PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Semi-individualised Chinese Medicine Treatment as an Adjuvant Management for Diabetic Nephropathy – A pilot add-on, randomised, controlled, multi-centre, open-label pragmatic clinical trial
AUTHORS	CHAN, Kam Wa; IP, Tai Pang; KWONG, Alfred; LUI, Sing Leung; CHAN, Gary; Cowling, Benjamin; YIU, Wai Han; WONG, Dickson; LIU, Yang; FENG, Yibin; TAN, Kathryn; CHAN, Loretta; LEUNG, Joseph; LAI, Kar Neng; TANG, Sydney

VERSION 1 - REVIEW

REVIEWER	Shih-Hua Lin Tri-Service General Hospital, Taipei, Taiwan
REVIEW RETURNED	17-Dec-2015

GENERAL COMMENTS	General comments: Dr. Chan and colleagues reported the protocol of their clinical trial focusing on the effect of a regimen of Chinese medicine (CM) for retarding diabetic nephropathy (DN). The trial design conforms to a conventional one, but more information is needed to clarify the detail. Specific comments: 1. The authors cited an observation study from Taiwan suggesting that use of Chinese medicine was associated with lower risk of end-stage renal disease (ESRD). However, in the subgroup analysis of that study, CM of different classes seemed to have contrary effect on the risk of precipitating renal failure. The authors carefully exclude those CMs with a reported higher risk of ESRD in the literature, in order to comply with the ethical requirement (their intervention group). 2. More rationale was needed to justify the use of these CMs in retarding diabetic nephropathy alone, instead of all patients with chronic kidney disease (CKD). This is not mentioned in the introduction. 3. The protocol on which the authors based their treatment decision was not accessible and raised concern (reference 18). 4. The enrollment criteria needed to be more specific. Should diabetic retinopathy be one of the criteria for recognizing diabetic nephropathy? Also, the rationale of enrolling CKD stage 2 to 3 patients only needed to be provided, since those with late stage CKD were more likely to have renal progression and the benefit accrued from CMs might be more prominent. Furthermore, stage 3 CKD patients are frequently subdivided into stage 3a and 3b based on their risk of renal progression. Should this be factored into

	consideration? 5. More data regarding herbal safety should be recorded instead of only collecting hepatotoxicity in this trial (page 14, herbal safety section). 6. The termination criteria were vague. The authors only described that the presence of severe adverse effect would prompt discontinuation of trial treatment in any specific patient, but the nature of the “severe adverse effect” was not provided. How did the authors grade the severity of given toxicity, including hepatotoxicity, marrow toxicity, and other? This was similarly found in the termination criteria of the entire trial. The serious adverse events were better pre-specified and collected while minor adverse events were narratively recorded. Minor comments: 1. Should there be an interim analysis, since certain CMs were known to increase the risk of ESRD? This was not mentioned in the protocol, nor is any safety committee assembling addressed in the protocol.

REVIEWER	Jwa-Kyung Kim Department of Internal Medicine & Kidney Research Institute, Hallym University Sacred Heart Hospital, Anyang, Korea
REVIEW RETURNED	08-Jan-2016

GENERAL COMMENTS	The objective of this study “Semi-individualised Chinese Medicine Treatment as an Adjuvant Management for Diabetic Nephropathy – A pilot add-on, randomized, controlled, multi-centre, open-labelled clinical trial” is to access the feasibility for a subsequent phase III RCT through preliminary evaluation of the effect of an adjuvant semi-individualized CM treatment to DN patients with stage 2 -3 with microalbuminuria. The study design is an assessor-blind, add-on, randomized, controlled, parallel, multi-centre, open-labelled pilot clinical trial. Primary end-points are the changes in renal function and the amount of albuminuria. Although the topic is novel and interesting, I have the follow questions: - Safety) Although Chinese Medicine is popular in some areas, Are they or their ingredients or formulas are safe? In general, the addition of herbal medicine or OTCs is prohibited in clinical practice especially in patients with decreased renal function. Is there no possibility that the addition of CM may be harmful? As shown in recent data of Lin MY (KI 2015;88:1365-73), some formulas were associated with increased ESRD risk. The CM in your study did not include the formula? Also, is there no interaction between CM and glucose control? If the addition of CM may affect serum glucose and HbA1c levels, the effect of CM may not clear. Also the relationship between CM and the changes of BMI should be clearly shown. Recently, most diabetic ESRD patients became obese. If the addition of CM influence BMI or insulin resistance, its effect may be confused. - Unclear rationale) The authors hypothesized that the add-on

