator for the diagnosis of early renal impairment in diabetes mellitus. For early-stage DKD, Urinary microalbumin creatinine ratio (ACR) will be a primary evaluation index. The change in ACR from baseline will be evaluated between the two groups, and ACR will be compared at baseline and treatment endpoint (24 weeks) in each group.

Secondary outcomes

1. Compare the ratio of progression into the clinical proteinuria period after intervention between the two groups
2. Urine microalbumin negative conversion rate. The ratio of normal urinary microalbumin (<20 ug/min) was compared between the two groups.

3. Change in GFR. Change in the D-value and ratio of glomerular filtration rate (GFR) before and after treatment were compared between the two groups. Glomerular rate filtration (GFR): using the simplified MDRD formula: GFR (ml/min·1.73m^2) =186×serum creatinine-1.154×age-1.154×[female×0.742] × [Chinese×1.233].
4. Doubling rate of baseline creatinine value will be compared between the two groups after intervention

Safety assessment

Adverse events（AEs）will be continuously monitored for 24 weeks at the beginning and end of the study period, and the incidence of AEs will be evaluated in each visit, including vital signs, ECG, liver function, renal function, Routine blood examination, routine urinalysis, and routine stool examination. In addition, AEs, such as signs and symptoms and other ailments, will also be documented truthfully at every study visit, including the occurrence time, severity, duration, effective measures and transfer. Each AE associated with the intervention drugs will be classified as mild, moderate and severe. Severe AEs will need to submit to the principal investigator and the ethics committee within 24 h. All AEs will be properly resolved. Criteria for the severity of adverse events, detailed requirements are as follows:

Mild: mild discomfort, subjects can endure no treatment, no special treatment is required, no effect on subjects' recovery.

Moderate: moderate discomfort, unbearable to the subject, requiring special treatment, has a direct impact on the subject's recovery.

Severity: severe discomfort, life-threatening, fatal or disabling, immediate emergency treatment is required.

Study visits and assessment

Intervention cycle will be 24 weeks, including a run-in period. After the research commences, visits will occur every four weeks during the study period. An overview of specific measurements and visit of time points for data collection can be shown in **Table.1.**

Quality control data collection

To maintain the high quality of this trial and assure its adherence to the protocol, all investigators and drug administrators participating in the research will be trained rigorously basis on a standardized operation practice (SOP) manual. Withdrawals or drop visits also will need to be explained in CRFs. All data will be documented in a standardized case report form (CRF) and instantly recorded in the database via the ClinResearch Electronic Data Capture System at http://www.tcmcec.net/crivrs/. The monitor will review the CRFs, check the inclusion, exclusion, and withdraw criteria, as well as ensure information of CRFs in accordance with those in the source medical records. Except for, Original CRFs will be reserved at the research centre for five years after completion of the study. The validity and authenticity of the

multicenter trial will be guaranteed by establishing three committees including the clinical trial guidance committee, the data and safety monitoring board and the outcome evaluation committee, hitch respectively being responsible for trial design and the executing process, monitoring the data collection process to control its quality and evaluating the outcomes. The diagram of enrollment, interventions, and assessments is shown in **Fig.1.**

Sample size

The sample size was estimated according to the relevant data from Cossar study published in *New England* [18,19]. These results showed that the proportion of patients with ACR reduced by 50% or more in the Cosszar group was 12.5% and the preliminary study of TSF data manifested that TSF as an add on study can improve 50% of patients. Estimating the proportion of patients with ACR reduced by 50% or more was 18.7%.The estimated sample size formula was tested by using the hypothesis of two population rates(See Formula 1 in the Supplementary Files)

(**n** is the sample size; p_1 and p_2 are the sample rate, and p= (p_1+p_2) /2 is the sample average rate, α is the type 1 error and β are the type 2 error, while u_α and u_β are the locus of the corresponding standard normal distribution). According to the unilateral test, u_α=1.64485, u_β=0.84162, and substitute it into above formula, **n**=274.46. Therefore, 275 patients were needed in each group. Considering the drop-rate of no more than 15%, the final sample size was estimated to be 632 in total.

