Peritoneal dialysis in older adults
Evaluation of clinical, nutritional, metabolic outcomes, and quality of life

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Abstract

The number of older adults requiring dialysis is increasing worldwide, whereas the use of peritoneal dialysis (PD) in this population is lower with respect to younger patients, despite the theoretical advantages of PD respect to hemodialysis. This is most likely due to the concern that older patients may not be able to correctly and safely manage PD.

We aimed to prospectively compare clinical, nutritional and metabolic outcomes and measures of quality of life between younger (<65 years old) and older (≥65 years old) patients on PD.

PD patients were enrolled and divided into 2 groups according to age (Group A < 65 years, Group B ≥ 65 years). Clinical and instrumental parameters, and quality of life were evaluated at baseline (start of PD) (T0) and at 24 months (T1). Technique survival, mortality, total number of hospitalizations, and the index of peritonitis (episodes of peritonitis/month) were also evaluated.

Fifty-one patients starting PD were enrolled. Group A included 22 patients (48.7 ± 8.3 years), and Group B consisted of 29 patients (74.1 ± 6.4 years). At baseline, the 2 groups showed no differences in cognitive status, whereas Group A showed higher total cholesterol ($P = .03$), LDL ($P = .03$), and triglycerides ($P = .03$) levels and lower body mass index ($P = .02$) and carotid intima media thickness ($P < .0001$) with respect to Group B. At T1 Group B showed, compared to baseline, a significant reduction in albumin ($P < .0001$) and phosphorus ($P = .045$) levels, while no significant differences on body composition, technique survival, total number of hospitalizations, index of peritonitis, and quality of life indices were observed.

Our data do not show clinically relevant barriers to use PD in older adult patients, supporting its use in this population. Nutritional and metabolic parameters should be carefully monitored in older PD patients.

Abbreviations: BMI = body mass index, BOLDE = Broadening Options for Long-Term Dialysis in the Elderly, CKD = chronic kidney disease, CRP = C-reactive protein, DBP = diastolic blood pressure, ESV = erythrocyte sedimentation velocity, FGF = fibroblast growth factor, HD = hemodialysis, HDL = high-density lipoprotein, IMT = intima-media thickness, iPTH = parathormone, KDQOL-SF = Kidney Disease Quality of Life Short Form, LDL = low-density lipoprotein, LVM = left ventricular mass, MF-BIA = multifrequency bioelectrical impedance analysis, nPCR = normalized protein catabolic rate, PD = peritoneal dialysis, RRF = residual renal function, RRT = renal replacement therapy, SBP = systolic blood pressure.

Keywords: older adults, peritoneal dialysis, peritonitis, quality of life, renal replacement therapy

1. Introduction

The prevalence of chronic kidney disease (CKD) is increasing in older adults worldwide [1,2] and the most prevalent causes of loss of kidney function are diabetes, hypertension, and vascular disease.[3] As cardiovascular disease is better managed in the general population, patients live longer and the risk to develop CKD in the older adults is significantly higher.[3] Therefore, the number of older adults needing renal replacement therapy (RRT) is steadily increasing.[4] Studies have shown that dialysis can significantly impact on life expectancy and quality of life, but only in a limited number of patients the peritoneal dialysis (PD) is considered as the first therapeutic option.[5,6] This is probably due to the concern that older adult patients may not be able to safely manage this home-based treatment or due to a perception that PD is associated with greater risk of complications.[7] From the other side, hemodialysis (HD) can be negatively perceived by patients because of the long time to be spent in the HD facilities.[8,9] A large number of older patients do not positively accept to start a HD treatment, while the proportion of older adults on PD is considerably lower with respect to younger patients. Despite different studies have shown that survival and quality of life of older adults on PD do not differ from HD, the option to implement PD is still largely lacking.[7–10] Furthermore, the North Thames Dialysis Study, a prospective study conducted on patients ≥ 70 years old, has shown that survival, hospitalization, and quality of life were similar between patients on HD and those on PD.[5,11,12] Older patients are often socially isolated and often depressed due to loss of independence. Moreover, the associated vascular disease results in a high risk of failure for vascular access for the HD treatment. This results in increased reliance on venous access, with higher risk of infection.[5,8,11–13] Failure of vascular access can necessitate frequent hospital admissions for radiological and surgical procedures. Also, cardiac disease can cause hypotension and arrhythmias during HD session and older adults often feel "weakened" after a HD treatment. In addition, many patients cannot reach the dialysis center independently and require transportation provided by the hospital.[8,12,14] The problem is to determine whether older adults can safely manage PD. Several older adults can be trained to do their own PD, although this may take longer time. Family members are often willing to help with all or part of the procedure, and in some parts of Europe, the use of community nurses enables older adults to be on PD in their own homes.[15–17] Furthermore, we should consider that PD is a less expensive treatment than HD.[18]

