

Protocol Kidney Cell: Use of Bioelectric Stimulation to Improve Kidney Function and Sarcopenia in Patients on Hemodialysis

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Location of Study: Hemodialysis unit of the Santa Clara Hospital of Irmandade Santa Casa de Miseric^ordia de Porto Alegre

Sponsor: KidneyCell, a subsidiary of Leonhardt Ventures

Brief Summary: Chronic kidney disease affects over 45 million people worldwide, and 2 million in Brazil. When it progresses to the most advanced stage, it requires use of hemodialysis on average three times per week for 3-4 hours per session, to remove toxins that are no longer cleared by the native kidney. Unfortunately, the prognosis of patients who require long term dialysis declines significantly with each year of support, and may be as high as 60% at five years. The only long-term solution for quality of life and survival is a kidney transplant. However, the waiting time for a kidney transplant can be years, as success is correlated with the number of antigens that are matched between the donor and recipient, and requires life-long use of multiple immunosuppressive drugs. The leading cause of kidney failure today are Diabetes Mellitus and Systemic Arterial Hypertension and the incidence of that disease is rising at alarming rates. There is a great need to find an alternative solution to avoid the inevitable progression to end stage kidney failure.

The Leonhardt protocol is a multi-component regimen that is scientifically based and cutting-edge approach to treat organ injury and chronic dysfunction by regenerating new normal organ tissue and function. It includes the non-invasive use of micro-current bioelectric using very precise signals to upregulate the expression in the target tissue of several pro-regenerative proteins that are

normally present in the kidney tissue and skeletal muscles, but in very low levels, leading to recovery of function. One protein, called α -Klotho, has been shown to be produced primarily by the kidney, and important in maintaining not only normal kidney function, but is also an anti-aging protein. Klotho is also produced in skeletal muscle, and has been shown to have potent regenerative effects in healing large volume muscle loss injuries when given exogenously.

The electric stimulation will be delivered at two locations, including first the muscle of the thigh to improve sarcopenia and then via simple patch electrodes placed on the skin directly anterior and posterior to the kidney for kidney regeneration. These signals stimulate native organ repair by multiple mechanisms including potent stem cell homing proteins such as SDF-1 which will attract stem cells, as well as proteins such as VEGF and PDGF, which enhance blood flow, and proteins such as IGF to induce cellular repair. The direct kidney stimulation will also include the signal for α -Klotho, as it is one of the key proteins needed for kidney regeneration.

Study Design: Randomized controlled trial

Number of Subjects: 20

Number of Study Sites: 1-2

Duration of Study: 8 weeks

Stimulation Frequency: 2 times/week

Number of sessions: 16 sessions

Duration of Stimulation: 20 minutes for the thigh muscles (motor stimulation) and 45 minutes for kidneys (sensory stimulation)

Simulator to be Used: FDA 510K approved Mettler model 240

PROTOCOL: To intervention group, the BES sessions will take place during the first and second hour of dialysis and will last for 20 minutes for thigh muscle stimulation followed by 45 minutes for the kidney stimulation. The patient will be positioned supine on the dialysis chair, the knees will be at 60° flexion through a foam wedge and ankles restrained by a band to make isometric exercise. The electrodes used to perform the electrical stimulation will be adhesive, disposable and hypoallergenic and provided by the sponsor. To thigh muscle stimulation the electrodes will be placed on the motor point of the quadriceps muscle and the distal electrode was placed perpendicular to the longitudinal axis of the thigh and above the upper border of the patella in both lower limbs. The neuromuscular electric stimulation will be applied by symmetrical biphasic pulsed current, at an 80 Hz frequency, 400 ms pulse width, 10 s contraction time, rest time decreasing as the protocol advanced (50s to 30s to 20s), at reciprocal mode, two times a week, for

8 weeks and session time of 20 minutes. The intensity will be individually adjusted to the patient's tolerance limit to produce visible muscle contraction. The detailed protocol is presented in the table 1 and the intervention for lower limbs in figure 1.

Table 1. Protocol to electrical stimulation neuromuscular on quadriceps

Week	Total time	Contraction-Rest	Nº of contractions
1	20 min	10-50	20
2	20 min	10-50	20
3	20 min	10-30	30
4	20 min	10-30	30
5	20 min	10-30	30
6	20 min	10-20	40
7	20 min	10-20	40
8	20 min	10-20	40



Figure 1

For direct stimulation of the kidneys the electrodes will be placed in the abdominal corresponding to the kidney anatomical site and dorsal region at the level of the 10th thoracic vertebra as the figure 2. Will be realized 5 minutes of VEGF protein stimulation (biphasic, frequency 50 PPS, 300 μ s, continuous mode), 15 minutes of SDF1 protein stimulation (biphasic, frequency 30 PPS, 100 μ s, continuous mode), 5 minutes of PDGF protein stimulation (biphasic, frequency 10 PPS, 200 μ s, continuous mode), 15 minutes of α -Klotho protein stimulation (symmetrical biphasic pulsed TENS current with frequency of 20PPS and 1000 μ s) and 5 minutes of IGF protein stimulation (microcurrents, 22 PPS, polarity +) (table 2). The control group will not receive any intervention.