Statistical analysis

The statistical analysis of this study was completed by independent statistician, and the detailed statistical analysis plan was formulated separately by statistician before the inception of this trial and determined with the principal investigator. Three analysis sets will be used for assessment of this study: the intention-to-treat set (ITT), the per-protocol analysis set (PPS) and the safety analysis set (SAS). ITT and PPS will be used to appraise the efficacy of TSF. If any given case exists missing a critical variable, the last observation used as the final results will be carried forward to the finial data. The changes of urinary microalbumin creatinine ratio, urinary microalbumin, GFR, creatinine and baseline information will be present after treatment. A paired t -test or the Wilcoxon signed-rank test will be employed to compare each group. Changes relative to baseline after treatment will be compared between groups using the t-test or the Wilcoxon rank-sum test. A P value < 0.05 will be deem as to show a statistically significant difference.

Bias analysis

The main evaluation outcomes of this trial are the rate of progression from microalbuminuria period into clinical proteinuria, which are very objective. Although ACEI and ARB drugs have achieved a recognized efficacy internationally, a certain proportion of patients still cannot delay the progression of DKD even using these two drugs. Therefore, the bias factors affecting the outcome evaluation include three

aspects：(1) blood glucose level (2) ACEI or/and ARB use and (3) Laboratory testing error of urine microalbuminuria.

These factors are solved as follows :(1) for the blood glucose level, the consistency of this factor in the two groups was ensured due to the random and double-blind study design method. (2) subjects only took cossar and no other ARB or/and ACEI drugs. (3) Central laboratories are used for the main outcomes.

Discussion

DKD is a part of the systemic microangiopathy and glomerular sclerosis caused by diabetes. In European and American countries, DKD is the primary cause of renal replacement therapy, accounting for about 1/2 and. It is the second common cause of ESRD in China after glomerular disease [1]. Compared to CKD caused by non-diabetic, DKD develops more rapidly into ESRD [35,36]. It is urgent to seek an effective preventive measure to delay DKD. CHMs have been common in treating DM [37-42]and its complications [43-45], including DKD. In modern times, many patients have turned to Chinese herbal medicine for treatment as a complementary and necessary combination-drug therapy for kidney disease in China due to its fewer adverse reactions and more effective interventions. Currently, research from Taiwan has demonstrated that patients with CKD who used CHM had a significantly reduced ESRD risk (60%) [46]. Relevant clinical observations have shown that TSF appears to prevent further development and deterioration of the disease, which includes the reduction of urinary albumin and the normalization of glomerular filtration rate [47-50]. Potential mechanisms have been proved that herbal medicine could regulate oxidative stress which is well recognized that oxidative stress plays a significant role in worsening of DKD [50-52]. However, there is less clinical research on delaying the progression of DKD, especially in the early stage as only period of reversing kidney lesion. This work has the potential function to delay the development of DKD. Therefore, we are implementing this study to evaluate efficacy and safety of TSF treatment for DKD. If successful, this work will provide an evidence-based medical evidence for a therapeutic approach of delaying the progress of DKD.

Trial status

Patient recruitments began on May 2018. It is expected to be completed in May 2022. At the time of manuscript submission,95 patients had been recruited and estimate time of recruitment completion is May 2020. Currently, we are still recruiting participants. Protocol version number (20160718 protocol02) and date (2016-10-18)

Abbreviations

Diabetic kidney disease (DKD)；End stage kidney disease(ESKD);chronic kidney disease(CKD);renin-angiotensin-aldosterone system (RAAS);angiotensin-converting enzyme inhibitors(ACEI);angiotensin II receptor blockers (ARB);Chinese herbal medicine (CHM);Tangshan Formula (TSF);National Kidney

Foundation Kidney Disease Outcomes Quality Initiative (NKF-KDOQI);the Kidney Disease Improving Global Outcomes (KDIGO);Urinary microalbumin excretion rate (UAER);albumin-to-creatinine ratio (ACR);serum creatinine (SCr);case report form (CRF);clinical research organization (CRO);Urinary microalbumin (MAlb);glomerular filtration rate (GFR);Adverse events（AEs）;Standardized operation practice (SOP); standardized case report form (CRF); intention-to-treat set (ITT); per-protocol analysis set (PPS); the safety analysis set (SAS);

Declarations

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Authors'contributions

De Jin drafted the protocol. Fengmei lian, QingNi, and Xiaolin Tong revised the protocol. WJH, QiBap, Yanan Ya, FY, and Meizhen Zhang recruited the patients. All of the authors participated in the design and read and approved the final manuscript.

Authors'information

XLT is a principal investigator at China Academy of Chinese Medical Sciences; FMl is researcher in Good Clinical Practice at Guang anmen hospital; QN is the head of endocrinology department in Guang anmen hospital.

