Rapid #: -14787642

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RESEARCH ARTICLE

Intradialytic neuromuscular electrical stimulation reduces DNA damage in chronic kidney failure patients: a randomized controlled trial

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ABSTRACT

Background: Chronic kidney failure (CKF) patients on renal replacement therapies exhibit elevated levels of DNA lesions and this is directly related to high mortality.
Objective: This study aimed to evaluate the effect of neuromuscular electrical stimulation (NMES) on genomic damage in CKF patients on conventional haemodialysis (HD).
Methods: Twenty-one patients with CKF on HD were randomized into control (CG =10) or neuromuscular electrical stimulation (NMESG = 11) groups. NMES was applied on the quadriceps muscle during the HD session, three times a week, for 8 weeks in NMESG. DNA damage in blood was evaluated by the alkaline comet assay prior to follow-up, after 4 and 8 weeks of intervention.
Results: Intradialytic NMES in CKF patients induced a significant decrease in DNA damage after four [49.9 (3.68) vs 101.5 (6.53); $p = 0.000$] than eight [19.9 (2.07) vs 101.5 (6.53); $p = 0.000$] weeks compared to baseline. Genomic damage was also significantly less after four [NMESG: 49.9 (3.68) vs CG: 92.9 (12.61); $p = 0.001$] than after eight [NMESG: 19.9 (2.07) vs CG: 76.4 (11.15); $p = 0.000$] weeks compared to CG.
Conclusions: This study demonstrates for the first time that intradialytic NMES is able to reduce DNA damage in blood of CKF patients.

Introduction

Chronic kidney disease (CKD) patients are at a great risk of cancer and cardiovascular diseases compared to a healthy population (Di Angelantonio et al. 2010, Stengel 2010). These patients exhibit signs of genomic instability and, consequently, extensive stress generation, besides the formation of other endogenous substances with genotoxic properties (Himmelfarb 2009, Corredor et al. 2015). Along these lines, increased genomic damage is a predictor of a worse prognosis in haemodialysis patients and therefore has a direct relationship with high mortality in this population (Corredor et al. 2015).

Physical activity has been inversely related to deoxyribonucleic acid (DNA) damage and production of reactive oxygen species (ROS), revealing a positive effect on the mitochondrial function of various organs and systems (Ascensão et al. 2003, Mota et al. 2010). In a recent study, the effect of a combined physical exercise training program (aerobic exercise, strength exercise and stretching) on healthy individuals, demonstrated that chronic exercise was able to reduce DNA damage and oxidative stress biomarkers, as well as, increase total antioxidant capacity, functional capacity and muscle strength of the subjects (Soares et al. 2015).

Neuromuscular electrical stimulation (NMES) is a technique commonly used to generate muscle contractions for the purpose of (re)training and it is applied through surface electrodes positioned on the skeletal muscles (Maffiuletti et al. 2014). The main benefits of this technique are improvements of: muscle strength of lower limbs (Sbruzzi et al. 2010, Dobsak et al. 2012), increase in muscle mass (Sillen et al. 2013, Vaz et al. 2013), functional capacity and maximum oxygen consumption (VO_2 peak) (Sbruzzi et al. 2010, Gomes Neto et al. 2016), endothelial function (Karavidas et al. 2013) and quality of life (Dobsak et al. 2012, Smart et al. 2013). It has been recognized that NMES provides important physiological and functional adaptations as well as benefits in clinical situations such as chronic heart failure (Sbruzzi et al. 2010, Smart et al. 2013), chronic obstructive pulmonary disease (Vivodtzev et al. 2008, Sillen et al. 2009, Vivodtzev et al. 2012) and orthopedic disorders (Sillen et al. 2013, Vaz et al. 2013). However, few studies used this therapeutic modality in CKD patients. It was recently demonstrated by Dobsak et al. (2012), Simó et al. (2015) and Schardong et al. (2017) that when NMES is applied during dialysis, it promotes an increase in quadriceps muscle strength and distance covered in the six-minute walk test, improving some aspects of quality of life as well as the effectiveness of dialysis in chronic kidney failure (CKF) patients. However, the studies that demonstrated increased muscle strength of the lower limbs after NMES did not evaluate the effects of this therapy on DNA damage levels on CKF patients.