At the baseline, after 4 and 8 weeks of treatment, both groups will be evaluated for the outcomes as specified below.

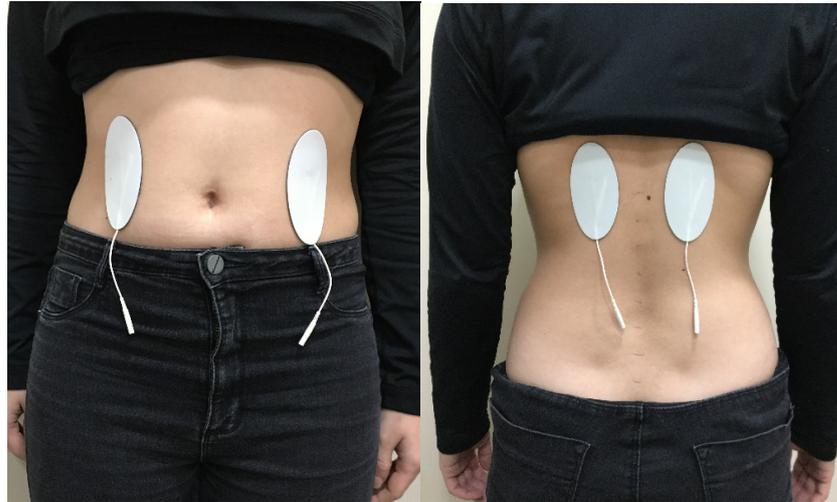


Figure 2

Table 2. Protocol to direct stimulation of the kidneys

Proteína	Corrente	Frequência	Larg. Pulso	Modo	Polaridade	Tempo (min)
VEGF	Bifásica	50 PPS	300 μ s	Contínuo	---	5
SDF1	Bifásica	30 PPS	100 μ s	Contínuo	---	15
PDGF	Bifásica	10 PPS	200 μ s	Contínuo	---	5
Klotho	TENS	20 PPS	1000 μ s	---	---	15
IGF	Microcorrente	22 PPS	---	---	Positiva	5
						Total: 45

Eligibility Criteria:

1. Age 18-80 yr of age;
2. On chronic hemodialysis for more than 3 months;
3. Clearance of urea during hemodialysis ($Kt/V \geq 1.2$ or $URR \geq 65\%$);
4. To be able to ambulate > 300 meters in 6 minutes;

Exclusion Criteria:

1. Cognitive impairment that prevents conducting evaluations, as well as inability to understand and sign the informed consent form;
2. Epidermal lesions at the site of application and/or intolerance stimulator and/or skin sensitivity change;
3. Patients with recent sequel of stroke that limits ambulation;

4. Disabling musculoskeletal disease;
5. Uncontrolled hypertension (Systolic blood pressure > 200 mmHg and diastolic blood pressure > 110 mmHg);
6. Grade IV heart failure (NYHA) or decompensated;
7. Uncontrolled diabetes (blood glucose > 300 mg/dL, HgbA-1-C > 10);
8. Unstable angina;
9. Fever and/or infectious disease;
10. Acute myocardial infarction within past two months;
11. Peripheral vascular disease in the lower limbs as deep vein thrombosis or obliterates thromboangitis that limits ambulation.

Primary Outcomes: Change in α -klotho protein expression and serum creatinine. [Time Frame: Baseline, 4 and 8 weeks].

Secondary Outcomes:

1. Muscle strength of the quadriceps. Muscle strength will be evaluated by dynamometry by a load cell [Time Frame: Baseline and 8 weeks].
2. Muscle strength of the lower limbs. Muscle strength of the lower limbs will be assessed by sit-and-stand test (SST) of 10 repetitions. [Time Frame: Baseline and 8 weeks].
3. Functional capacity. Functional capacity will be assessed by change in 6 minute walk test. [Time Frame: Baseline and 8 weeks].
4. Inflammatory profile. The inflammatory profile will be assessed through blood collection and analysis of immunological markers such as interleukin 6, interleukin 10 and tumor necrosis factor. [Time Frame: Baseline, 4 and 8 weeks].
5. Change in oxidative stress. Oxidative stress will be assessed by measurement of oxidative and antioxidant markers. [Time Frame: Baseline, 4 and 8 weeks].
6. Quality of Life. The quality of life will be assessed by questionnaire EuroQol-5D health questionnaire (EQ-5D) [Time Frame: Baseline and 8 weeks].
7. Incidence and type of adverse effects occurring during treatment. [Time Frame: Weekly].

Data Analysis: Data will be analyzed by a pre-selected individual who will be blinded as to treatment assignment. The results will not be made available to the sponsor until the Principal Investigator and Co-Investigator have reviewed and approved the analysis.