Osteosarcopenia in Patients with Non-Dialysis Dependent Chronic Kidney Disease

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ABSTRACT

Introduction: Chronic kidney disease (CKD) is associated with a reduction in bone mineral density (BMD), but less is understood regarding the relation between BMD and muscle mass, especially in non-dialysis dependent-CKD (NDD-CKD). The aim of this study was to explore the prevalence and association of low BMD (osteopenia and osteoporosis) with markers of muscle mass and function in patients with NDD-CKD.

Methodology: This cross-sectional observational study included patients with NDD-CKD. Routine biochemical parameters including those related to mineral and bone metabolism were evaluated. Body composition was assessed by dual energy x-ray absorptiometry (DXA) for BMD (g/cm²), total and trunk body fat (%), total lean soft tissue (LST; kg), and appendicular skeletal muscle mass (ASM; kg) as the sum of the LST from the limbs. The latter two variables were used as markers of muscle mass, together with its height indexed values: ASM/height² as ASM index (ASMI; kg/m^2), and LST/height² as LST index (LSTI, kg/m^2). Muscle quality index (MQI) was calculated as handgrip strength (HGS)/mean ASM_{arms} (kg/kg). Osteosarcopenia was defined according to referenced cut-points for patients presenting with low ASMI, HGS and BMD.

Results: Patients (n=257, 57.6% males) had a mean age=64.8±12.9 years, estimated glomerular filtration rate (eGFR)=30.1±12.9 ml/min and body mass index (BMI)=26.8±4.8 kg/m^2. Patients with low BMD (39.4%) presented with lower BMI, LST, LSTI, ASM and ASMI for both sexes. BMD was positively and significantly correlated with LST, LSTI, ASM, ASMI and HGS. Low ASM was associated with low BMD (odds-ratio-OR; 95% confidence interval-CI: males OR= 4.54, 2.02-10.21; females OR=4.45, 1.66–11.93). Linear multiple regression analysis (adjusted for sex and eGFR) showed significant associations between T-score with HGS (R^2=0.288, R^2 adjusted=0.272, standardized coefficient β=0.536, p<0.0001) and also with MQI (R^2=0.095, R^2 adjusted=0.075, standardized coefficient β=0.309, p=0.024).

Osteosarcopenia was present in about 7% of participants and similarly distributed between sexes.

Conclusion: Low BMD was prevalent, and associated with low markers of muscle mass and quality, in NDD-CKD patients of both sexes. In view of the known significance of these conditions, targeted interventions are needed to optimize body composition and functional status of these patients.

Keywords: bone mineral density, osteosarcopenia, muscle quality index, chronic kidney disease

INTRODUCTION

Chronic kidney disease (CKD) is a condition characterized by structural renal changes, with a progressive decline in its functions (1). As the kidneys contribute to the regulation of mineral metabolism, including modulation of calcium and phosphorus, parathormone (PTH) and vitamin D concentrations, CKD leads to mineral and bone disorders (2-6). Therefore, osteopenia and osteoporosis, conditions characterized by a low bone mineral density (BMD) (7), are reported in patients with non-dialysis dependent CKD (NDD-CKD) (8). Low BMD is a term widely used to combine both osteopenia (define as T-score $\leq$ -1.0) and osteoporosis (T-score $\leq$ -2.5) (4, 8-11). Low BMD is associated with higher cardiovascular risk, such as vascular calcification in patients with NDD-CKD (4, 12, 13), and in patients undergoing hemodialysis therapy (3, 14).

Low BMD often coincides with skeletal muscle loss in older adults; in fact, the metabolism of both tissues is similar and seem to be interconnected (15, 16). Low muscle mass can in turn occur concurrent to low strength and/or functional capacity, which defines sarcopenia. Sarcopenia is common in CKD, due to increased protein catabolism as a result of metabolic and nutritional abnormalities (17), and is associated with higher mortality in patients undergoing hemodialysis (3, 4).

Muscle quality reflects micro- and macroscopic aspects of muscle architecture and composition and is more strongly association with muscle function than with muscle mass (18, 19). Additionally, poor muscle quality can lead to adverse outcomes such as reduced physical function, lower quality of life and well-being, higher morbidity and mortality (19). Muscle strength and mass can be used to estimate muscle quality index (MQI), which has been shown as a reliable marker of muscle function (20, 21).

Interestingly, osteopenia/osteoporosis and sarcopenia, can co-exist in a syndrome called osteosarcopenia, a concept first introduced by Binkley and Buehring (22). However, this

syndrome has been poorly understood, partially due to the lack of diagnostic criteria (15, 23). Some studies have investigated the relationship between BMD and lean soft tissue (LST, which includes muscle mass) (24) and sarcopenia in patients undergoing hemodialysis (7, 25). However, to the best of our knowledge, this has not been previously explored in patients with NDD-CKD. Early detection of osteosarcopenia in the NDD-CKD population may improve prognosis and quality of life, reducing cardiovascular risk and mortality. As such, the aim of this study was to explore the prevalence and association of low BMD (osteopenia and osteoporosis) with markers of muscle mass, function, and osteosarcopenia in patients with NDD-CKD.