Ethics approval

Ethics committee of Guang anmen hospital of the China Academy of Chinese Medical Science has approved this trial for the participating centers (No.2016-093-KY-01) and Informed consent form must be obtained before randomization.

Consent for publication

All the participants have provided consent to share their individual medical information.

Competing interests

There are no competing interests in this work

References

1. United States Renal Data System. International Comparisons. In United States Renal Data System. 2014 USRDS annual data report: Epidemiology of kidney disease in the United States. Bethesda (MD): National Institutes of Health,National Institute of Diabetes and Digestive and Kidney Diseases 2014; 188–210.
2. King P,Peacock I,Donnelly R. The UK Prospective Diabetes Study (UKPDS): clinical and therapeutic implications for type 2 diabetes. Br J Clin Pharmacol 1999; 48: 643–8; DOI: http://dx.doi.org/10.1046/ j.1365-2125.1999.00092.x.
3. Dean J. Organising care for people with diabetes and renal disease. J Ren Care 2012; 38 Suppl 1: 23–9; DOI: http://dx.doi.org/10.1111/ j.1755-6686.2012.00272.x.
4. Ahmad J. Management of diabetic nephropathy:recent progress and future perspective. Diabetes Metab Syndr. 2015;9(4):343–58
5. Cho N H,Shaw J E,Karuranga S,et al. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045.[J]. Diabetes Research & Clinical Practice,2018,138:271.
6. American Diabetes Association.Glycemic targets: standards of medical care in diabetes—2018[J]. Diabetes Care,2018,41(Supplement 1): S55-S64.
7. Weiping JIA.Guidelines for prevention and treatment of type 2 diabetes in China (2017 edition)[J]. Chinese diabetes society,2017.
8. Li R, Bilik D, Brown M B, et al. Medical costs associated with type 2 diabetes complications and comorbidities[J]. The American journal of managed care, 2013, 19(5): 421.(PMID: 23781894)
9. Hajhosseiny R,Khavandi K,Jivraj N,Mashayekhi S,Goldsmith DJ,Malik RA. Have we reached thelimits for the treatment of diabetic nephropathy?Expert Opin Investig Drugs. 2014;23(4):511–22.
10. Dhodi JB,Mestry SN,Juvekar AR. Diabetic nephropathy-genesis,prevention,and treatment.Int J Pharm Pharm Sci. 2014;6(9):42–7.(Google Scholar)
11. Mogensen C E. Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes.[J]. N Engl J Med,1984,310(6):356-360.
12. Viberti G C,Hill R D,Jarrett R J,et al. Microalbuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus.[J]. Lancet,1982,1(8287):1430.
13. Marre M,Chatellier G,Leblanc H,et al. Prevention Of Diabetic Nephropathy With Enalapril In Normotensive Diabetics With Microalbuminuria[J]. Bmj,1988,297(6656):1092-1095.
14. Parving H H,Persson F,Lewis J B,et al. Aliskiren combined with losartan in type 2-diabetes and nephropathy-secondary publication.[J].New England Journal of Medicine,2008,358(23):2433.
15. Brenner B M,Cooper M E,De Z D,et al. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy.[J]. N Engl J Med,2001,11(1):861-869.
16. Tang J L,Liu B Y,Ma K W. Traditional Chinese medicine.[J]. Lancet,2008,372(9654):1938-1940.