	therapy of CM may be related with less severe decrease in eGFR than those without CM therapy. Then, it means the addition of CM retard eGFR decline rate? The absolute differences in eGFR may not reflect the true effect of CM on renal function. - Sample size) please provide the rationale for sample size and power calculation - Inclusion criteria) stable dose of ACEi or ARB was required in inclusion criteria, then how about other medication that has anti-proteinuric effect such as statin or vitamin D?

REVIEWER	Cheng Shi Hui Universiti Putra Malaysia Malaysia
REVIEW RETURNED	18-Apr-2016

GENERAL COMMENTS	This manuscript is regarding the effect of Chinese medicine treatment as an adjuvant treatment for diabetic nephropathy, this study is of potential interest, however, some points remain unclear and need to be addressed before a more definite evaluation of the paper is possible. Major points: 1) the authors indicate 5 different Chinese medicine but there is no further discussion as to how they choose the Chinese medicine and how each Chinese medicine would benefit the renal outcome. Besides, it is also not clear why the author divided the Chinese medicine treatment group into 5 subgroups. 2) there is no clear hypothesis in this study. 3) Also it is well known that several factors such as high blood glucose level, high blood pressure and high protein diet can affect the renal function test. How does this take into account? 4) there is not sufficient detail in the protocol to replicate the study, it is not clear how the authors measure the outcome measurements including urine albumin-to-creatinine ratio, FBG, HbA1c, liver function test, serum fibroblast growth factor 23 (FGF-23), urinary monocyte chemotactic protein 1 (MCP-1), urinary cystatin C, urinary nephrin, urinary transforming growth factor beta-1 (TGF-β1) and urinary vascular endothelial growth factor (VEGF). 5) it is not clear how the authors determine whether the patient has hypersensitivity towards Chinese medicine 6) it is not clear whether the sample size calculation is powered to achieve statistical significance. In the limitation, the sample size is not powered, while in the sample size calculation, power analysis is discussed. Minor points: 1) Some of the text have grammatical errors. Please proofread the whole paper. 2) Figure 1 is not clear. The 5 subgroup of Chinese Medicine is not explained in the figure.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1
Comment 1:
The authors cited an observation study from Taiwan suggesting that use of Chinese medicine was associated with lower risk of end-stage renal disease (ESRD). However, in the subgroup analysis of that study, CM of different classes seemed to have contrary effect on the risk of precipitating renal failure. The authors carefully exclude those CMs with a reported higher risk of ESRD in the literature, in order to comply with the ethical requirement (their intervention group)
Reply:
To comply with the ethics requirement, we have excluded all herbal medicines with known toxicity that is listed in Schedule 1, Chapter 549, Chinese Herbal Medicines, Chinese medicine Ordinance of Hong Kong. The herbal formulas that have been shown to increase the risk of end-stage renal failure in the captioned study have been excluded for safety concern.
Supplementary information has been inserted in the section 'Herbal Safety'

Comment 2:
More rationale was needed to justify the use of these CMs in retarding diabetic nephropathy alone, instead of all patients with chronic kidney disease (CKD). This is not mentioned in the introduction.
Reply:
The pathogenesis and clinical manifestation of diabetic nephropathy is different from other chronic kidney diseases. Hyperglycemia has been proven to participate in the pathogenesis of diabetic nephropathy via direct glycotoxicity, advance glycation end-products and reactive oxygen species. Clinically hyperglycemia plays a critical role in the development of diabetic nephropathy and tight glucose control has been shown in major RCTs to retard the progression of diabetic nephropathy. Therefore, we would like to evaluate the effect of Chinese medicine on diabetic nephropathy specifically.
Supplementary information has been inserted in the section 'Background'.