METHODS

Study design and population selection

A cross-sectional observational study was conducted in clinically stable NDD-CKD patients from the nephrology outpatient clinic at Pedro Ernesto University Hospital (Rio de Janeiro State University, Rio de Janeiro, Brazil). Eligible participants were adults (≥18 y) with estimated glomerular filtration rate (eGFR) <60 mL/min and undergoing standard medical and nutritional treatment for at least 6 month (nephrologist and renal dietitian). Routine appointments were scheduled for 4 to 6 visits per year, depending on the overall health status and CKD stage. As part of the nutritional counseling, all patients were advised to restrict their daily protein based on the guidelines recommendations for protein intake (0.6 g protein/kg body weight or 0.8 g protein/kg body weight in the presence of diabetes) and energy (25 to 30 kcal/kg daily) (26). As for eligibility, patients were excluded if presenting the following: active malignant disease, glomerulonephritis under immunosuppressive therapy, history of previous kidney or any other organ transplantation, human immunodeficiency virus infection, acute inflammation, chronic lung disease, liver failure, heart failure (class 3 or 4), apparent edema,

undergoing dialysis, using immunosuppressive and corticoid drugs, as well as pregnant or lactating women.

Protocol and data collection

The human ethics research committee of the Pedro Ernesto University Hospital approved the study, and all patients signed an informed consent before inclusion. Demographic, clinical/laboratorial, anthropometric, and body composition data were collected throughout 4 years.

Patients were advised to fast for 12h before the assessments, avoid strenuous physical activity, and to follow their usual prescribed diet. A blood sample was collected early in the morning, followed by anthropometric measurements performed by experienced dietitians, whereas a trained technician performed the dual energy x-ray absorptiometry (DXA) for the body composition analysis.

Laboratory parameters

Routine laboratory parameters for CKD patients (creatinine, urea, uric acid, hemoglobin, glucose, total cholesterol, high- and low-density lipoprotein (HDL and LDL) cholesterol, triglyceride, potassium, phosphorus, calcium, and albumin) were analyzed at the University Hospital Central Laboratory. Insulin, parathyroid hormone (parathormone) (PTH) and 25(OH)D (25-hydroxy vitamin D) were determined in blood samples from serum stored in a freezer at -70°C. Insulin was determined by radioimmunoassay using human-insulin kits (Millipore, Billerica, MA, USA), which determines insulin concentration with a sensitivity of 0.2 μU/mL. PTH and Vitamin D concentrations were measured by chemiluminescence enzyme immunoassay (Vitamin D was analyzed using DiaSorin Inc., Stillwater, MN, USA; sensitivity limit ≤ 10.0 ng/mL; linearity limits: 8.0-160.0 ng/mL; coefficient of variation: 1.4-3.7%). The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation (27) and CKD stages were defined according to eGFR: 45-59 (stage 3a), 30-44 (3b), 15-29 (4),

and < 15 (5) mL/min/1.73 m² (26). Fasting plasma glucose and insulin values were used to estimate the homeostasis model assessment of insulin resistance (HOMA-IR), which was calculated as follows (28): *HOMA-IR = (fasting insulin [mU/mL] x fasting plasma glucose [mmol/L])/ 22.5*.

Laboratory parameters of mineral and bone metabolism: The normal limits were based on recommended serum values: calcium (8.4-9.5 mg/dl) (29), phosphorus (3.5-5.5 mg/dl) (29), PTH (>200 pg/ml) (29, 30) and vitamin D (>20 ng/mL) (31).

Anthropometric and body composition assessment

Anthropometric measurements included body weight and height. Body weight and height values, to the nearest 0.1 kg and 0.5 cm, respectively, were measured on a balance-beam scale with a stadiometer attached to the platform Filizola® (São Paulo, Brazil), with patients wearing light clothing and no shoes, empty bladder, and standing straight with their head in the Frankfort plane. The mean of the three measurements was recorded. Body mass index (BMI) was calculated, for each patient, as weight (kg)/height (m^2).

Body composition was assessed using a Lunar iDXA densitometer and the enCore 2008 version 12.20 software (GE Healthcare). Body composition parameters included BMD, total and regional body fat (%), LST, and the sum of the LST from the limbs, often called appendicular skeletal muscle mass (ASM). Both LST and ASM were indexed to height in meters squared as a standard adjustment approach: LST index (LSTI, kg/m^2) and ASM index (ASMI, kg/m^2) (18). The load-capacity index (LCI) was calculated using total fat mass (kg) divided by total LST (kg) (32). The latter model considers fat mass as the "metabolic load" and LST as the "metabolic capacity". LCI has been proposed as a more sensitive approach for disease-risk prediction, integrating the effects of both adiposity and LST (26).