The aim of this study was to compare clinical, instrumental parameters, quality of life, and hospitalizations between younger (< 65 years old) and older (≥65 years old) patients on PD.

2. Patients and methods

The study protocol was approved by the Local Clinical Research Ethics Committee. The study conforms to the principles outlined in the Declaration of Helsinki and we obtained a written consent by all patients before the enrollment.

2.1. Study design

We performed a prospective study on clinically stable patients starting PD at the Peritoneal Dialysis Unit, Policlinico Umberto I, Sapienza University of Rome, Rome, Italy.

We enrolled consecutive patients from January 2009 to August 2014, eligible to perform PD.[6] Patients were divided into 2 groups in accordance to age: the first group (Group A) included patients aged < 65 years and the second group patients aged ≥ 65 years (Group B). Clinical, laboratory, instrumental parameters and quality of life were evaluated at baseline (start of PD) (T0) and at 24 months (T1). Kt/V urea and creatinine clearance (L/week/1.73 m2) were calculated. These values were measured by collecting the dialytic effluent over a 24-hour period and by a plasma sample. Residual renal function (RRF) (glomerular filtration rate; mL/minute) was calculated as mean of renal clearances of urea and creatinine, collecting all urine output over the same 24-hour period and dialysate creatinine clearance was obtained from 24-hour collections of dialysate. We also used normalized protein catabolic rate (nPCR) (grams per kilogram per day) calculated from dialysis kinetic modeling.[19–21] We evaluated the technique survival, the mortality, the "index of peritonitis," expressed as months free from peritonitis (episodes of peritonitis/months), and the number of hospitalizations at T0 and T1 in both groups. Peritoneal dialysis-associated peritonitis was defined as a symptom or sign (abdominal pain, fever, and turbid dialysate) combined with an effluent cell count of more than $100/\mu L$ leukocytes, with at least 50% polymorphonuclear neutrophilic cells.[13] The Kidney Disease Quality of Life Short Form (KDQOL-SF) was also administered in both groups.

2.2. Inclusion criteria

Patients aged >18 years starting PD.

2.3. Exclusion criteria

Patients with cancer, liver disease, human immunodeficiency virus, and those who had been on HD (before starting PD) for more than 30 days and patients with previous renal transplantation were excluded. Patients who refused to give consent and patients with missing clinical data were also excluded.

2.4. Anthropometric assessments

Patients were evaluated before the first replacement with empty peritoneum in the morning. During routine visits, height and weight were measured with the patient wearing indoor clothing before intraperitoneal dialysate. Body weight was determined to the nearest 0.1 kg using a calibrated digital scale. Body mass index (BMI) was calculated using the formula [weight (kg)/height (m^2)].

2.5. Laboratory measurements

Blood were collected in the morning after an overnight fasting (at least 12 hours). In all patients, the levels of fasting plasma glucose (mg/dL), total serum cholesterol (mg/dL), triglycerides (mg/dL), high-density lipoprotein (HDL) (mg/dL), low-density lipoprotein (LDL) (mg/dL), serum nitrogen (mmol/L), serum calcium (mg/dL), phosphate (mg/dL), sodium (mEq/L), potassium (mEq/L), serum uric acid (mg/dL), C-reactive protein (CRP) (mg/dL), and erythrocyte sedimentation velocity (ESV) (mm/H), were measured using standard automated techniques. Serum albumin (g/dL) was determined by bromcresol purple method. Parathormone (iPTH) (pg/mL) was measured using a 2-site assay that measures "intact" hormone. LDL-cholesterol was calculated using the Friedewald equation: LDL (mg/dL) = total cholesterol − HDL − (triglicerydes/5). Arterial blood gas analysis was performed using a blood gas analyzer (Nova Biomedical Corporation Waltham MA, USA).

