Research on Gut-kidney Axis Regulation of Diabetic Kidney Disease Based on Multi-omics and Bacterio-drug Interaction Control Mechanism

Research program

Version：V1.0

Version Date：2021-02-22

NCT number: _____

Title Code: U21A20411

Research Unit: The First Affiliated Hospital of Hunan university of Traditional Chinese Medicine

Department: Endocrinology, Nephrology

1 Background

Since the 21st century, the number of diabetes mellitus (DM) cases worldwide has increased rapidly. According to the International Diabetes Federation, in 2021, 537 million adults had diabetes globally, and it is expected that by 2045, this number will exceed 780 million. China has the highest number of diabetes-related deaths each year, approximately 10,000[1]. Nearly half of type 2 diabetes mellitus type 2 (T2DM) will develop into diabetic kidney disease (DKD) [2, 3]. DKD has the characteristics of high prevalence, many pathogenesis mechanisms, and lack of effective strategies for treatment and management.

In the past 30 years, DKD has become the most common cause of kidney failure and end-stage kidney disease (ESKD), and it is also the main cause of death in diabetics[4-6]. Early detection helps to overcome treatment inertia and achieve timely medical intervention to maximize the preservation of diabetes. The patient's renal function is essential to avoid ESKD and improve clinical outcomes. These measures are crucial for preventing patients from progressing to ESKD and improving clinical outcomes.

The gold standard for diagnosing DKD is renal biopsy, which offers the highest accuracy. However, due to the invasive nature of renal biopsy, patient acceptance is low, limiting its widespread applicability. It is primarily used when it is difficult to distinguish between DKD and non-diabetic kidney disease, and it is not the preferred diagnostic method for DKD.

Over the past decade, the advent and application of multi-omics technologies, including metabolomics, proteomics, and genomics, has led to a growing recognition of the pivotal roles played by intestinal dysbiosis and intestinal-derived metabolites in the pathogenesis of DKD[7, 8]. The prevailing view in modern medicine is that intestinal flora plays a pivotal role in the digestive process, influencing the synthesis, absorption, and metabolism of nutrients. Additionally, it forms a crucial component of the intestinal barrier, which serves as a vital line of defense against invading pathogens. This concept is also reflected in traditional Chinese medicine, where the spleen is regarded as the primary regulator of physiological functions. In light of these findings, the identification of DKD gut-derived metabolic markers and the restoration of gut flora homeostasis from the perspective of the intestinal flora through the use of multi-omics techniques may represent promising strategies for the prevention and management of DKD.

This study aims to employ microbial metabolomics to gain a deeper understanding of the Traditional Chinese Medicine (TCM) pathogenesis of DKD. It seeks to provide a scientific foundation for this field and to guide TCM theories in the clinical practice of DKD prevention and management.

2 Methods

2.1 Trial overview, ethical consideration, and patient recruitment

This study is an ongoing, single-center, cross-sectional observational clinical trial. The subjects were selected for observation and subsequent analysis for key biomarkers such as blood glucose, Urine Albumin-to-Creatinine Ratio(UACR), and renal function. Blood, urine, feces, and moss on the tongue samples were collected from the subjects and stored for subsequent analysis. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Hunan University of Traditional Chinese Medicine in accordance with the relevant ethical standards. The study is currently being conducted at the First Affiliated Hospital of Hunan University of Traditional Chinese Medicine and involves four groups of participants: type 2 diabetic kidney disease (DKD), type 2 diabetes mellitus (T2DM), chronic kidney disease (CKD), and healthy human. Subjects will be recruited from inpatients or outpatients of the Department of Endocrinology and the Department of Nephrology of the First Affiliated Hospital of Hunan University of Traditional Chinese Medicine. Additionally, healthy human subjects will be recruited from the general public through printed advertisements, invitations, etc. The total number of individuals proposed to be recruited in the four groups is 720.