Muscle function and quality

Muscle strength was analyzed in a subset of the studied patients (n=142, 55%) by handgrip strength (HGS) using a handheld dynamometer (Baseline® Smedley Spring Dynamometer; Fabrication Enterprises Inc.), according to the protocol recommended by the American Association of Hand Therapists (33). Participants were first familiarized with the device and were then evaluated seated, shoulders adducted and neutrally rotated, elbow flexed at 90°, forearm in neutral and wrist between 0 and 30° of dorsiflexion. Participants were instructed to grip the dynamometer with the maximum strength in response to a voice command. Measurements were repeated at 1 min intervals and obtained three times for each hand in a rotational way. The highest value of three measurements in each hand was considered and the mean value was used in the study analysis. Muscle quality was accessed as muscle quality index, using the HGS and the mean of LST from both arms ($MQI=HGS\ (kg)/ASM_{arms}\ (kg)$) as proposed by Lee and Dierickx (21).

BMD status

BMD reflects the weight (g)/area (cm^2) of the bones, and BMD abnormalities are assessed using the T-score, which is the number of standard deviations (SDs) of BMD compared to the mean BMD for a sex and ethnicity-matched young adult healthy population (34). In the present study, total body BMD was obtained from all included patients, and the lumbar spine (vertebrae L1-L4) in a subset of 30 patients. The L1-L4 T-score is one of the reference parameters for the diagnosis of low BMD (34). Thus, accuracy analysis between total BMD T-score and L1-L4 T-score was performed. Bland-Altman and concordance correlation coefficient (CCC) results showed a strong agreement (limits of agreement: 0.18; 95% CI: 0.23-2.00; CCC: 0.69; 95% CI: 0.52-0.81; accuracy: 0.80, $p<0.0001$). Thus, BMD status of the total sample was assessed according to total T-score values.

Definitions

BMD status: Normal BMD is defined as total T-score > -1.0, osteopenia as T-score ≤ -1.0 and > -2.5, and osteoporosis as T-score ≤ -2.5 (7). Patients were diagnosed according to these 3 BMD categories and selected analyses were performed by comparisons between 2 BMD status: normal and low (T-score ≤ -1.0; grouping patients with osteopenia or osteoporosis).

Adiposity, muscle mass and strength status: Low muscle mass was defined according to cut-points values proposed for older adults based on ASM < 20 kg for men and < 15 kg for women (35), and ASMI: < 7.0 kg/m^2 for men and < 5.5 kg/m^2 for women (36), as endorsed by the European Working Group on Sarcopenia in Older People (EWGSOP2) (18). Low muscle strength was defined according to cut-points values proposed for older adults: using HGS < 27 kg for men and < 16 kg for women (37). High adiposity (obesity) was defined as fat percentage > 25% for men and > 32% for women (38), and LCI was classified using age, sex and BMI reference curves percentiles (low < 0.15 percentile and high > 0.85 percentile) (32).

Osteosarcopenia: Low muscle mass and strength (according to ASMI+HGS) combined with low BMD was used to define four phenotypes: normal (normal muscle mass and strength + normal BMD), osteopenia/osteoporosis (normal muscle mass and strength + low BMD), sarcopenia (low muscle mass and strength + normal BMD), and osteosarcopenia (low muscle mass and strength + low BMD).

Muscle quality: Muscle quality index (MQI; kg/kg) was used to define 3 groups: normal (> 10.13 for men; > 11.95 for women), low (10.13 to 8.37 for men; 11.95 to 10.09 for women) and poor muscle quality (< 8.37 for men; < 10.09 for women) (21).

Statistical analyses

A minimum sample size of 157 patients was estimated based on the study conducted by Hyun et al (2020) (4) evaluating 2128 NDD-CKD patients, with a prevalence of 41% of low BMD (33% of osteopenia and 8% of osteoporosis); sampling analysis considered type I error -

alpha=0.05 and type II error - beta=0.10). The normality of continuous variables was tested by the Kolmogorov-Smirnov test, continuous variables were presented as mean±SD when normally distributed, and as median and interquartile interval when not normally distributed. Comparison of continuous variables were performed according to distribution pattern by ANOVA or Kruskal-Wallis tests (for > 2 groups, with Bonferroni post-hoc analysis), and by independent samples T-test or Mann-Whitney test (between 2 groups). Pearson's or Spearman's correlation coefficients (according to continuous variables distribution pattern) were calculated separately by sex to analyze the degree of association between two variables. Logistic regression analysis was performed to explore possible risk factors (as independent categorical variables) associated with low BMD (as categorical dependent variable). Linear regression analysis was performed to estimate the association between continuous variables. Unadjusted and adjusted analysis were performed (co-variates: eGFR, age) in males and females. Statistical significance was considered when $P < 0.05$. Statistical analyzes were performed with the statistical software package SPSSv.22.0 (IBM-SPSS Inc., USA).