This study hypothesizes that intradialytic NMES could be a treatment strategy able to reduce the DNA damage levels in

the blood of patients with CKF. Taking into account the lack of evidence, the aim of this study was to evaluate the effect of intradialytic NMES on genomic damage in CKF patients.

Therefore, the research question for this randomized controlled trial was: Is intradialytic NMES able to reduce DNA damage in CKF patients?

Clinical significance

- NMES when applied for 8 weeks on lower limbs and during conventional haemodialysis is able to reduce the DNA damage of patients with CKF and this is related to reduction of mortality.
- NMES is a feasible, safe and low-cost therapy to be implemented into the routine of haemodialysis units for rehabilitation of chronic kidney patients.
- NMES is a therapeutic strategy that could improve the prognosis of patients in the final stage of CKD.

Methods

Design

This study consists of a randomized controlled trial with blinding of the outcome evaluator conducted in CKF patients undergoing conventional HD treatment. DNA damage of the systemic blood was considered the primary outcome of the study and it was measured at three time points: baseline, after 4 and 8 weeks of intervention. NMES therapy was applied for 8 weeks.

Ethic

The project was reviewed and approved by the Ethics Committee in Human Research of Irmandade Santa Casa de Misericórdia de Porto Alegre (ISCMPA) hospital, and Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA) (reports number 436.347 and 467.789, respectively). The study was also registered in the clinical trials system (ClinicalTrials.gov; NCT02336776 identifier) and written informed consent was obtained from all patients prior to their inclusion in the study and they were informed about the guarantee of confidentiality of their data.

Participants and setting

Initially, there was an oral invitation to all patients and, if they showed interest in participating in the research, their electronic medical records were consulted to determine the eligibility criteria for the study.

The inclusion criteria were: patients of both sexes, with CKF on conventional HD for at least three months and urea reduction ratio (URR) ≥65% (Hemodialysis Adequacy 2006 Work Group 2006). Patients who had cognitive impairment and were unable to understand and sign the written informed consent were excluded. Patients with recent effects of stroke (less than three months); osteoarticular or disabling musculoskeletal disorders; uncontrolled hypertension (systolic blood pressure >230 mmHg and diastolic blood pressure >120 mmHg); heart failure class IV (New York Heart Association) (McMurray et al. 2012); uncontrolled diabetes (blood glucose >300 mg/dL); unstable angina; presence of an infectious disease; recent acute myocardial infarction (less than two months); active smokers; patients with peripheral vascular disease in the lower limbs such as deep vein thrombosis as well as those with one of the following contraindications to the use of NMES: epidermal lesions at the stimulation site, intolerance to electric current or skin sensitivity changes were also excluded.

Clinical information was obtained by consulting the patients' electronic records and an interview was performed before starting any evaluation or intervention. Identification data; CKF risk factors; demographic data; HD time; underlying disease; routine laboratory tests and medications used by the patients were recorded.

The randomized clinical trial was performed in the haemodialysis unit of the Santa Clara Polyclinic of ISCMPA hospital. The alkaline comet assay was performed in the Genetic Toxicology laboratory of UFCSPA. Both procedures were conducted between March 2015 and December 2015.

Randomization

The patients were randomized into two groups: control group (CG) and neuromuscular electrical stimulation group (NMESG) by www.random.org online software (1:1 allocation ratio). The sequence of numbers was generated by a researcher "blind" to the study (he wasn't a member of the data collection team) and it was stored in the computer of this researcher until the beginning of the interventions. The random numbers only were disclosed to the physiotherapist responsible for applying NMES prior to the start of the protocol, in order to ensure the allocation sequence concealment and avoid selection bias.

Intervention

All patients were submitted to conventional HD (3 times per week, for 4 hours). The equipment used for dialysis was the Fresenius Medical Care machine (4008 S model) and the dialysator (filter) used was the FX100 classix (Fresenius Helixone® High Flux). As dialysis buffer the patients received 900 g of sodium bicarbonate (bibag®, Fresenius Medical Care).