2.6. Blood pressure measurements

Systolic and diastolic blood pressure (BP) levels were measured in patients on PD during routine visits. Clinic BP measurements were made 3 times after 10 minutes of rest in a seated position using a standard automated sphygmomanometer and cuffs adapted to the arm circumference, according to the British Hypertension Society guidelines. The mean values for systolic BP (SBP) and diastolic BP (DBP) were calculated for all participants.[22] The systolic and diastolic BP levels were taken as the points of appearance and disappearance of Korotkoff sounds, respectively. Hypertension was defined as SBP >140 mmHg or DBP >90 mmHg on repeated measurements.

2.7. Bioelectrical impedance analysis

Multifrequency bioelectrical impedance analysis (MF-BIA) (Akern, Florence, Italy) was performed using an impedance plethysmograph that emits an 800-μA, 50-kHz alternating current. Measurements were taken with patients being in a supine position for 5 minutes, according to the manufacturer's guidelines. The analysis of the entire body involves the placement of 2 electrohydraulic injectors at the back of the hands and feet at the distal ends of metacarpals and metatarsals, and 2 measuring electrodes were placed on the dorsal surfaces of the wrists and ankles. We proceeded to the recording of impedance, resistance, reactance, and phase angles and the subsequent transformation into estimates of lean body mass, body fat, cell mass, total body water, and intracellular water.[23,24]

2.8. Common carotid intima-media thickness (IMT)

At T0 and T1, right and left carotid ultrasound was blindly performed by an experienced sonographer who was unaware of the characteristics of the patients under examination. Participants were studied with the high-resolution B-mode ultrasound machine Toshiba Aplio XV (Toshiba AplioxV, Toshiba American Medical Systems, Inc., Tustin, CA) equipped with a 5- to 12-MHz linear transducer with a 0.01-mm resolution, following a standardized vascular protocol. Three different longitudinal views (anterior oblique, lateral, and posterior oblique) and a transverse view were obtained. IMT was measured at three points on the far walls of both left and right distal common carotid arteries, carotid bulb, and the proximal portion of the internal carotid arteries. Images were captured in end diastole triggered by electrocardiographic recording. The mean IMT was computed as the average IMT on both sides. The value of IMT was considered normal when between 0.55 and 1 mm.[25,26]

2.9. Echocardiography

Transthoracic echocardiography after emptying the peritoneal cavity was performed. M-mode 2D echocardiographic examinations by a single experienced sonographer in the echocardiography laboratory and using a standard institutional protocol were completed.[27,28] Commercially available instruments (Toshiba AplioxV, Toshiba American Medical Systems, Inc., Tustin, CA) equipped with 2.25- to 7.5-MHz imaging transducers were used; the patients were in the left decubitus position, and the sonographer was blinded to all clinical details of the patients. All echocardiographic data according to the guidelines of the American Society of Echocardiography were recorded.[29] The end-diastolic and end-systolic left ventricular internal diameter, interventricular septum thickness, and posterior wall thickness were measured. The left ventricular mass (LVM) was estimated following Devereux's formula normalized by body surface area and height.[30]

2.10. Kidney Disease Quality of Life Short Form

The KDQOL-SF is a self-report measure developed for individuals with kidney disease and those on dialysis. It is a shorter version of a measure developed by the same authors. It includes 43 kidney disease-targeted items, such as the effects of the disease of activities of daily living, work status, and social interaction, and 36 items that provide a measure of physical and mental health. The 80 items take about 16 minutes to complete. The item role physical, emotional well-being, and weight of kidney disease is assigned a score from 0 to 4; emotional role instead corresponds to the item a score from 0 to 3. The total score of the questionnaire ranges from 0 to 15, associating at the greater value the lower perceived well-being.[31]

2.11. Statistical analysis

All continuous variables were expressed as mean ± standard deviation, and categorical variables were expressed as numbers (percentage). Student t-tests and ANOVA were performed to determine differences between groups, as appropriate. Binomial test or χ^2 test was used for the comparison of categorical data. A probability value of $P < .05$ was considered to be statistically significant. Data management and analysis were performed using IBM SPSS Statistics 17 for Windows software (IBM Corporation, New Orchard Road Armonk, New York, NY).