RESULTS

A total of 257 NDD-CKD patients were evaluated (57.6% males, eGFR of 30.1±12.9 ml/min and BMI of 26.8±4.8 kg/m^2). Mean age was 64.8±12.9 years (women: 65.8±12.7, median: 66.5; interquartile intervals: 57.5-76.0; men: 63.3±13.1 years, median: 65.0, interquartile intervals: 54.8-74.0). Most patients presented with CKD stages 3b (31.6%) and 4 (43.3%); 14.2% had stage 3a and 10.9% had stage 5. The main underlying disease was hypertension (43.6%, n= 112), followed by other diseases including diabetes mellitus (14.0%, n= 36), chronic glomerulonephritis, tubulointerstitial nephritis, and polycystic kidney disease (24.5 %, n=63) and unknown etiologies (17.9%, n= 46). The studied NDD-CKD patients were under regular interdisciplinary treatment for 5.1 ± 3.3 years. Patients' appointments are

routinely scheduled with the nephrologist and renal dietitian for around 4-6 visits per year, depending on CKD stage and overall health status, thus comprising a clinically stable patient population. The median proteinuria (measured in urine sample as protein:creatine ratio) was 500.6 mg/g (interquartile interval: 153.4-1190.0 mg/g). Of note, patients with active glomerulonephritis were not included in the study. Prevalence of osteopenia and osteoporosis was 30.4% (n=78) and 9% (n=23), respectively.

Analysis of bone mineral metabolism parameters

Mean calcium (9.13±0.62 mg/dl) and phosphorus (3.85±0.82 mg/dl), and median vitamin D concentration (29.7 ng/dl, interquartile interval: 22.6 - 42.4) were within non-deficient limits. The frequency of hyperphosphatemia was 19% (n=49) these patients presented mean serum phosphorus of 5.1±0.6 mg/dl. No patients were using phosphate binders. Vitamin D deficiency was present in 17% (n=44) of the patients with mean serum vitamin D of 15.8±4.8 ng/dl. Only 7.4% (n=19) of the patients used vitamin D supplements. T-score values (used as the variable do define normal and low BMD) were similar between patients using (T-score= -0.53±1.61) and not using (T-score= - 0.64±1.26) (p= 0.787) vitamin D supplements.

Hyperparathyroidism frequency was 62% (n=159), the median PTH values of these patients was 118.7 pg/ml (interquartile interval: 92.1-182.2), and mean T-score values of these patients (-0.58±1.39) were not significantly different to patients without hyperparathyroidism (-0.66±1.17). Univariate correlation analysis between T-score values with calcium, phosphorus, PTH and vitamin D were not significant.

Comparison analysis among groups according to BMD

A comparison of patients by BMD status is shown in Table 1. There were no differences in routine laboratory variables, including those related with renal function and parameters of bone mineral metabolism. Patients with normal BMD presented with higher values related to glucose metabolism and triglycerides compared to osteopenia and osteoporosis groups (Table

1). T-score was similar between patients with diabetes (14%, n=36) (T-score= - 0.61±1.26) and those without this condition (T-score= -0.83±1.34) (p=0.442). BMI was similar between males and females in each group of BMD status. Both males and females in the normal BMD group presented with higher mean BMI values compared to those in the osteopenia and osteoporosis groups (p<0.0001), while BMI were similar (p=0.410) between patients in the osteopenia group compared to those in osteoporosis group for both sexes.

As expected, males presented with higher LST and muscle parameters than females, but adiposity parameters were higher in females (p<0.0001), for LSTI males had higher values compared to females in the normal BMD and osteopenia (but not osteoporosis) groups (Table 1). Patients in the osteopenia and osteoporosis groups presented with lower LST, LSTI, and ASM compared to participants in normal BMD group, regardless of sex. Males with normal BMD had higher ASMI values, compared to those in the osteopenia and osteoporosis groups; females in normal BMD group had higher ASMI compared to those in the osteopenia group, but similarly to those in the osteoporosis group; ASMI was similar between the osteopenia and osteoporosis groups for both sexes. Adiposity parameters (total and trunk body fat, and LCI) were lower only in males with osteopenia or osteoporosis compared to males with normal BMD. In females, adiposity parameters were similar among the three groups (Table 1).