The interventions occurred between the 2nd and the 3rd hour of the haemodialysis (Dobsak et al. 2012). The intradialytic intervention with NMES occurred three times per week, during 8 weeks, totalling 24 sessions. The CG did not receive any intervention and it was only assessed at initiation and reassessed after 4 and 8 weeks of follow-up. NMES was applied through a calibrated electrical stimulator (Neurodyn II, model N53, IBRAMED, São Paulo/SP, Brazil) using rectangular symmetrical biphasic pulsed current. NMES sessions occurred in the supine position, with lower limbs resting on a foam wedge and knees flexed at 60° (Hoy et al. 1990). Also, a nylon velcro band was used to attach the ankles of the patient to the wedge and dialysis armchair, to perform

concomitant isometric recruitment. Electric current was applied through two hypoallergenic, self-adhesive electrodes, size 7.5 × 13 cm (ValuTrode, CF7515 model, São Paulo/SP, Brazil). The proximal electrode was 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 (Vaz et al. 2012).

The motor point of the quadriceps muscle was determined using a 3.2 cm diameter electrode (ValuTrode, model CF3200, São Paulo/SP, Brazil), rectangular symmetrical biphasic pulsed current and the same parameters adopted for intervention, but with enough intensity to cause tetanic contraction. Punctual stimulation was applied near the middle portion of the muscle belly of the quadriceps, and the motor point was determined as the point where the strongest contraction of the quadriceps was obtained. This was confirmed by visual inspection of the contraction of the muscle belly and by the confirmation of the patient that this point corresponded to the site where the stimulation was felt with greater intensity (Vaz et al. 2012).

NMES was applied by a trained physiotherapist or physical therapy student in both lower limbs simultaneously: at 80 Hz frequency, 400 μs pulse width, 10 seconds contraction time, rest time decreasing with the advance in protocol (starting with 50 seconds and reducing 10 seconds every two weeks), 3 times a week, during 8 weeks, and increasing session time (starting with 20 minutes and increasing 2 minutes per week). The intensity adopted was the same in both lower limbs and it varied in each session according to the patient tolerance (Vaz et al. 2013), since they were treated individually. As a safety measure in NMESG, the patients' vital signs were checked before and at the end of each session.

DNA damage evaluation

Chemicals: Phosphate-buffered saline (PBS) was obtained from Gibco-BRL (Grand Island, NY). Low melting-point agarose (LMP), normal melting-point agarose (NMP), sodium chloride (NaCl), ethylenediaminetetraacetic acid (EDTA), dimethyl sulfoxide (DMSO) and sodium hydroxide (NAOH) were purchased from Sigma (St. Louis, MO). All other reagents were of analytical grade and purchased from local commercial suppliers.

Technique: systemic blood samples were obtained prior to follow up, after 4 and 8 weeks of intervention, always before the 2nd HD session of the week in appropriate EDTA tubes. The evaluation of DNA damage levels occurred by alkaline comet assay, which measures single and double DNA strand breaks, and was performed as described by Singh et al. (1988) and Hartmann and Speit (1997), in accordance with general guidelines for use of the comet assay (Tice et al. 2000). In brief, aliquots of 20 μL of whole blood were suspended in 90 μL of LMP agarose and spread onto a glass microscope slide pre-coated with NMP agarose. Slides were placed in ice-cold lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris pH 10.0 with 10% DMSO and 1% Triton X-100) at 4 °C for at least 1 day for to remove cell proteins and leave

DNA as "nucleoids". After the lysis-buffer procedure, the slides were covered with fresh alkaline buffer (300 mM NaOH and 1 mM EDTA, pH 13.0) for 15 minutes to allow DNA unwinding and then, electrophoresis was performed for 15 minutes (25 V; 300 mA; 0.9 V/cm). Slides were neutralized with 0.4 M Tris (pH 7.5), washed in bi-distilled water and stained using a silver nitrate staining protocol (Nadin et al. 2001). After drying at room temperature overnight, the samples were analyzed using an optical microscope. One hundred cells (from each of the two replicate slides) were selected and analyzed. Cells were visually scored according to tail length and received scores from 0 (no migration, undamaged, without a tail) to 4 (maximal migration). Therefore, the damage index (DI) was calculated, ranging from 0 (completely undamaged: 100 cells ×0) to 400 (with maximum damage: 100 cells ×4) (Tice et al. 2000, Burlinson et al. 2007). The slides were analyzed by a blinded evaluator.

Statistical analysis

The sample size was calculated using the Lee online software and it was based on rates of: alpha error: 5% and power: 80%. We used the concept of the muscle strength improvement to calculate the participants' needs to be treated by NMES. The maximum isometric strength improvement found by Dobsak et al. (2012) after NMES therapy (37 ± 44.8 N) was used to calculate the sample size that results in nine patients per group.

