Respiratory Medicine 134 (2018) 103–109

Contents lists available at ScienceDirect

Respiratory Medicine

journal homepage: www.elsevier.com/locate/rmed

Effects of respiratory muscle training on endothelium and oxidative stress biomarkers in hemodialysis patients: A randomized clinical trial

Nataly Gurgel Campos[a], Débora Fortes Marizeiro[a], Ana Carolina Lins Florêncio[a], Ítalo Caldas Silva[a], Gdayllon Cavalcante Meneses[b], Gabriela Freire Bezerra[b], Alice Maria Costa Martins[b], Alexandre Braga Libório[a,c,*]

[a] Medical Sciences Post-graduate Program, Department of Clinical Medicine, Universidade Federal do Ceará, Brazil
[b] Department of Clinical and Toxicological Analysis, Faculty of Pharmacy, Federal University of Ceara, Fortaleza, Ceara, Brazil
[c] Medical Sciences Post-graduate Program, Centro de Ciências da Saúde, Universidade de Fortaleza – UNIFOR, Brazil

ARTICLE INFO

Keywords:
Pulmonary function
Glycocalix
Hemodialysis

ABSTRACT

Introduction: Hemodialysis (HD) patients have altered pulmonary function and this is associated with impaired endothelial function and cardiovascular events. Respiratory muscle training (RMT) has the potential to improve cardiovascular outcomes in patients undergoing maintenance HD. Here, we evaluated the effects of RMT on endothelium/glycocalyx, oxidative stress biomarkers and pulmonary function test in HD patients.

Methods: This is a randomized controlled clinical trial including 41 patients undergoing thrice-weekly maintenance HD. Patients were randomly assigned at a 2:1 ratio to receive or not RMT during HD sessions for 8 weeks. Main outcomes were changes in levels of the biomarkers related to endothelium activation (vascular cell adhesion molecule 1, VCAM-1, and intercellular adhesion molecule 1, ICAM-1), glycocalyx derangement (syndecan-1), aberrant angiogenesis (angiopoietin-2) and oxidative stress (malondialdehyde) compared to baseline. Also, maximal inspiratory/expiratory pressure (MIP, MEP), Forced vital capacity (FVC) and forced expiratory volume in the first second (FEV1) were evaluated. Other outcomes included changes in functional capacity and pulmonary function test. We also performed a *post-hoc* analysis of plasma endothelin-1 levels.

Results: Of 56 randomly assigned patients, 41 were included in the primary final analyses. RMT increased all pulmonary function parameters evaluated and significantly reduced plasma syndecan-1 levels at 8 weeks compared to placebo (between-group difference: −84.5; 95% CI, −148.1 to −20.9). Also, there was a reduction in plasma levels of angiopoietin-2 (between-group difference: −0.48; 95% CI, −1.03 to −0.097). Moreover, there was a significant reduction in mean blood pressure at rest (between-group difference: −12.2; 95%CI, −17.8 to −6.6) associated with a reduction in endothelin-1 levels (between-group difference: −0.164; 95% CI, −0.293 to −0.034). There was no difference regarding biomarkers of endothelial activation or oxidative stress.

Conclusion: A short-term RMT program ameliorate FVC, FEV1 and reduces syndecan-1 and angiopoietin-2 biomarker levels. Finally, better blood pressure control was attained during training and it was associated with a reduction in endothelin-1 levels.

1. Introduction

Chronic kidney disease (CKD) patients, mainly those undergoing maintenance renal replacement therapy (peritoneal dialysis or hemodialysis - HD), have a complex syndrome with various effects on the cardiovascular, nervous, respiratory, musculoskeletal, immune, endocrine, and metabolic systems. In these patients, altered pulmonary function [1] may be the direct/indirect result of circulating uremic toxins, volume overload, muscle atrophy, malnutrition, anemia, inflammation, oxidative stress, extraosseous calcification and others [2].

Several studies have described an association between reduced lung function, irrespective of previous smoking status, and cardiovascular events/mortality [3–5].It has also been demonstrated that patients with reduced pulmonary function also have impaired endothelial function [6–8].Because the endothelium and its glycocalyx have a central role in all phases of the atherosclerotic process [9], a causal sequence of events, although controversial [10], has been suggested: reduced lung function leads to endothelial alterations and, consequently, to cardiovascular disease [11].

Cardiovascular diseases are, collectively, the main cause of death in

* Corresponding author. Av. Abolição, 4043 Ap 1203, Ed. Jangada, CEP: 60165-082, Fortaleza, Cerá, Brazil.
E-mail address: alexandreliborio@yahoo.com.br (A.B. Libório).

https://doi.org/10.1016/j.rmed.2017.12.005
Received 25 October 2017; Received in revised form 4 December 2017; Accepted 6 December 2017
Available online 07 December 2017
0954-6111/

patients undergoing hemodialysis [12].Besides hypertension, these patients have endothelial glycocalyx derangement [13], altered endothelial function [14], dysregulated angiogenesis [15] and endothelial cell activation [16]. Moreover, they are have increased oxidative stress [17]. All these processes are associated, directly or indirectly, to the atherosclerotic process.

In this study, we hypothesized that respiratory muscle training (RMT) would lead to improvements in Ref. [1] endothelial activation [2], glycocalyx derangement [3]; aberrant angiogenesis and [4] oxidative stress biomarkers in patients undergoing maintenance HD. We also assessed effects of RMT on respiratory function, respiratory muscle strength, functional capacity, blood pressure and endothelin-1 levels, a potent vasoconstrictor.