Table 1. Clinical and nutritional parameters between BMD groups of patients with non-dialysis dependent chronic kidney disease (NDD-CKD; n=257)

	Normal BMD (N=156, 60.70%)	**Low BMD (N=101, 39.3%)**	
		Osteopenia (N=78, 30.4%)	**Osteoporosis (N= 23, 9.0%)**
Clinical and Laboratory routine variables			
Creatinine (mg/dl)	2.5±1.1	2.5±1.1	2.4±0.8
eGFR (ml/min)	30.7±13.1	29.8±12.7	27.0±12.3
Urea (mg/dl)	83.7±34.4	84.3±38.9	83.3±35.8
Uric acid (mg/dl)	7.8±2.0	7.3±1.9	7.0±1.5
Potassium (mEq/L)	4.8±0.6	4.8±0.5	4.7±0.5
Calcium (mg/dl)	9.5±0.5	9.4±0.7	9.5±0.5

Phosphorus (mg/dl)		3.9±0.9	3.9±0.7	4.1±1.5
PTH (pg/ml)		82.8 (51.8-131.4)	81.8 (54.1-124.5)	77.6 (45.9-141.0)
Vitamin D (ng/ml)		29.4 (22.8-40.1)	30.4 (22.4-49.5)	30.6 (24.4-42.5)
Hemoglobin (g/dl)		12.2±1.7	11.9±1.6	11.5±1.2
Albumin (g/dl)		4.3±0.3	4.3±0.4	4.3±0.3
Glucose (mg/dl)		112.6±45.9[a]	99.4±21.8[b]	94.4±12.5[b]
Insulin (µU/ml))		8.9 (5.6-15.0)[a]	6.0 (4.1-8.7)[b]	4.9 (4.3-12.1)[b]
HOMA-IR		2.3 (1.4-3.7)[a]	1.5 (0.9-2.4)[b]	1.4 (1.0-2.5)[b]
Triglycerides (mg/dl)		147.0 (102.0-194.0)[a]	115.5 (90.0-167.5)[b]	93.5 (85.0-124.0)[b]
Total cholesterol (mg/dl)		170.6±58.5	164.0±50.7	169.1±56.9
LDL-cholesterol (mg/dl)		108.7±40.9	101.6±42.2	100.9±34.1
HDL-cholesterol (mg/dl)		41 (35.0-53.5)	45.5 (38.0-54.0)	61.5 (46.0-82.0)
BMI and Body Composition				
BMD (g/cm^2)		1.2±0.1[a]	1.0±0.4[b]	0.8±0.1[c]
	♀	1.2±0.1[a]	1.0±0.05[b]	0.9±0.03[c]
	♂	1.1±0.1[a]	0.9±0.04[b]	0.8±0.03[c]
T-score		0.20±0.85[a]	-1.57±0.40[b]	-2.98±0.31[c]
	♀	0.23±0.91[a]	-1.56±0.50[b]	-3.07±0.35[c]
	♂	0.13±0.94[a]	-1.58±0.50[b]	-2.89±0.45[c]
BMI	♂	28.0±4.2[a]	24.3±4.0[b]	22.1±3.6[b]
	♀	28.3±5.5[a]	25.2±4.1[b]	25.7±4.8[b]
Total body fat (%)*	♂	31.4±6.9[a]	28.2±8.4[b]	27.6±8.6[b]
	♀	40.6±7.5	39.7±6.1	38.5±6.8
Trunk fat (%)*	♂	35.9±9.9[a]	31.2±11.4[b]	27.9±11.8[b]
	♀	43.2±9.8	41.0±7.2	39.1±10.2
Load Capacity Index (kg/kg)*	♂	0.48 (0.40-0.52)[a]	0.42 (0.29-0.50)[b]	0.39 (0.30-0.55)[b]
	♀	0.69 (0.56-0.83)	0.66 (0.57-0.79)	0.67 (0.46-0.80)
Lean Soft Tissue (kg)*	♂	51.4±7.4[a]	44.0±4.4[b]	39.2±4.2[b]
	♀	39.1±6.1[a]	34.3±4.7[b]	34.4±5.1[b]
Lean Soft Tissue Index (LSTI) (kg/m^2)*	♂	18.1±2.1[a]	16.4±1.6[b]	15.3±1.8[b]
	♀	16.0±2.1[a]	14.6±1.8[b]	15.2±1.6[b]
Appendicular Skeletal Muscle Mass (kg)*	♂	24.2±5.2[a]	20.6±3.5[b]	17.5±1.9[b]
	♀	18.5±3.7[a]	15.2±2.5[b]	15.9±3.5[b]
Appendicular Skeletal Muscle Index (kg/m^2)*	♂	8.5±1.5[a]	7.5±1.4[b]	6.8±0.8[b]
	♀	7.6±1.4[a]	6.5±0.9[b]	6.9±1.3[a,b]

Presented as mean±SD for normal distributed variables and as median (interquartile interval) for non-normal variables.
eGFR: estimated glomerular filtration rate; HOMA-IR: homeostasis model assessment of insulin resistance; LDL: low density lipoprotein; HDL: high density lipoprotein; BMD: bone mineral density; PTH: parathyroid hormone.
Symbols: ♀ for Female patients; ♂ for Male patients.
ANOVA (one-way analysis for normally distributed variables) for comparison among groups of BMD. Statistical difference ($p<0.01$). Pairwise comparisons by post-hoc Bonferroni analysis: values that do not share similar letter are statistically different
*Independent T-test for comparisons between sexes: total body fat, trunk fat and load capacity index were lower in males versus females ($p<0.05$); lean soft tissue (LST), skeletal muscle mass, appendicular muscle mass (ASM) and ASM index (ASMI) were higher in males versus females ($p<0.05$), in each of the three groups; LSTI was higher in males vs. females in normal BMD and osteopenia groups, but similar in osteoporosis group.