17. Pathak JV,Dass EE. A retrospective study of the effects of angiotensin receptor blockers and angiotensin converting enzyme inhibitors in diabetic nephropathy. Indian J Pharmacol. 2015;47(2):148–52.
18. Fried LF,Emanuele N,Zhang JH,Brophy M,Conner TA,Duckworth W,et al. Combined angiotensin inhibition for the treatment of diabetic nephropathy. N Engl J Med. 2013; 369(20):1892–903.
19. Montero de Francisco A,García-Luque A,Fernandez M,Puerro M. Side effects of angiotensin converting enzyme inhibitors and angiotensin II receptor antagonists: are we facing a new syndrome. Am J Cardiol.2012; 110(10):1552–3.
20. Roberts JR,Lee JJ,Marthers DA. Angiotensin-converting enzyme (ACE) inhibitor angioedema: the silent epidemic. Am J Cardiol. 2012; 109(5):774–5
21. Liu J,Henkel T. Traditional Chinese medicine (TCM): are polyphenols and saponins the key ingredients triggering biological activities?[J]. Current Medicinal Chemistry,2002,9(15):-.
22. Letasiová S,Jantová S,Cipák L,et al. Berberine-antiproliferative activity in vitro and induction of apoptosis/necrosis of the U937 and B16 cells.[J]. Cancer Letters,2006,239(2):254.
23. Bang J Y,Kim K S,Kim E Y,et al. Anti-angiogenic effects of the water extract of HangAmDan (WEHAD),a Korean traditional medicine[J]. Science China Life Sciences,2011,54(3):248-254.
24. Wedge D E,Gao Z,Tabanca N,et al. Chemical composition and biological activities of two Angelica essential oils from China[J]. Planta Medica,2009,75(4):422.
25. Wang F L,Tang L Q,Yang F,et al. Renoprotective effects of berberine and its possible molecular mechanisms in combination of high-fat diet and low-dose streptozotocin-induced diabetic rats.[J]. Molecular Biology Reports,2013,40(3):2405-2418.
26. Zhao X F,Su S J,Yun G,et al. Mortality and recurrence of vascular disease among stroke patients treated with combined TCM therapy[J]. Journal of Traditional Chinese Medicine,2012,32(2):173.
27. Chen H,Jing G,Zhao X,et al. Retrospective analysis of the overt proteinuria diabetic kidney disease in the treatment of modified Shenzhuo formula for 2 years[J]. Medicine,2017,96(12):e6349.
28. Guo Jing,Chen Hongdong,Zhao Xiyan,et al. Diabetic kidney disease treated with a modified Shenzhuo formula derived from Traditional Chinese Medicine: a case report[J]. Journal of Traditional Chinese Medicine,2017,37(6):854-861.
29. Tian J,Zhao L,Zhou Q,et al. Efficacy of Shenzhuo formula on diabetic kidney disease: a retrospective study.[J]. Journal of Traditional Chinese Medicine,2015,35(5):528.
30. Moher D, Hopewell S, Schulz K F, et al. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials[J]. Journal of clinical epidemiology, 2010, 63(8): e1-e37.
31. Boutron I,Altman D G,Moher D,et al. CONSORT Statement for Randomized Trials of Nonpharmacologic Treatments: A 2017 Update and a CONSORT Extension for Nonpharmacologic Trial Abstracts[J]. Annals of Internal Medicine,2017,167(1):40-47.

32. Moher D,Chan A. SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials)[M]// Guidelines for Reporting Health Research: A User's Manual. John Wiley & Sons,Ltd,2014:56-67.
33. National Kidney Foundation. KDOQI clinical practice guideline for diabetes and CKD: 2012 update. Am J Kidney Dis. 2012;60(5):850–86.
34. Levin A, Stevens P E, Bilous R W, et al. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease[J]. Kidney International Supplements, 2013, 3(1): 1-150. (Google Scholar)
35. Mogensen CE,Christensen CK,Vittinghus E. The stages in diabetic renal disease. With emphasis on the stage of incipient diabetic nephropathy. Diabetes. 1983;32(Suppl 2):64-78
36. Piccoli GB,Grassi G,Cabiddu G,Nazha M,Roggero S,Capizzi I,et al.Diabetic kidney disease: a syndrome rather than a single disease. Rev Diabet Stud.2015;12(1-2):87-109.
37. Tong X,Xu J,Lian F. Structural Alteration of Gut Microbiota during the Amelioration of Human Type 2 Diabetes with Hyperlipidemia by Metformin and a Traditional Chinese Herbal Formula: a Multicenter,Randomized,Open Label Clinical Trial[J]. Mbio,2018,9(3).
38. Yu X,Xu L,Zhou Q,et al. The Efficacy and Safety of the Chinese Herbal Formula,JTTZ,for the Treatment of Type 2 Diabetes with Obesity and Hyperlipidemia: A Multicenter Randomized,Positive-Controlled,Open-Label Clinical Trial.[J]. International Journal of Endocrinology,2018,(2018-4-1),2018,2018(1):9519231.
39. Tian J,Lian F,Yu X,et al. The Efficacy and Safety of Chinese Herbal Decoction in Type 2 Diabetes: A 5-Year Retrospective Study[J]. Evid Based Complement Alternat Med,2016,2016(9):5473015.
40. Lian F,Tian J,Chen X,et al. The Efficacy and Safety of Chinese Herbal Medicine Jinlida as Add-On Medication in Type 2 Diabetes Patients Ineffectively Managed by Metformin Monotherapy: A Double-Blind,Randomized,Placebo-Controlled,Multicenter Trial[J]. Plos One,2015,10(6):e0130550.
41. Xu J,Lian F,Zhao L,et al. Structural modulation of gut microbiota during alleviation of type 2 diabetes with a Chinese herbal formula[J]. Isme Journal,2015,9(3):552-562.
42. Ji L,Tong X,Wang H,et al. Efficacy and safety of traditional chinese medicine for diabetes: a double-blind,randomised,controlled trial[J]. Plos One,2013,8(2):e56703.
43. Chen H,Jing G,Zhao X,et al. Retrospective analysis of the overt proteinuria diabetic kidney disease in the treatment of modified Shenzhuo formula for 2 years[J]. Medicine,2017,96(12):e6349.
44. He L,Wang H,Gu C,et al. Administration of Traditional Chinese Blood Circulation Activating Drugs for Microvascular Complications in Patients with Type 2 Diabetes Mellitus[J]. J Diabetes Res,2016,2016(10):1-9.
45. Lian F,Wu L,Tian J,et al. The effectiveness and safety of a danshen-containing Chinese herbal medicine for diabetic retinopathy: A randomized,double-blind,placebo-controlled multicenter clinical trial[J]. Journal of Ethnopharmacology,2015,164:71-77.
46. Lin MY,Chiu YW,Chang JS,Lin HL,Lee CT,Chiu GF,Kuo MC,Wu MT,Chen HC,Hwang SJ. Association of prescribed Chinese herbal medicine use with risk of end-stage renal disease in patients with chronic kidney disease.Kidney Int. 2015;88(6):1365–73