Comment 3:
The protocol on which the authors based their treatment decision was not accessible and raised concern (reference 18).
Reply:
The clinical protocol could be accessed through the China National Knowledge Infrastructure database. Since the protocol is written in Chinese, a detailed Chinese Medicine subgroup diagnosis and treatment section are extracted and presented in the manuscript. The dosage of medication depends on the clinical decision and we have adapted the protocol by fixing the dose based on the recommendation of Chinese medicine expert and China pharmacopoeia.
Supplementary information on the diagnostic criteria has been inserted in the section 'Intervention and control'

Comment 4:
The enrollment criteria needed to be more specific. Should diabetic retinopathy be one of the criteria for recognizing diabetic nephropathy? Also, the rationale of enrolling CKD stage 2 to 3 patients only needed to be provided, since those with late stage CKD were more likely to have renal progression and the benefit accrued from CMs might be more prominent. Furthermore, stage 3 CKD patients are frequently subdivided into stage 3a and 3b based on their risk of renal progression. Should this be factored into consideration?
Reply:
Macroalbuminuria is a strong indicator of chronic kidney disease related to diabetes. Diabetic retinopathy, although highly associated with diabetic nephropathy alone, provides varied and controversial additional specificity on top of macroalbuminuria. Based on the recommendation from KDOQI guideline, we decided to include macroalbuminuria rather than diabetic retinopathy into

inclusion / exclusion criteria and we will report the rate of diabetic retinopathy of individual subgroups. Currently, there is limited high quality data from prospective interventional study evaluating the effect of Chinese medicine on diabetic nephropathy. To ensure safety of patients, we decided to include only stage 2 to stage 3 patients in this pilot since they are more stable clinically. We will consider including later CKD stages in the subsequent full study depending on the trend of change in renal function and liver function. We will also report the rate of GFR attrition and perform subgroup analysis on stage 3a and 3b patients.

Comment 5:
More data regarding herbal safety should be recorded instead of only collecting hepatotoxicity in this trial (page 14, herbal safety section).
Reply:
Different from new drug development, this pilot trial is evaluating an additional Chinese medicine programme using marketed and well-established Chinese herbal medicine prescribed by a licensed Chinese medicine physician. According to the recommendation from World Health Organisation, unlike conventional investigational new drug development, a full range of toxicological tests is not necessary for well-used traditional medicines. For the assessment of safety, we follow post-marketing surveillance strategy to evaluate untoward events including all-cause mortality, cardiovascular death and cancer incidence. Since hepatotoxicity and nephrotoxicity are the most encountered forms of herbal induced toxicity locally, we collect data on hepatotoxicity to monitor liver function. We will also include brian natriuretic peptide test to monitor cardiotoxicity as cardiovascular event is common among patients with DN. We have excluded herbal medicines that are suspected to have genotoxicity and carcinogenicity and we have excluded patients with pregnancy. We also evaluate vital signs and blood picture for patients to monitor the general condition and hematological toxicity. Further investigation will be performed if the reading is deranged and safety is concerned.