The distribution of the data was tested with the Shapiro-Wilk normality test. To compare the baseline variables, the Student's *t*-test was used for normal distribution data and the Mann-Whitney *U* test for non-parametric data. To evaluate the effect of the intervention between the groups the Generalized Estimating Equation (GEE) was used, followed by Bonferroni *post-hoc* test. Data were expressed as mean ± SEM; median and interquartile range, and frequency. A *p* value <0.05 was considered significant. The analysis was performed per-protocol on the patients included in the study.

Results

Thirty-three CKF patients on HD were evaluated according to the eligibility criteria for a possible admission in this study, and thereafter 21 patients were included. Figure 1 shows the flowchart of patient selection and composition of the groups.

Table 1 represents demographic data and physical and clinical characteristics: anthropometric, laboratory analysis and risk factors for CKF. The groups did not differ for any of these variables at a pre-intervention time nor for levels of DNA damage.

In order to verify the intradialytic effects of NMES therapy on systemic blood DNA integrity of CKF patients, an analysis of DNA damage by alkaline Comet assay was performed. This assay detects (repairable) DNA single and double-strand breaks and alkali-labile sites. It can be seen in the representative images of blood from NMESG patients that therapy induced a decrease in DNA damage (Figure 2). The genomic damage index showed a significant decrease after NMES
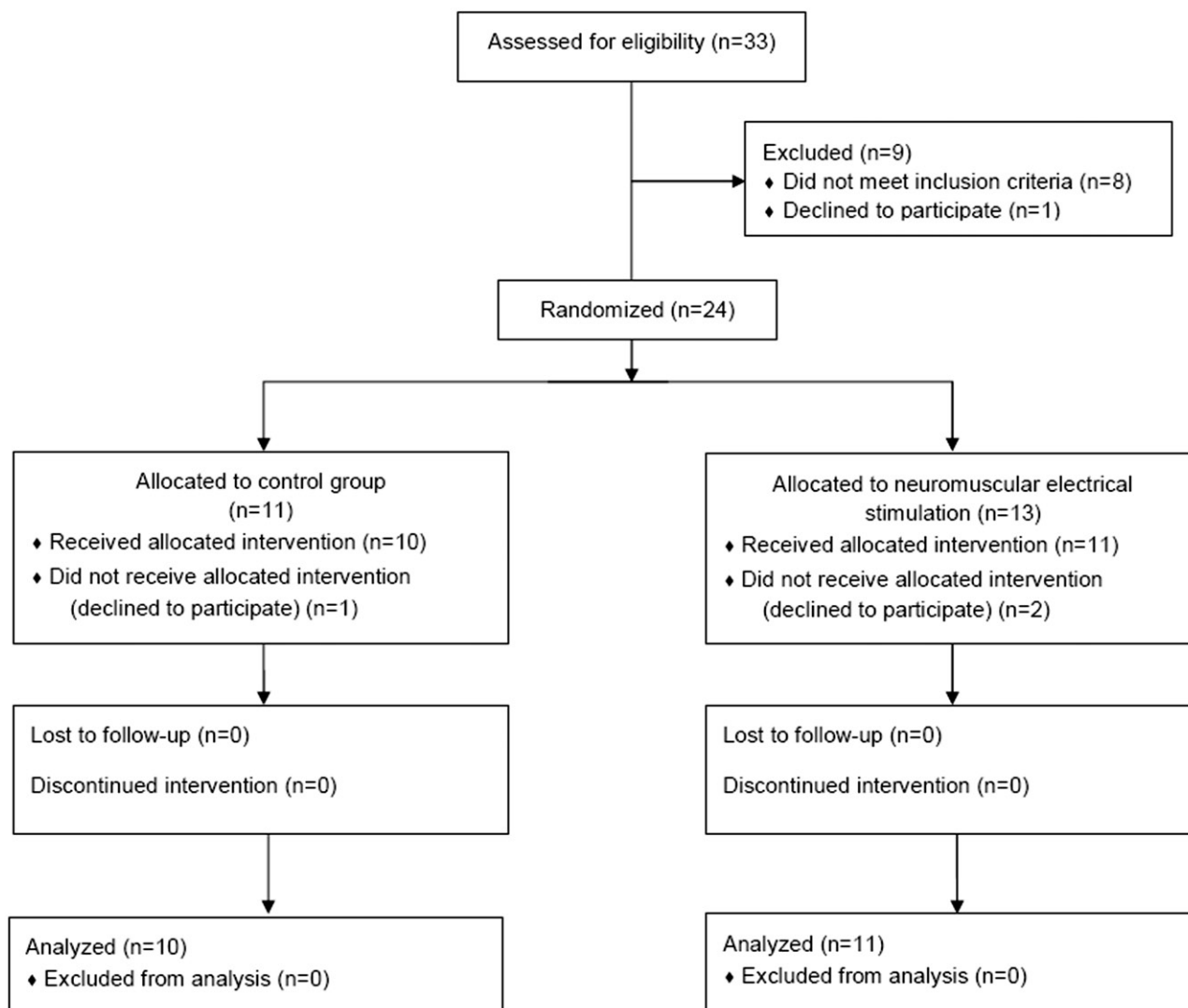
Figure 1. Flowchart of patients' admission and composition of groups.