47. Fan X M,Huang C L,Wang Y M,et al. Therapeutic Effects of Tangshen Formula on Diabetic Nephropathy in db/db Mice Using Cytokine Antibody Array.[J]. Journal of Diabetes Research,2018,(2018-2-22),2018,2018(3):1-10.
48. Zhao T T,Sun S F,Zhang H J,et al. Therapeutic Effects of Tangshen Formula on Diabetic Nephropathy in Rats[J]. Plos One,2016,11(1):e0147693.
49. Huang M, Zhu C, Liang Q L,et al. [Effect of tangshen formula on phospholipids metabolism in diabetic nephropathy patients.[J]. Acta Pharmaceutica Sinica,2011,46(7):780. (PMID:22010346)
50. Fan X M, Huang C L, Wang Y M, et al. Therapeutic Effects of Tangshen Formula on Diabetic Nephropathy in db/db Mice Using Cytokine Antibody Array[J]. Journal of diabetes research, 2018.
51. Miranda-Díaz A G,Pazarín-Villaseñor L,Andrade-Sierra J. Oxidative Stress in Diabetic Nephropathy with Early Chronic Kidney Disease[J]. Journal of Diabetes Research,2016,(2016-7-20),2016,2016(1).
52. Kashihara N,Haruna Y,Kondeti V K,et al. Oxidative Stress in Diabetic Nephropathy[J]. Current Medicinal Chemistry,2010,17(34):4256-4269.

Table

Table.1. Measurement items and point of data capture

Visit Project	Screening period / baseline	Visit 1-2, 4-5	Visit 3	Visit 6
Visiting time	-7□0 days	Medication 4,8,16,20 weeks ± 7 days	Medication 12 weeks ± 7 days	Medication 24 weeks ± 7 days
Collect basic medical history				
Sign informed consent	×			
Fill in the general information	×			
Medical history and treatment history	×			
Determine inclusion and exclusion criteria	×			
Vital signs	×	×	×	×
Physical examination	×	×	×	×
Co-morbidity and medication records	×	×	×	×
Monitoring and inspection				
Urine pregnancy test	×			
Glycosylated hemoglobin, Blood lipids	×		×	×
Fasting blood sugar and blood pressure	×	×	×	×
Effectiveness observation				
Urinary microalbumin creatinine ratio	×	×	×	×
Urinary microalbumin excretion rate	×	×	×	×
Serum creatinine	×	×	×	×
Glomerular rate filtration	×	×	×	×
24h urinary microalbumin and 24h urinary microalbumin quantification	×		×	×
Physic-chemical examination				
Routine blood test and routine urine test	×		×	×
Stool routine examination	×		×	×
Vital signs	×	×	×	×
ECG, liver and kidney function	×		×	×
Adverse event		×	×	×
Other work				
Random grouping	×			
Distribute drug and patient journal cards	×	×	×	
Recovery drugs, quantity statistics		×	×	×
Recycle patient's diary card		×	×	×