Comment 6:
The termination criteria were vague. The authors only described that the presence of severe adverse effect would prompt discontinuation of trial treatment in any specific patient, but the nature of the “severe adverse effect” was not provided. How did the authors grade the severity of given toxicity, including hepatotoxicity, marrow toxicity, and other? This was similarly found in the termination criteria of the entire trial. The serious adverse events were better pre-specified and collected while minor adverse events were narratively recorded.
Reply:
An on-going local service programme implementing similar Chinese medicine prescriptions for 40 diabetic kidney disease patients recorded no serious adverse events for a period of 48 weeks. For serious adverse events, we will examine all hospitalised subjects or subjects at the risk of hospitalisation. Such events may include death, severe cardiac disorders (acute coronary syndrome, cardiac arrest, acute cardiac failure, acute congestive heart failure, cardio-respiratory arrest, cardiogenic shock, myocardial infarction), severe renal disorders (acute kidney injury, renal failure), severe blood and lymphatic disorder (disseminated intravascular coagulation, anemia requiring transfusion), severe gastrointestinal disorders (gastrointestinal hemorrhage, gastric perforation, intestinal ischemia, intestinal perforation), severe infections (pneumonia requiring hospitalization, sepsis), severe metabolism disorder (metabolic acidosis), severe neurological disorders (cerebral haemorrhage, cerebrovascular accident, encephalopathy, intracranial aneurysm, subarachnoid haemorrhage), severe psychiatric disorders (schizophrenia, bipolar disorder, completed suicide), severe respiratory disorders (acute respiratory distress syndrome, pleural effusion, pulmonary embolism, pulmonary oedema, respiratory failure), severe vascular disorder (shock) and any form of neoplasms.
For the grading of adverse events, we record the adverse events based on the Common Terminology Criteria for Adverse Events version 4.03 and follow the FDA guideline to categorise adverse events into 5 grades: Grade 1) mild; asymptomatic or mild symptoms; clinical or diagnostic observations only;

no intervention indicated; Grade 2) moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living; Grade 3) severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living; Grade 4) life-threatening consequences; urgent intervention indicated; and Grade 5) death related to adverse events. For hepatotoxicity, the trial will be terminated for a patient if he/she has 1) a AST or ALT level higher than 8 times of upper limit of normal; or 2) a AST or ALT level higher than 5 times of upper limit of normal for 2 weeks; or 3) a AST or ALT level higher than 3 times of upper limit of normal and a total bilirubin level higher than 2 times of upper limit of normal or an INR level higher than 1.5; or 4) a AST or ALT level higher than 3 times of upper limit of normal and presented with fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia. We follow the guideline of Council for International Organisations of medical Sciences to evaluate causality of hepatotoxicity.
Supplementary information on adverse event management has been inserted in the section 'Herbal safety', 'Termination criteria', 'Outcome measurement' and 'Data analysis'.

Comment 7:
Should there be an interim analysis, since certain CMs were known to increase the risk of ESRD? This was not mentioned in the protocol, nor is any safety committee assembling addressed in the protocol.
Reply:
There will be an interim analysis after 24 weeks of the recruitment of the last patient to evaluate the effect of the Chinese medicine programme. This study is under the supervision of the Clinical Trials Centre of the University of Hong Kong and the Institutional Review Board of the University of Hong Kong / Hospital Authority Hong Kong West Cluster. The review panel of Clinical Trials Centre and Institutional Review Board are composed of independent experts and lay members and require regular safety and efficacy data monitoring and instant reporting of serious adverse events. The Institutional Review Board reserves the right to terminate the trial which functions as the data monitoring board.
Supplementary information on trial management has been inserted in the section 'Organisational structure and responsibilities'.