3. Results

A total of 51 patients (28 males) were enrolled. Group A included 22 patients (mean age: 48.7 ± 8.3 years), 21% were on automated PD. Group B consisted of 29 patients (mean age of 74.1 ± 6.4 years), 72% are on continuous ambulatory PD. The etiology of end-stage renal disease (ESRD) was autosomal dominant polycystic kidney disease in 4 patients (18%) in Group A and 2 patients (7%) in Group B; chronic glomerulonephritis in 4 patients (18%) in Group A and 6 patients (21%) in Group B; diabetic nephropathy in 2 patients (9%) in Group A and 6 patients (21%) in Group B; chronic pyelonephritis in 2 patients (9%) in Group A and 1 patient (3%) in Group B; hypertensive nephrosclerosis in 6 patients (27%) in Group A and 12 patients (41%) in Group B; and unknown in 4 patients (18%) in Group A and 2 patients (7%) in Group B.

3.1. Baseline characteristics

Patient's characteristics at baseline (T0) are shown in Table 1. In summary, there were no significant differences between the 2 groups, except for higher values in Group A with respect to Group B of total cholesterol (mg/dL) (195.4 ± 43.3 vs 172.2 ± 32.3; $P = .03$), LDL (mg/dL) (125.2 ± 34.3 vs 103.9 ± 31.1; $P = .03$), and triglycerides (mg/dL) (137.8 ± 51 vs 110.1 ± 35.5; $P = .03$), while Group A, with respect to Group B, had a significant lower BMI (kg/m²) (23.45 ± 3.08 vs 26.05 ± 3.50; $P = .02$), and carotid IMT (mm) (0.97 ± 0.32 vs 1.54 ± 0.31; $P < .0001$). The prevalence of comorbidities, including diabetes and cardiovascular diseases, was not statistically different between the 2 groups, as shown in Table 2.

At T0 the 2 groups appeared relatively homogeneous on the items General Health, Role Physical, Role Emotional, and Social

Table 1

Patient's characteristics at baseline (T0).

Patient's characteristics	Group A (n = 22)	Group B (n = 29)
Body mass index, kg/m^2	23.45 ± 3.8	26.05 ± 3.5*
APD, %	36%	27%
CAPD, %	64%	73%
Hb, g/dL	11.12 ± 1.7	10.9 ± 1.6
Albumin, g/L	4.05 ± 0.6	4.08 ± 0.3
Serum creatinine, mg/dL	6.4 ± 1.6	6.1 ± 2.3
BUN, mg/dL	153.5 ± 42.1	137.6 ± 44.2
Natrium, mEq/L	138.4 ± 11.3	140.8 ± 4.3
Potassium, mEq/L	4.7 ± 0.7	4.6 ± 0.5
Calcium, mg/dL	9.4 ± 0.8	9.3 ± 0.8
Phosphorus, mg/dL	4.8 ± 0.8	5.02 ± 1.3
Ipth, pg/mL	231.6 ± 154.6	212.8 ± 111.4
Total cholesterol, mg/dL	195.4 ± 43.3	172.2 ± 32.3†
HDL, mg/dL	51.2 ± 16.8	48.4 ± 16.4
LDL, mg/dL	125.2 ± 34.3	103.9 ± 31.1†
Triglycerides, mg/dL	137.8 ± 51	110.1 ± 35.5†
Serum iron, μg/dL	66.2 ± 28.2	53.5 ± 16.0
Serum ferritin, ng/mL	147.5 ± 116	131.1 ± 107.7
Serum transferrin, mg/dL	217.7 ± 43	207.8 ± 51.1
Fibrinogen, mg/dL	460.2 ± 108.1	500.2 ± 116.1
CRP, mg/dL	1.13 ± 0.85	0.84 ± 0.23
CIMT, mm	0.97 ± 0.32	1.54 ± 0.31‡
LVMI, g/m^2	111.2 ± 23.1	123.8 ± 29.4
KT/V	2.81 ± 0.97	2.85 ± 1.52
RRF	9.6 ± 9.85	10.11 ± 9.95

Data are show as mean ± standard deviation or %.

* P = .02.

† P = .03.

‡ P < .0001.

APD = automated peritoneal dialysis, BUN = blood urea nitrogen, CAPD = continuous ambulatory peritoneal dialysis, CIMT = carotid intima-media thickness, CRP = C-reactive protein, eGFR = estimated glomerular filtration rate, Hb = hemoglobin, HDL = high-density lipoprotein, iPTH = intact parathormone, LDL = low-density lipoprotein, LVMI = left ventricular mass index, RRF = residual renal function.