No statistical difference in pairwise comparisons of body adiposity parameters, LST, LSTI and muscle mass parameters by post-hoc analysis were observed between the groups with osteopenia and osteoporosis. As such, subsequent analysis considers low BMD as one group (n= 101, 39.30%).

The mean age was higher in the low BMD group compared to normal BMD (70.6±11.0 vs. 61.0±13.0 years; p<0.0001). CKD stages were similarly distributed between groups with low and normal BMD (CKD3a: 13.5% vs. 14.6%; CKD3b: 30.2% vs. 32.5%; CKD4: 45.9% vs. 41.7%; CKD5: 10.4% vs. 11.2%), p=0.953. Sex distribution was similar between groups according to BMD status (normal BMD group: males=60.3%, females=39.7%; low BMD group: males=53.5%, females=46.5%; chi^2-p=0.344).

Correlation and association analysis among T-score and parameters of fat and muscle masses

Adiposity parameters (total body fat, trunk fat and LCI) had a direct correlation with T-score only in males, while LST, LSTI, ASM and ASMI were correlated with T-score in both sexes (Table 2).

Table 2. Correlations analysis among T-score and parameters of fat and muscle masses of patients with non-dialysis dependent chronic kidney disease (NDD-CKD; n=257)

T-score versus		Adiposity parameters			Muscle mass parameters			
		Body fat (%)	Trunk fat (%)	LCI (kg)	LST (kg)	LSTI (kg/m^2)	ASM (kg)	ASMI (kg/h^2)
	♂	**0.253****	**0.302****	**0.235****	**0.632*****	**0.440*****	**0.494*****	**0.372*****
	♀	**0.170**	**0.180**	**0.192**	**0.479*****	**0.354*****	**0.439*****	**0.362*****

Pearson correlations: *** p< 0.0001; **p<0.005; * p<0.05.
LCI: load capacity index, LST: lean soft tissue, LSTI: lean soft tissue index, ASM: appendicular muscle mass, ASMI: appendicular muscle mass index.

Low ASM was associated with a higher risk for low BMD in both sexes, while high body adiposity and LCI was associated as a protective factor for low BMD in men, but not in women (Table 3).

Table 3. Odds ratio (95% CI) for low bone mineral density according to the presence of possible risk factors related to body composition in non-dialyzed patients with chronic kidney disease (n= 257)

Low BMD Risk factors		Unadjusted OR (95% CI)	Adjusted[#] OR (95% CI)
Low ASM	♂	5.32 (2.54 – 11.27)***	4.54 (2.02 – 10.21)**
	♀	4.44 (1.87 – 10.57)***	4.45 (1.66 – 11.93)**
High total body fat	♂	0.23 (0.10 – 0.52)**	0.18 (0.07 – 0.48)**
	♀	0.72 (0.25 – 2.10)	0.45 (0.13 – 1.55)
High LCI	♂	0.52 (0.30 – 0.88)*	0.47 (0.26 – 0.84)*
	♀	0.64 (0.34 – 1.18)	0.65 (0.3 – 1.33)

[#]adjusted for age and eGFR. P model for regression analysis coefficients: ***p<0.0001;**p<0.005; *p<0.05
BMD: bone mineral density; ASM: appendicular muscle mass; LCI: load capacity index,
Low ASM: men <20 kg and women <15 kg) (35)
High total body fat: men >25% and women >32% (38)
High LCI: >0.85 percentile of sex and age-matched normal population (32).

Associations of muscle strength and muscle quality index with bone mineral density