Reviewer: 2
Comment 1:
Although Chinese Medicine is popular in some areas, Are they or their ingredients or formulas are safe? In general, the addition of herbal medicine or OTCs is prohibited in clinical practice especially in patients with decreased renal function. Is there no possibility that the addition of CM may be harmful? As shown in recent data of Lin MY (KI 2015;88:1365-73), some formulas were associated with increased ESRD risk. The CM in your study did not include the formula?
Reply:
According to the observational study from Taiwan (Lin MY et al. 2015), prescribed Chinese medicines are generally safe. We have excluded all herbal medicines with known toxicity that listed in Schedule 1, Chapter 549, Chinese Herbal Medicines, Chinese medicine Ordinance of Hong Kong. The herbal formulas that have been shown to increase the risk of end-stage renal failure in the captioned study have been excluded for safety concern.
Supplementary information has been inserted in the section 'Herbal Safety'

Comment 2:
Also, is there no interaction between CM and glucose control? If the addition of CM may affect serum glucose and HbA1c levels, the effect of CM may not clear. Also the relationship between CM and the changes of BMI should be clearly shown. Recently, most diabetic ESRD patients became obese. If the addition of CM influence BMI or insulin resistance, its effect may be confused.

Reply:
We do not exclude the possibility that the use of CM may affect blood glucose, BMI and insulin resistance. We will control the effect of CM on blood glucose, BMI and insulin resistance with regression analysis. Adjusted mean of primary outcome measures (GFR and UACR) will be presented. We will also measure the C-peptide and fasting insulin level of patients to examine the insulin resistance.
Supplementary information on the control of confounding factors has been inserted in the section 'Outcome measurement' and 'Data analysis'

Comment 3:
The authors hypothesized that the add-on therapy of CM may be related with less severe decrease in eGFR than those without CM therapy. Then, it means the addition of CM retard eGFR decline rate? The absolute differences in eGFR may not reflect the true effect of CM on renal function.
Reply:
Estimated GFR is a conventional estimation of kidney function. GFR and UACR have been shown to be independently related to renal progression and all-cause mortality.

Comment 4:
Please provide the rationale for sample size and power calculation
Reply:
Currently, there is no consensus on sample size calculation for pilot studies. Previous studies suggested a sample size of 12-30 per arm. Since the primary objective of this trial is to obtain estimates of key clinical outcomes and explore the feasibility of a subsequent main study, we calculate the sample size based on the control of inflation factor (IF) to the estimation of sample size for the subsequent main study as follow:

IF = Sucl / Sobs = sqrt [(n-1))/ χ^2 1-α, n-1]
Nadj/Nunadj ≈ IF2 ≈ nunadj * IF^2

N ≈ [2(□1- á/2+□1- ß')2(IF*s)^2]/(u1- u2)^2] = [2(□1- á/2+□1- ß)s^2]/(u1- u2)2]
□1- ß' = □1- á/2(IF-1-1) + □1- ß* IF-1

where IF = Inflation factor
Sucl = Standard deviation of upper confidence interval
Sobs = Observed standard deviation in pilot study
á = Chosen confidence level
ß = Nominal power set for main study
ß' = Actual power achieved for main study by using pilot SD for sample size calculation
n = Sample size of pilot study
N = Sample size of main study
Nunadj = Sample size of main study with no adjustment on standard deviation
Nadj = Sample size of main study with adjustment on standard deviation
Since the standard deviation used for sample size calculation for main study is often underestimated by pilot studies, an inflation factor should be used for adjustment. IF is calculated based on the size of the pilot study and the confidence level of achieving at least the desired power in the main study. The actual achieved power of the main study depends on the nominal power set for the main study and the IF.
In order to be 95% confident (two-sided) that the subsequent main study actually achieve a power of 80% with nominal power set at 90% (i.e., a 10% power forfeit), the IF should be less than 1.15. At IF = 1.15, a sample size of 80 is therefore needed to have 95% one-sided confidence that the main study will achieve at least the nominal power to test the hypothesis that an add-on Chinese medicine treatment programme could be more effective in stabilising the glomerular filtration rate among

diabetic nephropathy patients when compared to having standard care alone. For subgroup analysis, a sample size of 25 patients per each subgroup could achieve 80% one-sided confidence that the effect of stabilising glomerular filtration rate is different within subgroups of similar CM clinical pattern. With 5 subgroups, a sample size of 148 patients is therefore needed in this pilot trial to allow a 15% attrition rate.