Function of the KDQOL-SF test. Also, the items cognitive function (87.18 ± 11.42 vs 89.67 ± 14.72; P = .51) and Physical Function (63.24 ± 24.30 vs 53.75 ± 21.49; P = .15) did not show statistically significant differences between the 2 groups at T0.

3.2. Follow-up results

At T1 in Group B, a significant reduction in albumin (P < .0001) and phosphorus levels was observed (P = .045) (Table 3). KT/V, RRF and nPCR were not different between the 2 groups (3.0 ± 1.2 vs 2.5 ± 0.80, P = .08; 7.53 ± 4.21 vs 10.21 ± 5.59, P = .28; 1.05 ± 0.17 vs 0.95 ± 0.19, P = 0.29, respectively). No significant changes in body composition parameters were shown (Table 3) and no differences in the index of peritonitis (episodes of peritonitis/month) were registered (Group A: 0.03 ± 0.001 vs

Table 2

Comorbid conditions in Group A and Group B.

	Group A (n = 22)	Group B (n = 29)	P
Comorbid condition, n (%)	20 (91)	29 (100)	.35
Arterial hypertension, N (%)	15 (68.1)	24 (82.7)	.38
Diabetes, N (%)	2 (9)	6 (21)	.46
Ischemic, hypertensive or valvular heart disease, N (%)	3 (13.6)	9 (31)	.26
Peripheral vascular disease, N (%)	2 (9)	7 (24.1)	.30
Transient ischemic attack or stroke, N (%)	1 (4.5)	5 (17.2)	.34

Group B: 0.03 ± 0.002; P = .99) In addition, the survival of the technique during the follow-up was of 77.2% (n = 17) and 72.4% (n = 21) (P = .54) and the patient's survival rate at the end of the follow-up was 95.4% (n = 21) in Group A and 86.2% (n = 25) in Group B (P = .05). We reported 9 hospitalizations in Group A and 11 in Group B (P = .94). The reasons for hospitalization were comparable between the 2 groups (including cardiovascular and cerebrovascular events). Both groups did not differ regarding the item cognitive functions of the KDQOL-SF (Group A 86.27 ± 11.42 vs Group B 91.25 ± 9.34; P = .09). And they appeared relatively homogeneous on the items General Health, Role Physical, Role Emotional, Social and Physical Function and Satisfaction care. Also, Group A had a higher score for Energy /Fatigue (Group A 59.4 ± 13.1 vs Group B 54.1 ± 23.9; P = .35) and Sleep Quality (Group A 67.7 ± 13.3 vs Group B 60 ± 23.2; P = .17), although not statistically significant.

4. Discussion

Chronic kidney disease is a common condition among older adults and only a few patients with ESRD are treated with PD.[32–34] The Broadening Options for Long-Term Dialysis in the Elderly (BOLDE) study has shown that older patients can successfully manage PD, and that in 2 closely matched demographic groups of older dialysis patients on PD and HD, quality of life was similar, but with significantly less perception of intrusion of the disease in their lives in the PD group.[12,35,36] In our study, there were also no statistically significant differences in the index of peritonitis and in term of mean survival of the technique between younger and older patients. In HD, complications of the vascular access, intradialytic hypotension, and impaired autonomic function with hemodynamic instability, bleeding, and amyloidotic arthropathy represent the most relevant limitations, especially in older adults.[7,8,37] Authors suggested that patients on PD perceived lower distress and higher psychological well-being compared with patients undergoing other replacement therapies.[38,39] Moreover, several studies indicated that the psychological impact

Table 3

Biochemical, inflammatory and body composition variables in Group A and Group B at T0 and T1.