2.2 Study outcomes

Primary outcome: By employing a combination of microbiomic and metabolomic technologies, we conducted a comprehensive analysis of the intestinal flora and serum metabolic profiles associated with DKD. This approach enabled us to identify potential enterogenous disease markers of DKD and to investigate the metabolic profiles that may be linked to disease progression.

Secondary outcome: The objective of this study is to analyze the interaction between intestinal flora and intestinal-derived metabolites in the disease process of DKD. Additionally, the aim is to provide new disease diagnostic strategies and progression risk assessment indicators for diabetic nephropathy, which can be applied to early identification, precise diagnosis, and prognosis of the disease.

Exploratory outcomes ： In accordance with the test results, each group will select four to six remaining fecal samples as the donor source for allogeneic fecal bacteria transplantation. Subsequently, the correlation between intestinal flora and enteric metabolites in diabetic nephropathy will be investigated through the method of human-mouse allogeneic flora transplantation.

2.3 Study population

This study primarily recruited Type 2 diabetic kidney disease (DKD),Type 2 diabetes mellitus (T2DM), Chronic kidney disease (CKD), and healthy human.

2.3.1 Patient inclusion criteria：

(1) Age ≥18 years old and ≤75 years old, gender is not limited.

(2) Complete demographic data.

(3) Meet the diagnostic criteria of the Chinese Guidelines for the Prevention and Treatment of Diabetic Kidney Disease (2021 edition), the Chinese Guidelines for the Prevention and Treatment of Type 2 Diabetes Mellitus (2020 edition), and the Guidelines for the Screening, Diagnosis, Prevention and Treatment of Chronic Kidney Disease (2017 edition).

(4) The patient is informed of the study as approved by the ethics committee.

2.3.2 Healthy population inclusion criteria:

(1) The patient has no known history of infection or risk factors including HIV hepatitis B or C virus syphilis etc. and no current systemic infection;

(2) No obesity (body mass index >30) and/or diabetes no history of chronic kidney disease;

(3) Approved by the ethics committee patients were informed about the study.

2.3.3 Exclusion criteria：

(1) A history of infection or other acute disease within one month prior to enrollment.

(2) People who are in a state of stress.

(3) Other unstable chronic diseases;

(4)A history of acute and chronic gastrointestinal diseases, including acute gastroenteritis, functional gastrointestinal diseases, inflammatory bowel disease, celiac disease, and other chronic gastrointestinal diseases.

(5) Individuals who have received systemic antibiotics, immunosuppressants, chemotherapy, or chronic treatment with proton pump inhibitors within the past three months will be excluded from participation.

(6) Pregnant or lactating women.

(7) Individuals who have participated in other drug clinical trials within the past three months.

(8) Individuals who suffer from mental illness, intellectual disability, confusion, or other conditions that may interfere with their ability to cooperate with the completion of relevant information collection will also be excluded.

In order to ensure the rigor and reliability of the study, subjects who met one or more of the aforementioned criteria were excluded.

2.4 Study procedures

2.4.1 Questionnaire to collect information

(1) General items: name, gender, age, BMI, education level, labor intensity, dietary preferences, etc;

(2) TCM symptoms, tongue and pulse;

(3) Clinical information: disease duration, other complications (e.g., diabetic peripheral neuropathy, diabetic retinopathy, diabetic cardiomyopathy, etc.), concomitant diseases (hypertension, coronary artery disease, hyperlipidemia, cerebrovascular disease, and hyperuricemia, etc.), past medical history, history of smoking, alcohol, family history, and history of drug use.

2.4.2 Outcome Measures

Indicators of safety: Physical examination (including general condition, blood pressure, heart rate, heart rhythm, body temperature, respiratory rate, etc.) was

performed before taking the samples to assess the basic condition of the subjects.

Primary Outcome Measures : Urine Albumin-to-Creatinine Ratio(UACR), Estimated Glomerular Filtration Rate(eGFR),blood urea nitrogen(BUN), serum creatinine(Scr), fasting blood glucose(FBG), Glycosylated Hemoglobin, Type A1C(HbA1c), glucosylated serum protein(GSP) , etc. Test data were obtained from test results conducted within one month of enrollment for the purpose of evaluation.