Comment 5:
Stable dose of ACEi or ARB was required in inclusion criteria, then how about other medication that has anti-proteinuric effect such as statin or vitamin D?
Reply:
We consider ACE-inhibitors and ARBs are major effect modifiers of proteinuria and therefore we have included stable ACE-inhibitor or ARB dose in the inclusion / exclusion criteria. The effect of statins and other medication is relatively less well-proven, mild and controversial. For instance, both pro-proteinuric (R Agarwal, JASN 2004) and anti-proteinuric (Athyros VG et al., Expert Opin Pharmacother 2015) effect of statins have been reported. Likewise, the effect of vitamin D on human is unclear. We will report the use of medications between groups for analysis.

Reviewer 3
Comment 1:
The authors indicate 5 different Chinese medicine but there is no further discussion as to how they choose the Chinese medicine and how each Chinese medicine would benefit the renal outcome. Besides, it is also not clear why the author divided the Chinese medicine treatment group into 5 subgroups.
Reply:
The clinical protocol is synthesized based on expert consensus and literature review. The five clinical subgroups are the most prevalent subgroups of diabetic nephropathy based on the expert consensus and other observational studies. The five corresponding formulas are derived from expert consensus. Currently, there is no evidence-based clinical guideline for the use of Chinese medicine on diabetic nephropathy and this protocol contains the best available evidence of clinical management strategy. We would like to explore the clinical effect of this add-on Chinese medicine treatment programme with proper interventional study.
There are emerging evidence from in vitro and in vivo studies supporting the therapeutic effect of some Chinese herbal medicines in managing diabetic nephropathy, in particular to the key herbs used in our protocol. In general, it has been shown that the herbal medicine used in the formula could reduce proteinuria and alleviate decline of glomerular filtration rate by regulating transforming growth factor-beta (TGF-beta, an important cytokine in the pathophysiology of diabetic nephropathy) and podocyte apoptosis (an important risk factor for subsequent glomerular filtration rate decline) through some of the important pathways including Smad, MAPK and NF-kappa B signaling pathway. A neighbour group led by Zhang YB recently found that Yuye Decoction, a Chinese medicine formula with the same key herbs as our protocol (Astragali Radix and Dioscoreae Rhizoma) has a comparable effect in reducing insulin-induced glucose consumption as metformin in HepG2 cells. Chen J et al. showed that Astragaloside, an extract from one of the key herbs of our protocol, can increase podocyte density and reduce proteinuria in streptozotocin induced diabetic rats. Chen Y et al. showed that Astragaloside IV can reduce ER-stressed podocyte apoptosis via PERK-ATF4-CHOP pathway. Similar effect was observed by Gui D et al. and it was shown that serum TNF-alpha, MCP-1, ICAM-1 can be reduced by Astragaloside, suggesting a suppression on NF-kappa B activation. Nie Y et al. demonstrated a reduced level of TGF-ß1, Smad3, TGFßR-1 by astragalus injection to KKAy mice. Similar effect was observed in Sprague Dawley rats by Lee B et al. by applying Rehmannia, another key herb of the protocol. The clinical effect of improving renal clearance and reducing proteinuria was also demonstrated in some case series and a cohort study.

Comment 2:
There is no clear hypothesis in this study.
Reply:
The aim of this study is to optimise parameters and access the feasibility for a subsequent phase III randomised controlled trial through preliminary evaluation on the effect of the adjuvant semi-individualised CM treatment protocol on type 2 diabetic patients with stages 2 to 3 chronic kidney disease and macroalbuminuria. Since pilot studies are not hypothesis testing in nature, we decided not to include a hypothesis statement to avoid over-emphasis on hypothesis testing.
In the sample size calculation, we have formulated the hypothesis of the subsequent main study as add-on Chinese medicine treatment programme could be more effective in stabilising the glomerular filtration rate among diabetic nephropathy patients when compared to having standard care alone.