End of study summary				×

Figures

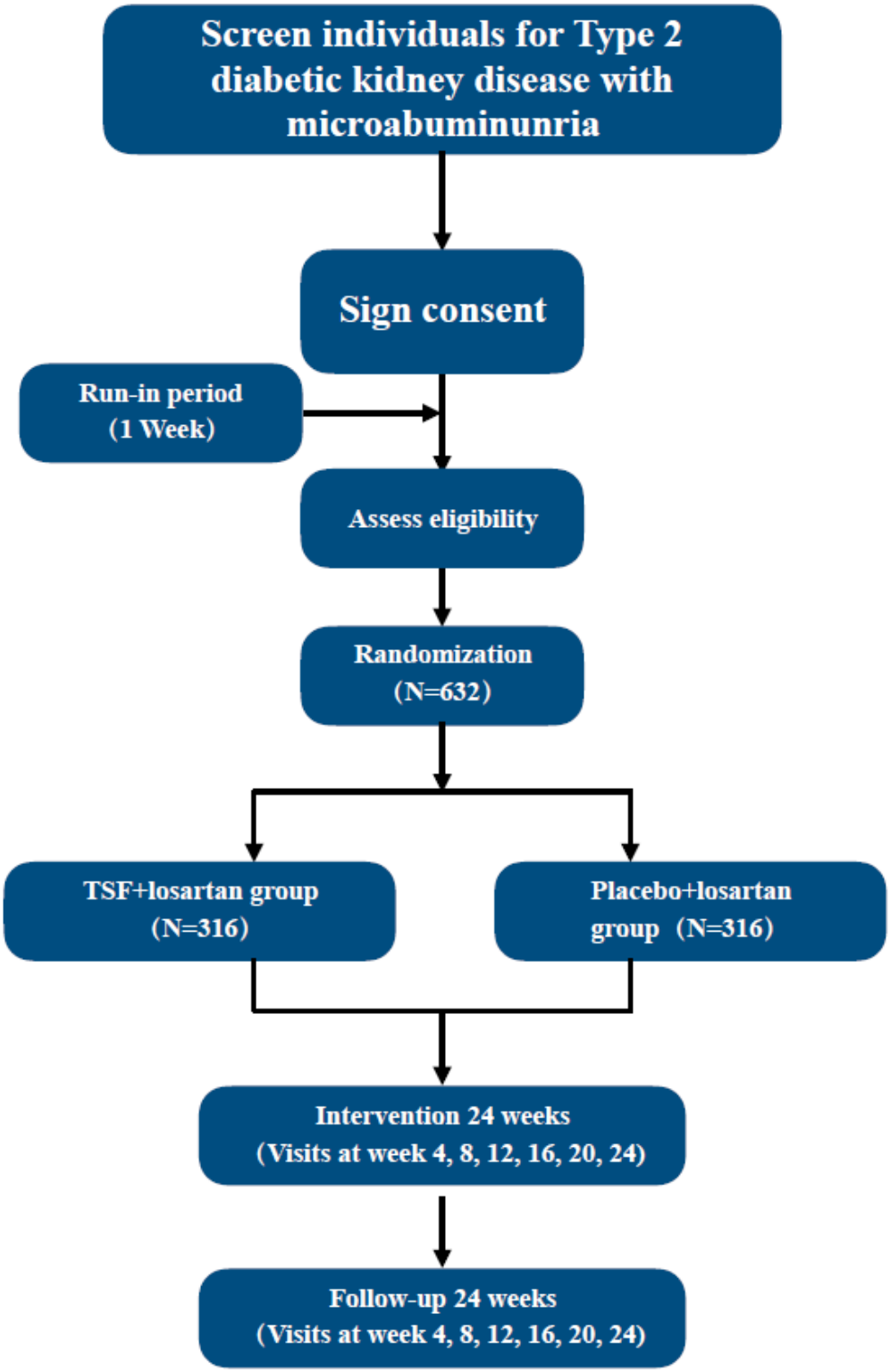

Figure 1

The diagram of enrollment, intervention, and assessments

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SPIRIT2013Checklist1.pdf
- Formula1.jpg