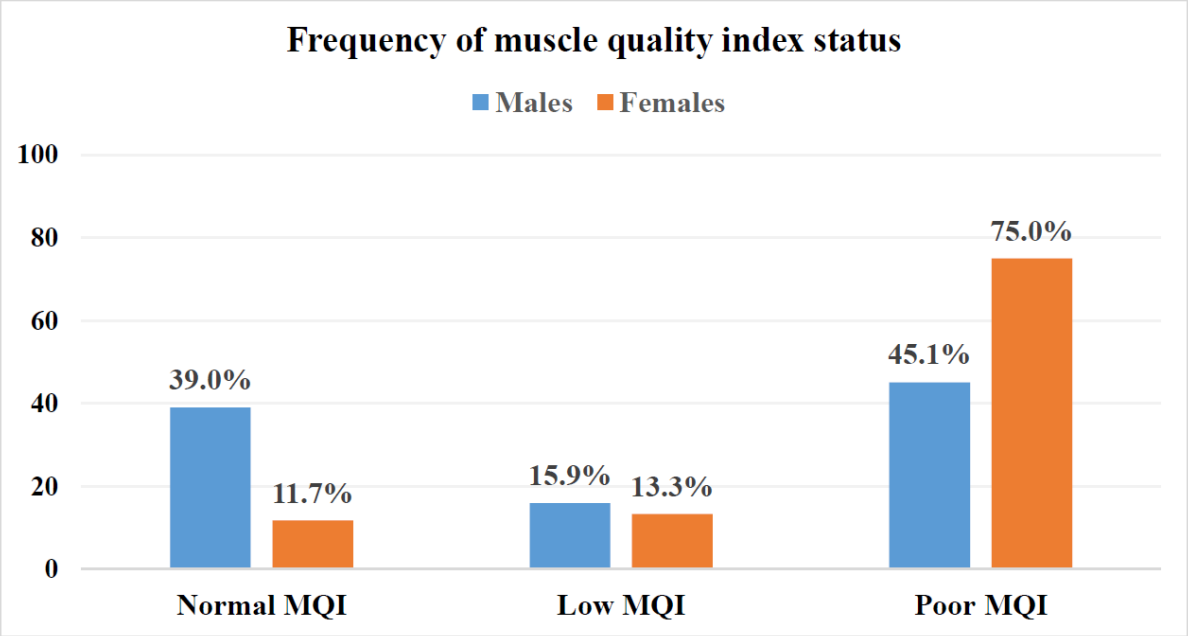
Patients whose muscle strength were assessed (n=142: 57.7% males, n=82 and 42.3% females, n=60) presented similar age (64.5±11.9 years) and BMI (26.6±4.6 kg/m^2) compared to the total sample (n=257), and same sex distribution. Males in the low BMD group presented with lower HGS (23.60±7.95 kg) compared to those in the normal BMD group (vs. 32.14±9.53 kg; p<0.0001), and females in the low BMD group had lower HGS (14.41±5.78 kg) than in those with normal BMD (vs. 19.30±7.26 kg; p=0.006). The HGS was higher in males compared to females in both BMD groups (p<0.0001).

The association between T-score (dependent variable) and HGS (independent variable) in the linear multiple regression analysis, adjusted for sex and eGFR, was significant with R^2=0.288, R^2 adjusted=0.262, standardized coefficient β=0.548, p<0.0001 (i.e., higher HGS protects from low T-score). The standardized beta coefficients for sex and eGFR were 0.156 (p=0.082) and 0.122 (p=0.121), respectively.

The mean MQI for all patients was 8.5±3.2 kg/kg and similar between sexes: males=8.9±3.0 versus females=8.0±3.4 (p=0.066). Most patients presented with poor MQI (n= 82, 57.7%) compared to low (n=21, 14.8%) and normal (n=39, 27.5%) MQI (p<0.0001) (Figure

1). The association between T-score (dependent variable) and MQI (independent variable) in the linear multiple regression analysis, adjusted for sex and eGFR, was significant with R^2=0.095, R^2 adjusted=0.075, standardized coefficient β=0.309, p=0.024 (i.e., higher MQI protects from low T-score. The standardized beta coefficients for sex and eGFR were -0.143 (p=0.091) and 0.158 (p=0.060), respectively

Figure 1. Frequency of male and female patients according to muscle quality status criteria of non-dialyzed patients with chronic kidney disease (CKD-NDD; n=142)



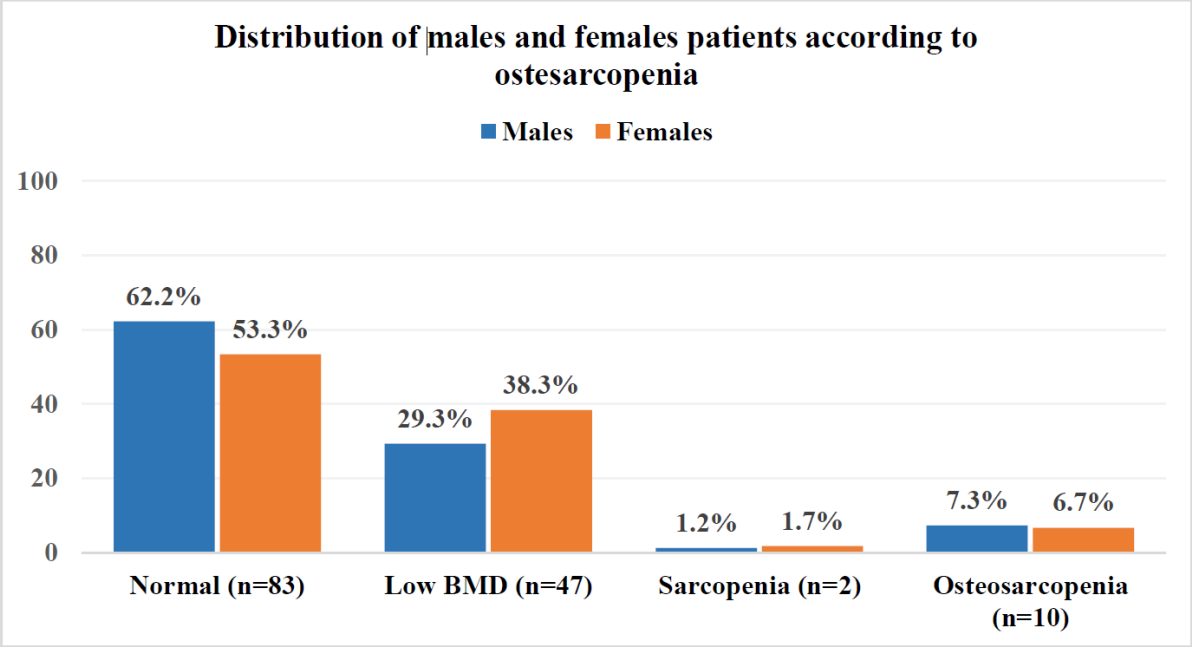
*Chi-square test for comparisons among 3 groups of MQI (normal, low and poor): males p=0.003; females p<0.0001. Abbreviation: MQI (muscle quality index)