Comment 3:
Also it is well known that several factors such as high blood glucose level, high blood pressure and high protein diet can affect the renal function test. How does this take into account?
Reply:
We will control the effect of CM on blood pressure, blood glucose and insulin resistance with regression analysis. Adjusted mean of primary outcome measures (GFR and UACR) will be presented. All patients will be given same advice on diet.
Supplementary information on the control of confounding factors has been inserted in the section 'Outcome measurement' and 'Data analysis'

Comment 4:
There is not sufficient detail in the protocol to replicate the study, it is not clear how the authors measure the outcome measurements including urine albumin-to-creatinine ratio, FBG, HbA1c, liver function test, serum fibroblast growth factor 23 (FGF-23), urinary monocyte chemotactic protein 1 (MCP-1), urinary cystatin C, urinary nephrin, urinary transforming growth factor beta-1 (TGF-β1) and urinary vascular endothelial growth factor (VEGF).
Reply:
Outcomes including urine albumin-to-creatinine ratio, FBG, HbA1c, liver function test, serum fibroblast growth factor 23 (FGF-23), urinary monocyte chemotactic protein 1 (MCP-1), urinary cystatin C, urinary nephrin, urinary transforming growth factor beta-1 (TGF-β1) and urinary vascular endothelial growth factor (VEGF) are measured with commercially available essay kit following manufacturers' instruction. The trial is on-going and we will publish the result with detailed material used after completing the study.

Comment 5:
It is not clear how the authors determine whether the patient has hypersensitivity towards Chinese medicine.
Reply:
Hypersensitivity towards Chinese medicine is determined similarly as other drug hypersensitivity which involves Type I, II, III and IV. Clinical presentation may include history of fever, rash, urticaria, angioedema, bronchospasm, pruritus, vomiting, diarrhea, hemolytic anemia, neutropenia, thrombocytopenia, arthralgia, lymphadenopathy, vasculitis, dermatitis after taking Chinese medicine.

Comment 6:
It is not clear whether the sample size calculation is powered to achieve statistical significance. In the limitation, the sample size is not powered, while in the sample size calculation, power analysis is discussed.

This pilot is powered to provide estimates of treatment effect, variance and other feasibility parameters but not powered for hypothesis testing of whether add-on Chinese medicine treatment

programme could be more effective in stabilising the glomerular filtration rate among diabetic nephropathy patients when compared to having standard care alone.

Comment 7:
Some of the text have grammatical errors. Please proofread the whole paper.
Reply:
Thank you for the reminder. Please refer to the updated manuscript.

Comment 8:
Figure 1 is not clear. The 5 subgroup of Chinese Medicine is not explained in the figure.
Reply:
Thank you for the reminder. Please refer to the updated figure.

VERSION 2 – REVIEW

REVIEWER	Shih-Hua Lin, MD Division of Nephrology Department of Medicine Tri-Service General Hospital National Defense Medical Center Taipei, Taiwan
REVIEW RETURNED	22-Jun-2016

GENERAL COMMENTS	The authors have significantly revised and improved their manuscript. Some minor suggestion is still suggested for completeness. 1. The content in the introduction part is still meager and needs second thought. The authors wrote in their response letter that hyperglycemia has multiple mechanisms of action leading to diabetic nephropathy (DN), but they did not specify whether there are existing studies, experimental or clinical ones, suggesting that Chinese Medicine (CM) can be a viable option for those with DN in the revised introduction section (page 9). I would expect a more robust rationale for this trial if possible. 2. In the response letter, the authors described that “only stage 2 or stage 3 with macroalbuminuria” were enrolled, and this is due to the proposition that they are more stable clinically and in order to ensure patient safety. This argument seems to imply that CM can have more negative influence on those with “unstable renal function”, such as those with stage 4 or 5 CKD. I would expect the authors make more comment on this issue and explain their rationale more clearly in the corresponding part of the revised method section. 3. It would be better if the authors exclude such influence a priori by assuring that the CM regimen they provided would not have any influence upon the factors such as lipid and blood pressure and blood sugar.

