UNIVERSIDAD AUTÓNOMA DE NUEVO LEÓN
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Research protocol:

Fecal microbiota transplantation as a therapeutic strategy in the progression of chronic kidney disease

Monterrey, Nuevo Leon; to July 26, 2018

Version 3.0 protocol
July 26, 2018

1. Background:

Chronic kidney disease (CKD) at its different stages, has a prevalence of 8 to 16% in the world's population (1) being diabetes mellitus, essential hypertension and glomerulonephritis the leading causes (1). The increasing incidence of this disease has raised for health systems unsustainable renal replacement therapies including peritoneal dialysis, hemodialysis and kidney transplantation (1). Once CKD is set the main treatment goal is to slow the progression and cardiovascular complications due to traditional risk factors such as hypertension, atherosclerosis, among others and non-traditional as the chronic inflammatory state described in CKD patients (2).

In recent years information regarding intestinal microbiota (IM) and its modulatory influence in inflammatory conditions has increased (3). It's been found it to influence systemic diseases such as; multiple CKD, metabolic diseases, autoimmune pathologies, neuro-psychiatric diseases, obesity, chronic ulcerative colitis, *Clostridiodes difficile* infection etc. (4, 5). The IM unbalance due quantitative and qualitative changes in its composition and metabolic activities is called dysbiosis (6). This disruption in the symbiotic relationship with the host is associated with the onset and development of complications and progression of many diseases (4). In CKD compounds such as indoles, phenols and ammonia are produced by the fermentation of IM undigested products that are then absorbed by the host to subsequently be excreted by the kidneys and in CKD accumulate due the decreased renal clearance (7,8).

Uremic toxins generated in the intestine favor oxidative stress in addition to inflammatory conditions present in these patients such increased indoxyl sulfate (IS) and p-Cresol sulfate (PCS) have been associated with a greater number of cardiovascular events and increased mortality in patients with CKD as well as persistent anemia, mineral metabolism disorders and increase in the progression of CKD (9, 10). It is increasingly clear that local and systemic consequences of kidney damage may result largely from changes in IM (7). Ramezani et. al. proposed understanding the pathological effects of dysbiosis on the IM as a therapeutic target in order to restore symbiosis (6). Studies have shown that administration of synbiotics, probiotics and prebiotics show a significant decrease in the levels of PCS and IS sulfate in adults with CKD in pre dialytic stage after altering stool microbiome (11, 12, 13, 14).

Fecal microbiota transplantation (FMT) from healthy donors is an alternative therapy with high success rates to correct dysbiosis. So far, this therapy is recommended by European guidelines as a medical therapy for relapsing *C. difficile* infections or refractory to standard medical treatments with an IA

evidence level. (15). Because of the exponential increase of people with CKD and the lack of effective measures to stop the progression of the disease it is necessary to study simple alternatives that could positively modify its natural course. The FMT is a safe simple measure able to reduce the systemic inflammatory process of CKD patients and therefore its progression. Once installed the CKD's main treatment's objective is to stop the decline in glomerular filtration rate (GFR). The inflammatory status of patients with CKD increases the progression of the disease, decreasing the GFR ranges from 1 to 5 ml / min /1.73 m^2 per year; according to KDIGO the average is 3 ml / min / 1.73 m^2 and ≥5 ml / min / 1.73 m^2 per year or a 25% GFR decreased from the baseline in those with rapid progression. This is a reason why our randomized double-blind clinical trial seeks to identify the impact of the FMT on the progression of chronic kidney disease and to evaluate the modification of intestinal microbiome in patients with chronic before kidney disease and after being subjected to FMT. A browse in PubMed database showed article in which FMT had been used to address this matter up to date June 2017, demonstrating the originality of the project.

2. Overall objective:

Identify the impact of intestinal microbiota transplantation in the progression of chronic kidney disease

3. Hypothesis:

Modification of intestinal microbiome in CKD patients by FMT decreases the CKD progression

4. Null Hypothesis:

Modification of intestinal microbiome of CKD patients by FMT doesn't lessens the CKD progression

5. Specific objectives:

- Evaluate changes of bowel microbiome in CKD patients before and after undergoing FMT
- Evaluate the behavior of CKD progression markers in patients undergoing FMT
- Evaluate whether a decrease of inflammation markers in CKD patients is after FMT treatment is developed

6. Materials and methods:

A) Methodological Study design:

Observational	☐	or	Experimental	X
Cross	☐	or	Longitudinal	☐
Comparative	☐	or	Descriptive	☐
Prospective	**X**	or	Retrospective	☐
Not blind	☐	or	Blind	X

B) Type of study:

1)	Survey or cross cohort	☐
2)	Cases and controls	☐
3)	Cohorts	☐
4)	Controlled Clinical Trial	X
5)	Study Diagnostic Test	☐

C) Population Studies:

- Number of patients: Outpatient Nephrology
- Number of subjects to include and basis of calculation: 28
- Population characteristics: kidney disease stage 4

A. Inclusion criteria:

- Being diagnosed with CKD, creatinine clearance less than 60 mL/minute, secondary hypertension and / or diabetes
- Older than 18 years of age

B. Exclusion criteria:

- Malignancies' treatment within the last 5 years
- Antibiotic treatment for any reason during the month prior to enrollment
- Use of probiotics in the last 3 months
- Diagnosed *Clostridiodes difficile* infection in the last year
- Previous FMT treatment
- Exacerbations of CKD during the 3 months prior or present at the time of enrollment