therapy at the 4th [49.9 (3.68) vs 101.5 (6.53); $p = 0.000$] and 8th [19.9 (2.07) vs 101.5 (6.53); $p = 0.000$] week when compared with prior to follow-up. DNA damage was also significantly less in the 4th [NMESG: 49.9 (3.68) vs CG: 92.9 (12.61); $p = 0.001$] and 8th [NMESG: 19.9 (2.07) vs CG: 76.4 (11.15); $p = 0.000$] weeks compared to the CG, as shown in Figure 3.

With regard to the safety of this therapeutic modality, no adverse effects or complications related to the electrical current during and after the stimulation session were observed.

Discussion

This was the first study to evaluate the effect of NMES therapy (carried out in the intradialytic period) on genomic damage of CKF patients. NMES therapy, performed for eight weeks, reduced DNA damage in the systemic blood of these patients, and so revealed relevant clinical information about a new rehabilitation approach able to decrease a high-risk factor in the mortality observed in the CKF population. The genomic injury in CKF patients is an independent predictor of mortality (Corredor et al. 2015), therefore, reducing the damage to DNA can to imply a possible increase of survival.

In a recent study, researchers (Soares et al. 2015) found that combined physical exercise (aerobic exercise, strength exercise and stretching) when performed three times a week, for 16 weeks, improved physical performance and it also reduced DNA damage in blood and biomarkers of oxidative stress in healthy men. The authors relate this finding to the increase of antioxidant defense capacity generated by physical exercise.

Another group (Franzke et al. 2015) evaluated the effect of 24 weeks of elastic band resistance training for major muscle groups (legs, back, abdomen, chest, shoulder and arms), twice a week, totaling 48 sessions on chromosomal damage in institutionalized elderly. The authors observed as Soares et al. (2015), a positive effect on the increase of resistance against genomic instability in subjects' blood. Our study demonstrated that DNA damage in the blood of a severely compromised population (McIntyre et al. 2006), can be reduced with the use of intradialytic NMES, and for an intervention period of only 8 weeks, less time than the studies cited.

The observed response can be explained by the type of muscle contraction induced by the NMES, which is typically of low to moderate intensity, reaching about 20–40% of the maximum voluntary contraction (Sbruzzi et al. 2011). Thus,

during exercise, low levels of formation of reactive oxygen species (ROS) can stimulate adaptive mechanisms, with increased activity or expression of antioxidant enzymes, leading to a reduction of oxidative damage (Gomez-Cabrera et al. 2008) and reduction of DNA damage, since, free radicals are mediators of the regulation of the enzymatic activity of DNA repair (Radák et al. 2003, Radak et al. 2008).

It is well documented that regular and moderate-intensity physical activity is associated with several benefits, including

Table 1. Characteristics of patients admitted to the study.