Variables	Group A (T0)	Group A (T1)	P	Group B T0	Group B T1	P
Albumin, g/dL	4.05 ± 0.61	4.0 ± 0.54	.77	4.08 ± 0.39	3.70 ± 0.44	<.0001
Phosphorus, mg/dL	4.85 ± 0.85	5.38 ± 1.19	.10	5.02 ± 1.30	4.32 ± 1.30	.045
nPCR, g/kg/day	1.1 ± 0.14	1.05 ± 0.17	.29	0.98 ± 0.20	0.95 ± 0.19	.56
CRP, mg/dL	1.13 ± 0.85	1.21 ± 0.32	.68	0.84 ± 0.23	1.07 ± 0.85	.17
FFM, %	76.1 ± 6.9	78.5 ± 8.6	.31	73.4 ± 8.7	70.5 ± 11.6	.28
FM, %	23.9 ± 6.9	21.5 ± 8.6	.31	28.07 ± 9.02	29.5 ± 11.6	.60
ECF/TBW	0.40 ± 0.05	0.42 ± 0.06	.23	0.41 ± 0.05	0.41 ± 0.03	.57

CRP = C-reactive protein, ECF = extra-cellular fluid, FFM = fat free mass, FM = = fat mass, nPCR = normalized protein catabolic rate, TBW = total body water.

of a disease can affect compliance with medical treatments.[40,41] A study showed that the non-adherence to medical prescriptions is a consistent concern in patients on PD causing major difficulties to manage their treatments,[41] that may include the adherence to the dietetic regimen. This aspect should be taken into consideration to effectively improve patient's outcomes. In fact, previous studies showed that patients on renal replacement therapy presented lower adherence to therapy, contributing to a greater morbidity and earlier mortality.[42] Our study showed significant modifications in serum phosphorus levels during the follow-up, suggesting a possible lower adherence to the therapy and to the dietetic regimen in younger patients and better acceptance of the specific food restriction in older adults. Patients with ESRD present a markedly increased cardiovascular risk and increased serum phosphate levels can have several negative effects on organ functions being associated with increased cardiovascular events and mortality.[43,44] The relationship between serum phosphorus and cardiovascular mortality has been also evaluated in patients in the REIN and NANHES III study,[45,46] suggesting that phosphorus is an independent predictor of cardiovascular mortality. Moreover, the phosphate-regulating hormone fibroblast growth factor (FGF)-23 is directly associated with left ventricular hypertrophy. Yamamoto et al[47] have shown that higher serum phosphorus intake is associated with higher left ventricular mass. Short-term studies have also shown that dietary phosphate reduction effectively decreases FGF-23 levels.[48] Moreover, our study showed a significant reduction of albumin levels in older patients, suggesting an increased risk of protein-energy wasting in this population, although albumin is a poor marker of nutrition, since it also represents an indirect measure of inflammation. Indeed, PD patients lose albumin in the dialysate, which could also cause hypoalbuminemia, and presumably older patients are not able to replace the losses, due to reduced liver function.[49] Other clinical and instrumental data, including body composition, showed similar results in both groups during the 24-month follow-up, suggesting an equally effective maintenance of most of the clinical and metabolic parameters during PD. Although Group B showed higher BMI with respect to Group A, we did not find differences in term of body composition, in particular regarding lean body mass. In fact, we were expecting a significant reduction of muscle compartment, which is a common feature among older patients. Interestingly, we observed a stable hydration status in patients of both groups. Also, echocardiographic indexes, including LVM index, which represents a clinical parameter to monitor cardiac hypertrophy [50] and possibly skeletal muscle mass modifications,[51] remained unchanged during the follow-up in the 2 groups. We did not observe modifications in atherosclerosis indexes (carotid IMT), although they are age-related and no significant differences in the number of hospitalizations. The 2 groups were comparable regarding specific items of the KDQOL-SF and group B showed no physical or cognitive disabilities that may justify the lack of use of PD.

We acknowledge the limitations of our study. In particular, we enrolled a relatively small, selective cohort of PD patients and a larger population appears necessary to confirm our results. Furthermore, as shown in our results, a significant proportion of patients were on several medications with potential impact on different metabolic indices that may have possibly confounded the results.

Our study showed similar results in term of clinical outcomes between younger and older patients treated with PD. We did not observe increased risk of peritonitis between groups and we found a good control of different clinical parameters and perception of general health during the 24-month follow-up. Our data support the expansion of PD procedure in older adults. Patients' preferences, after a precise information regarding dialysis techniques, should be elicited and carefully considered by healthcare professionals, considering the lower costs of PD compared to HD.[8,9] Larger randomized clinical trials are needed to confirm the results here presented.

4.1. Compliance with ethical standards

The authors declare that they have no conflict of interest.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from all individual participants included in the study.

The authors alone are responsible for the content and writing of the article.

The article has been seen and approved by all the authors.

The article is not under consideration for publication elsewhere.

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