C. Elimination criteria:

- Failure to comply the structured patient monitoring
- Non-delivery of stool samples at set times
- The patient decides to no longer participate in the study

D.- Landmark and recruitment method:

- Medical consult at the Nephrology Department, University Hospital "Dr. José Eleuterio González "

D) Description of the design (describing the methodology to be performed for each of the specific objectives):

After being selected and randomized patients who meet the criteria for inclusion and exclusion, will be assigned to a group to start treatment; one group with FMT-C (Frozen capsules of fecal microbiota) or another with placebo capsules which shall consist of an excipient harmless to the body (capsules frozen saline), treatment and follow-up of both groups will be developed in the service Infectología.

Both groups receive frozen for ingestion orally capsules (comprised of FMT or placebo according to the randomisation) with a frequency of 15 capsules each 12hrs for 4 doses on days 1, 10 and 30 of the study. Each capsule must be ingested over a period no longer than 1 hour.

measurements characteristic factors of the progression of kidney disease day 0,10, 30, 60, 90, 120 and 180 be made consisting of:

- Proteins in urine 24 hours
- Creatinine clearance 24 hours
- CBC
- serum creatinine
- Urea Nitrogen
- Urea
- Glucose
- Uric acid
- IS
- venous gases

Blood samples were taken by puncture of peripheral vein by laboratory personnel to assess renal function, urine samples will be collected by the patient at home and transported to the laboratory, none of these samples will be used for genetic analysis, only samples of feces they underwent genomic

analysis, collection of stool samples will days 0, 5, 10 30, 90 and 180 (on 10, 30, 90 and 180 with a range of +/- 2 days).
adverse effects questionnaires on days 1, 5, 30 and 60 is performed and quality of life assessment on days 0, 10, 30, 90 and 180.
Monitoring will face on a weekly basis to register if they have submitted infections, adverse effects and whether changes have received treatment. Visits will be made in the epidemiology and the Regional Center for Kidney Diseases University Hospital

Fecal microbiota sample preparation

Fecal microbiota donors will be chosen based on their medical history, weight, lack of risk behaviors, or medication with antibiotics and/or proton pump inhibitors, as well as no record of trips in the last 3 months or diarrhea in the last 6 months. Absence of chronic infections such as hepatitis B, C and HIV will be determined by immunoassay.
The first step involves combining the sample with recombinant antigens and diluent, the antibodies present in the sample will bind to the antigen coated microparticles after washing. The second step is to add human acridinium-labeled conjugate after a second wash cycle to subsequently add pre-activating and activating solutions to the mixture. The resulting chemiluminescent reaction is measured as units of reactive light in the ARCHITECT equipment. If the sample is greater chemiluminescent the cutoff point will be considered reactive. Feces also should be analyzed to rule *Entamoeba spp., Campylobacter jejuni, Yersinia enterocolitica, Salmonella spp., Shigella spp.,* enterohemorrhagic *E. coli., Giardia lamblia*, cestodes and nematodes by feces culture. the presence of rotavirus by agglutination of latex particles and Helicobacter pylori stool antigen test is detected.

All samples will be mixed with 10% glycerol and frozen at -70° C within a 60-minute period after their collection. They will be mixed and then suspended in a 0.9% saline solution. The final mixture will be filtered to remove particles bigger than 330 microns, finally, glycerol will be added as bacterial cryo-protector. Once this mixture is finished, the encapsulation will be performed with a 50 wells capsule filler using two sizes of sterile capsules for enteral release. The capsules will be filled with the feces mixture and sealed with its counterpart, then sealed capsule will be encapsulated in a second capsule. The final product will be stored frozen until 60 minutes prior use. The administration will be oral.

Randomization

The scrambling process will be performed by a random number generator which correspond to the even numbers and odd arm to another. This randomization by foreign medical staff will intervention, monitoring and treatment of patients.

7. Calculation of the sample size:

In order to demonstrate a difference in the progression of renal failure as measured by the cup of glomerular filtration rate in patients with renal insufficiency treated with transplantation of fecal microbiota, a formula for hypothesis testing and difference of two halves was used, with an average expected difference of at least 5 ml / min with a zα 1.96 significance level of 95% for two tails, and zβ value of 0.84 with a power of 80%, a sample of 15 participants per group was obtained. Moreover, as the second main objective ratio correction dysbiosis is compared. Using a formula for hypothesis testing and difference of two proportions, with a difference of expected proportions of at least 50% with zα 1.96 significance level of 95% for two tails,

8. Statistical analysis:

Descriptive statistics were used with measures of central tendency and dispersion. Categorical variables for use Chi square test or Fisher exct. For continuous variables T Student or Mann Whitney is used. Multivariate analysis be performed by ANOVA. We consider an equal or lesser value to 0.05 as statistically significant.

9. Activities PhD student in conducting this research:

- Recruitment and clinical monitoring of patients
- Explanation and signing of informed consent
- Coordinate administration of FMT / Placebo and collection of blood samples, feces and urine
- Analysis of results and statistical analysis of the variables
- Determining changes in the progression of kidney disease
- Analysis of changes in the gut microbiome (together with microbiologists)
- Write a manuscript for publication

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