	CG ($n = 10$)	NMESG ($n = 11$)
Sex (F/M) n, (%)	2/8 (20/80)	2/9 (18.2/81.8)
Age (years)	64.5 (57.50–67.75)	59 (45.00–72.00)
Height (cm)	171.5 (159.50–173.00)	172 (166.00–176.00)
Dry weight (kg)	71.8 (3.63)	77.5 (2.58)
Wet weight (kg)	73.9 (3.74)	79.7 (2.71)
BMI (kg/m²)	25.4 (0.9)	26.8 (0.9)
HD time (months)	28 (10.50–85.50)	24 (9.00–108.00)
URR (%)	70.5 (51.30–88.48)	86.7 (48.69–99.70)
Primary disease n, (%)		
Hypertension	2 (20)	4 (36.4)
Diabetes mellitus	2 (20)	1 (9.1)
Glomerulonephritis	2 (20)	1 (9.1)
Autoimmune	1 (10)	3 (27.3)
Cancer	1 (10)	1 (9.1)
Polycystic kidney disease	0 (0)	0 (0)
Unknown	2 (20)	1 (9.1)
Risk factors n, (%)		
Smoking	0 (0)	0 (0)
Ex-smoker	4 (40)	7 (63.6)
Hypertension	10 (100)	11 (100)
Sedentary lifestyle	8 (80)	11 (100)
Diabetes mellitus	2 (20)	2 (18.2)
Heart disease	7 (70)	7 (63.6)
FH heart disease	5 (50)	5 (45.5)
Peripheral vascular disease	5 (50)	4 (36.4)
Serum biochemistry		
Pre dialysis urea (mg/dL)	170.8 (7.36)	181.3 (12.82)
Post dialysis urea (mg/dL)	44.1 (4.84)	56.3 (3.61)
Creatinine (mg/dL)	8.9 (5.00–10.93)	9.9 (7.73–13.45)

CG: control group; NMESG: Neuromuscular electrical stimulation group; F: female; M: male; BMI: body mass index; HD: haemodialysis; URR: urea reduction ratio; FH heart disease: family history of heart disease. Data are expressed as mean ± SEM, median and interquartile range and frequency.

reducing the risk of cardiovascular diseases, diabetes, cancer and other lifestyle-related diseases (Blair et al. 1995, Hamman et al. 2006, Kruk and Aboul-Enein 2006, Radak et al. 2008). The intervention type and the NMES parameters adopted in this study mimic low to moderate intensity exercise training, where it was possible to observe reduction in DNA damage levels in the blood of CKF patients after 8 weeks of intervention.

Further, studies have shown that more debilitated patients tend to have a greater benefit when compared to less severe patients or healthy individuals (Meuleman et al. 2000). This may partially justify the results obtained in a shorter time period in our study.