2. Methods

2.1. Study design and participants

This was a randomized clinical trial comparing patients undergoing an 8-week RMT program using a variable pressure device (Threshold PEP®) with a control group. It was performed at an outpatient dialysis facility in the city of Fortaleza, Brazil. This center was chosen because it has infra-structural facilities for research and previous experience with clinical studies. All patients undergoing HD (n = 118) in that service were screened for eligibility (December 2016 to January 2017) by study coordinators. Data collection and study intervention were initiated in all patients by February 2017. Criteria for study participation included the following: patients receiving thrice-weekly maintenance HD for at least 3 months, age older than 18 and younger than 70 years, adequate cognitive and physical capacity to perform the study protocol procedures and to provide informed consent. Exclusion criteria included any chronic respiratory disease (patients with established diagnosis of chronic obstructive pulmonary disease – COPD, interstitial lung disease, asthma in treatment, chronic pleural effusion, pleurostomy, bronchiectasis and others) and patients with acute coronary syndrome, decompensated heart failure, major infectious processes, or hospitalization within the last 3 months. The study is registered at www.clinicaltrials.gov under number NCT 03041155.

A total of 84 patients were assessed for eligibility, and 56 gave their consent and were randomly allocated using a computer-generated random numbers, at a 2:1 ratio, to the RMT or the control group. Prior to starting the study interventions, 8 participants voluntarily chose not to participate in the study, 3 participants were hospitalized because cardiac disease and another had an infectious complication – Fig. 1. During the study period, one patient in the RMT group voluntary gave up participating in the study; additionally, one patient assigned to the control group was submitted to renal transplantation and another was removed from the study because he missed more than one dialysis session during study period. Thus, 41 patients completed the study (RMT group, 29; control group, 12) and were included in the analysis.

2.2. Study procedures

Participants were screened for eligibility criteria and clinical data were collected from the dialysis facility records. Before the midweek dialysis session, blood was collected for biomarker analysis, centrifuged, and the plasma aliquoted for storage at −80 °C until sent for analysis. Participants underwent pulmonary function tests: forced expiratory volume in 1 s (FEV_1); forced vital capacity (FVC); maximal inspiratory pressure (MIP); and maximal expiratory pressure (MEP). All pulmonary function tests were obtained before the midweek dialysis session and performed according to American Thoracic Society recommendations [18]. In addition, patients performed the 6-min walk test (6MWT) in the corridor of the dialysis facility, which is 30 m long. After resting on a chair for at least 10 min, we encouraged the patients to walk back and forth along the corridor for 6 min. We measured the total distance walked in 6 min. Blood pressure, pulse oxygen saturation (SpO2, using a Nellcor PM10N Handheld Pulse Oximeter), heart and respiratory rates were measured before and immediately after the 6MWT. Dyspnea was assessed according to the Borg modified scale from 0 (no dyspnea) to 10 (maximal dyspnea) [19].Oxygen desaturation was considered in the presence of a decrease greater than 4% from the baseline saturation [20]. All procedures described were performed before and after the study intervention (described below).

2.3. Study intervention

RMT was undertaken thrice weekly during 8 weeks using the threshold load training method supervised by a qualified trainer. Training was provided in the dialysis facility during hemodialysis sessions. Participants were required to breathe in (inspiratory training) or breathe out (expiratory training) against the resistance using a commercially available device (Threshold PEP; Respironics, Parsippany, NJ). Training consisted firstly of 12 sessions lasting 30 min each and resistance of 15 cmH_2O; the following 12 sessions lasted 40 min each and resistance was set at 20 cmH_2O. In the first half of each session, the resistance was against breathing in and in the last half, against breathing out.

2.4. Outcomes

The primary outcome was change in levels of the plasma markers of glycocalyx derangement (syndecan-1). Secondary outcomes included plasma markers of endothelium activation (VCAM and ICAM), aberrant angiogenesis (angiopoietin-2) and oxidative stress (malondialdehyde) compared to baseline. Other variables included changes in blood pressure and heart rate before and after the 6MWT; changes in functional capacity and changes in pulmonary function test and pulmonary muscle strength. We also decided to perform a *post-hoc* analysis of plasma endothelin-1 to investigate its role in blood pressure reduction.

2.5. Biomarker measurements

Syndecan-1 (Abcam, Cambridge, MA, USA), ICAM-1 and VCAM-1 (Life Technologies Brasil, São Paulo, Brazil) and angiopoietin-2 (Abcam, Cambridge, MA, USA) were measured using commercially available enzyme-linked immunosorbent assay kits. Malondialdehyde was measured using a TBARS Assay Kit (Cayman Chemical, Ann Arbor, MI). To measure ET-1, an ELISA kit from Biomedica Medizinprodukte GmbH & Co KG Divischgasse 4 1210 Vienna, Austria (cat. No. BI-20052) was used.

2.6. Statistical analysis

The study was designed to include 48 (2:1) patients to provide a 90% power to detect a difference of 40% in baseline-adjusted values between the intervention and control groups in the primary outcome. The power calculation assumed a common SD of 0.40 and two-sided $\alpha = 0.05$. All analyses were performed on an intention-to-treat basis.

All variables were tested for normal distribution. Results were expressed as mean ± standard deviation for normally-distributed continuous variables, median and range for skewed variables, and frequency and percentage for categorical variables. Comparisons of baseline characteristics of the intervention *versus* control groups were conducted using *t*-test or χ^2 tests, depending on data type. Changes from baseline to week 8 for each study outcome were assessed using linear regression - ANCOVA [21]. All models included baseline values, age, gender, race, dialysis vintage, body mass index, presence of diabetes mellitus, cause of end-stage renal disease, current or previous (last 6 months) use of corticosteroids, hemoglobin, serum parathormone, serum calcium and serum phosphorus as covariates. Mean between-group difference was used for comparison of within-group changes