Secondary Outcome Measures: 16S ribosomal DNA(16S rDNA) identification, metagenomic Next Generation Sequencing, Non-targeted and targeted Metabolomics technology detection , etc.

2.5 Data analysis

SPSS 26.0 will be applied for statistical analysis of clinical indicators. Data that exhibited a normal distribution were expressed as the mean ± standard deviation(`x±s), and an independent samples t-test was employed for comparison between two groups. Data that did not conform to a normal distribution were expressed as the median and interquartile spacing [M(IQP)], and a Mann-Whitney rank-sum test (U test) was used for comparison between two groups. In the case of count data, these were expressed as frequencies or percentages. In the event of unordered categorical count data, comparisons between two groups were performed using either the four-cell table or the column table c^2 test. The Pearson c^2 test or Fisher's exact probability method was employed in accordance with the sample size and the size of the theoretical frequency. In the case of ordered categorical count data, comparisons between two groups were performed using the U test. A two-sided test was employed for the normality test and variance chi-square test, with an alpha level of 0.1. This resulted in the rejection of the original hypothesis, indicating that the data are not normally distributed or that there is no significant variance. For other significance tests, an alpha level of 0.05 was used, leading to the rejection of the original hypothesis and the conclusion that the difference is statistically significant.

An integrated correlation analysis will be performed using a variety of analytical techniques, including intestinal flora species diversity analysis, Spearman's correlation analysis, network analysis, Procrustes analysis, MaAsLin analysis, ERMANOVA analysis, random forest analysis of differential intestinal flora with differential metabolites between groups, and efficacy evaluation.

3 Discussion

DKD is a common complication in the late stage of diabetes. What's more, it's a common cause of renal failure.By introducing the factors of gut microbiota and metabolites through multi-omics techniques, we conduct an analysis on the correlation of the onset of diabetic kidney disease to look for marker microbiota or metabolites and find relevant risk factors.This not only helps to explore the pathogenesis of DKD, but also provides a scientific basis for the early diagnosis, prevention and treatment of DKD. Even it is beneficial for delaying the progression of the disease, for drug research and development, and for changing potential treatment

strategies in the follow-up.

References:

[1] Saeedi P, Petersohn I, Salpea P, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9(th) edition[J]. Diabetes Res Clin Pract, 2019,157:107843.

[2] Thomas M C, Cooper M E, Zimmet P. Changing epidemiology of type 2 diabetes mellitus and associated chronic kidney disease[J]. Nat Rev Nephrol, 2016,12(2):73-81.

[3] Gheith O, Farouk N, Nampoory N, et al. Diabetic kidney disease: world wide difference of prevalence and risk factors[J]. J Nephropharmacol, 2016,5(1):49-56.

[4] Fu H, Liu S, Bastacky S I, et al. Diabetic kidney diseases revisited: A new perspective for a new era[J]. Mol Metab, 2019,30:250-263.

[5] Li H, Lu W, Wang A, et al. Changing epidemiology of chronic kidney disease as a result of type 2 diabetes mellitus from 1990 to 2017: Estimates from Global Burden of Disease 2017[J]. J Diabetes Investig, 2021,12(3):346-356.

[6] Rawshani A, Rawshani A, Franzen S, et al. Risk Factors, Mortality, and Cardiovascular Outcomes in Patients with Type 2 Diabetes[J]. N Engl J Med, 2018,379(7):633-644.

[7] Wang P, Wang T, Zheng X, et al. Gut microbiota, key to unlocking the door of diabetic kidney disease[J]. Nephrology (Carlton), 2021,26(8):641-649.

[8] Lin J, Wang Z, Sun J, et al. Gut microbiota and diabetic kidney diseases: Pathogenesis and therapeutic perspectives[J]. World J Diabetes, 2022,13(4):308-318.