VERSION 2 – AUTHOR RESPONSE

Reviewer 1

Comment 1:

The content in the introduction part is still meager and needs second thought. The authors wrote in their response letter that hyperglycemia has multiple mechanisms of action leading to diabetic nephropathy (DN), but they did not specify whether there are existing studies, experimental or clinical ones, suggesting that Chinese Medicine (CM) can be a viable option for those with DN in the revised introduction section (page 9). I would expect a more robust rationale for this trial if possible.

Reply:

There is emerging evidence from in vitro and in vivo studies supporting the therapeutic effect of some Chinese herbal medicines in managing diabetic nephropathy, in particular to the key herbs used in our protocol. In general, it has been shown that the herbal medicine used in the formula could reduce proteinuria and alleviate decline of glomerular filtration rate by regulating transforming growth factor-beta, podocyte apoptosis and podocyte detachment through some of the important pathways including Smad, MAPK and NF-kappa B signaling pathway. A neighbouring group led by Zhang YB recently found that Yuye Decoction, a Chinese medicine formula with the same key herbs as our protocol (Astragali Radix and Dioscoreae Rhizoma) has a comparable effect in reducing insulin-induced glucose consumption as metformin in HepG2 cells. Chen J et al. showed that Astragaloside, an extract from one of the key herbs of our protocol, can increase podocyte density and reduce proteinuria in streptozotocin induced diabetic rats. Chen Y et al. showed that Astragaloside IV can reduce ER-stressed podocyte apoptosis via PERK-ATF4-CHOP pathway. Similar effect was observed by Gui D et al. and it was shown that serum TNF-alpha, MCP-1, ICAM-1 can be reduced by Astragaloside, suggesting a suppression on NF-kappa B activation. Nie Y et al. demonstrated a reduced level of TGF-ß1, Smad3, TGFßR-1 by astragalus injection to KKAy mice. Similar effect was observed in Sprague Dawley rats by Lee B et al. by applying Rehmannia and some other key herbs of the protocol. The clinical effect of improving renal clearance and reducing proteinuria was also demonstrated in some case series and a cohort study.

Supplementary information has been inserted in the section ‘Introduction’.

Comment 2:

In the response letter, the authors described that “only stage 2 or stage 3 with macroalbuminuria” were enrolled, and this is due to the proposition that they are more stable clinically and in order to ensure patient safety. This argument seems to imply that CM can have more negative influence on those with “unstable renal function”, such as those with stage 4 or 5 CKD. I would expect the authors make more comment on this issue and explain their rationale more clearly in the corresponding part of the revised method section.

Reply:

Although supported by existing epidemiological (observational) and experimental evidence, the clinical effect (including efficacy and safety) of Chinese medicine on diabetic kidney disease patients is still unclear. Therefore we would like to start with a pilot trial involving more clinically stable patients to ensure patient safety. We will consider recruiting patients with later stage of chronic kidney diseases after gathering the data from this pilot.

Supplementary information has been inserted in the section ‘Herbal safety’.

Comment 3:

It would be better if the authors exclude such influence a priori by assuring that the CM regimen they provided would not have any influence upon the factors such as lipid and blood pressure and blood sugar.

Reply:

We do not exclude the possibility that the use of CM may affect lipid, blood glucose, BMI and insulin

resistance. We will control the effect of CM on blood glucose, BMI and insulin resistance with regression analysis. Adjusted mean of primary outcome measures (GFR and UACR) will be presented.

VERSION 3 – REVIEW

REVIEWER	Shih-Hua Lin, MD Tri-Service General Hospital National Defense Medical Center
REVIEW RETURNED	14-Jul-2016

GENERAL COMMENTS	The reviewer completed the checklist but made no further comments.