According to Thomas et al. (1997) and Hooker et al. (1992), cardiac output is frequently increased during NMES interventions, since, this is driven by the volumetric loading imposed by increased venous return of leg muscles and not by neural regulation of the heart rate. Dobsak et al. (2012) observed that NMES improved the clearance of uremic toxins and attribute this to increased blood flow in overloaded muscles and then to increased displacement of toxic substances into plasma (Dobsak et al. 2012). The reduction of levels
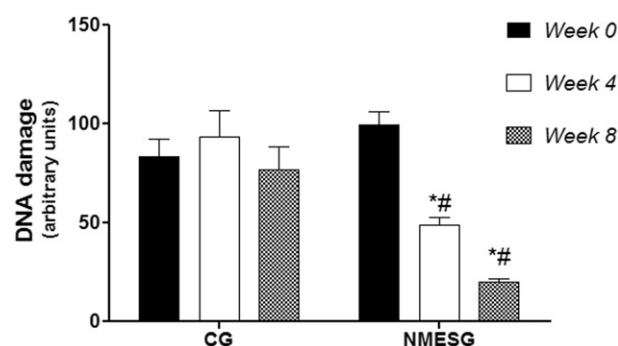
Figure 3. Effects of intradialytic neuromuscular electrical stimulation (NMES) on blood DNA damage of CKF patients after 4 and 8 weeks. CG: control group; NMESG: neuromuscular electrical stimulation group. Values are mean ± SEM. Asterisk indicates significant difference ($p < 0.05$) over time and fence indicates significant difference between groups.
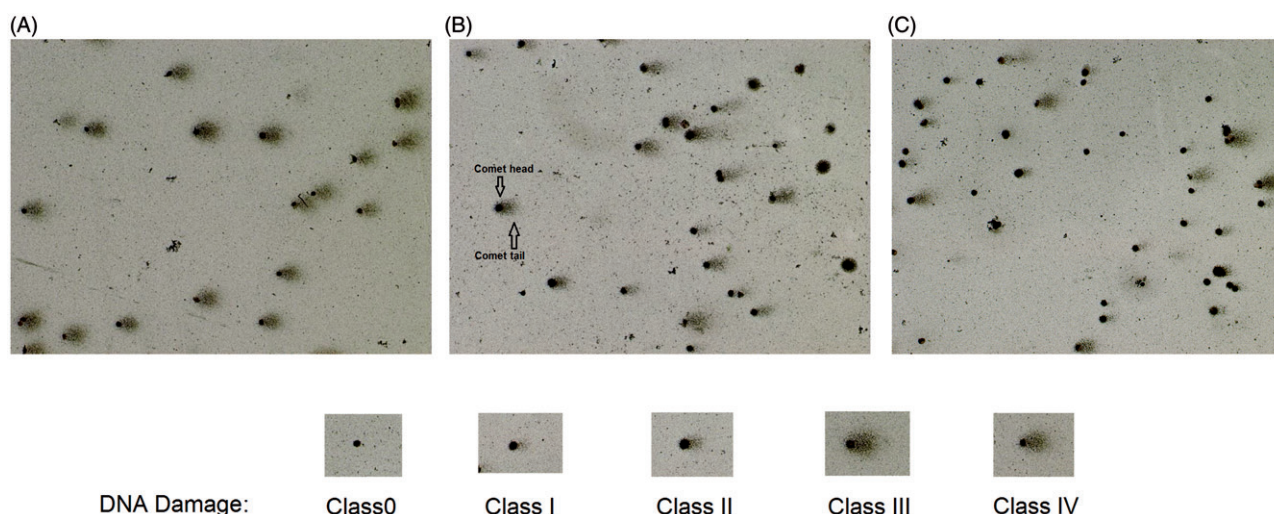
Figure 2. Representative images of DNA damage in blood of NMESG patients: prior to follow-up or week 0 (A); after 4 (B) and 8 (C) weeks of NMES therapy. One hundred cells were scored according to tail length into five between classes 0 undamaged = without a tail and 4: comets with no head = almost all DNA in tail. These images were represented as damage index (DI) of DNA, which ranged from 0 (completely undamaged: 100 cells ×0) to 400 (with maximum damage: 100 cells ×4). The images were obtained with a digital colour camera (Olympus IMS DP72) for microscope. Mag. 10×.

of genomic damage in CKF patients in HD is believed to be due to increased quadriceps blood flow and the improvement of venous return as a result of NMES. This possibly optimizes the clearance of uremic toxins and contributes to the reduction of reactive oxygen species and consequently of the DNA damage.

The limitations of the study are: the nonperformance of biochemical measures of markers of oxidative stress and antioxidant defenses, as well as inflammatory markers. These analyses could reinforce our findings and help elucidate the mechanisms of NMES in reducing the DNA damage found. In addition, other factors such as medications, diet and physical activity may also interfere with these results, however, patients were instructed to maintain their lifestyle and inform researchers of any major changes related to these issues. Still, no other studies used the alkaline comet assay to evaluate the effect of NMES on genomic damage in this population, therefore our findings are valid, but further trials are needed to confirm them.

Despite the limitations, it is important to emphasize that this protocol was also tested by our group in the same population on clinical variables such as lower limb strength and muscle architecture, functional capacity and endothelial function and positive results were obtained (Schardong et al. 2017). This information strengthens the findings of this study, despite this being a small randomized clinical trial.

Furthermore, NMES is a technique that is easy to apply and inexpensive. No adjustments are required in the structure of the haemodialysis unit, allowing that NMES can be implanted in the routine of CKF patients as an initial rehabilitation strategy, also optimizing the time they remain in HD.

Anyway, the results are relevant and positive for the population in the study, since, if untreated, the prognosis of disease outcome is bad. Therefore, intradialytic NMES therapy can be a promising strategy or an alternative of treatment to decrease common outcomes due to the accumulation of uremic toxins such as DNA damage, and increase the quality of life and survival of these patients.

Conclusions

Intradialytic NMES decreases the DNA damage levels in the systemic blood of CKF patients. Moreover, the detailed protocol in our study can be applied safely and effectively in CKF patients during the HD session.

Disclosure statement

The authors report no conflicts of interest.

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