Osteosarcopenia frequency

Osteosarcopenia was present in 7.0% (n=10) of patients and was similarly distributed between sexes (males: 7.3%, n=6; females: 6.7%, n=4) (Figure 2). Sarcopenia was present in two patients, one of each sex, thus comparisons analysis was performed among the three other groups (normal, low BMD, osteosarcopenia) (Table 4). T-score was similar between low BMD and osteosarcopenia groups, and was lower compared to normal group, for both males and

females. Mean ASMI values were lower in males and females with osteosarcopenia, compared to those with low or normal BMD. As presented in Table 4, males and females with low BMD were older compared to the other groups, and BMI was lower in the ostesarcopenia and low BMD groups, compared to the normal group. The eGFR were similar among the three osteosarcopenia groups for both sexes. Adiposity mean values were significantly lower in males with low BMD compared to those with normal BMD, while in females no differences were observed.

Figure 2. Frequency of osteosarcopenia criteria by sex in non-dialyzed patients with chronic kidney disease (CKD-NDD; n=142)



*Chi-square test for comparisons among 3 groups of MQI (normal, low and poor): males p<0.0001; females p<0.0001. Abbreviation: BMD (bone mineral density)

Table 4. Comparisons among groups of non-dialyzed patients with chronic kidney disease (CKD-NDD according to osteosarcopenia status (n=140)

Variables		Normal (n=83)	Low BMD (n=47)	Osteosarcopenia (n=10)
T-score	♂	0.20±0.79[a]	-1.76±0.71[b]	-2.08±0.73[b]
	♀	0.05±0.81[a]	-2.04±0.71[b]	-2.83±0.85[b]

Age (years)	♂	62.8±10.6[a]	69.9±11.4[b]	65.8±12.8[a]
	♀	59.6±12.4[a]	69.5±11.5[b]	68.8±11.1[a]
eGFR (ml/min)	♂	29.3±10.3	26.6±11.4	24.2±10.9
	♀	31.3±13.2	31.2±12.3	20.3±6.9
BMI (kg/m^2)	♂	28.4±4.5[a]	23.2±3.13[b]	22.3±3.5[b]
	♀	28.2±4.2[a]	25.9±4.1[b]	21.6±1.3[b]
ASMI (kg/m^2)	♂	8.78±1.39 [a]	7.73±0.84[b]	6.12±0.69[c]
	♀	7.8±1.5[a]	7.0±1.0[b]	5.4±0.5[c]
Total body fat (%)	♂	31.0±7.0[a]	27.3±7.35[b]	29.9±11.3[a]
	♀	39.9±6.9	39.1±6.0	35.5±3.1
MQI (kg/kg)	♂	9.3±3.0	8.6±3.0	7.4±3.3
	♀	8.2±3.5	7.7±3.1	6.2±3.1

Data are presented as mean±SD for normal distributed variables.
BMD: bone mineral density; eGFR: estimated glomerular filtration rate; BMI: body mass index; ASMI: appendicular skeletal muscle mass; MQI: muscle quality index.
Symbols: ♀ for Female patients; ♂ for Male patients.
ANOVA (one-way analysis for normally distributed variables) for comparison among groups. Statistical difference ($p<0.01$).
Pairwise comparisons by post-hoc Bonferroni analysis: values that do not share similar letter are statistically different.

Table 5. Two-way ANOVA for the impact of BMD status, sex and the interaction between BMD and sex on body composition parameters in patients with non-dialysis dependent chronic kidney disease (NDD-CKD; n=257)

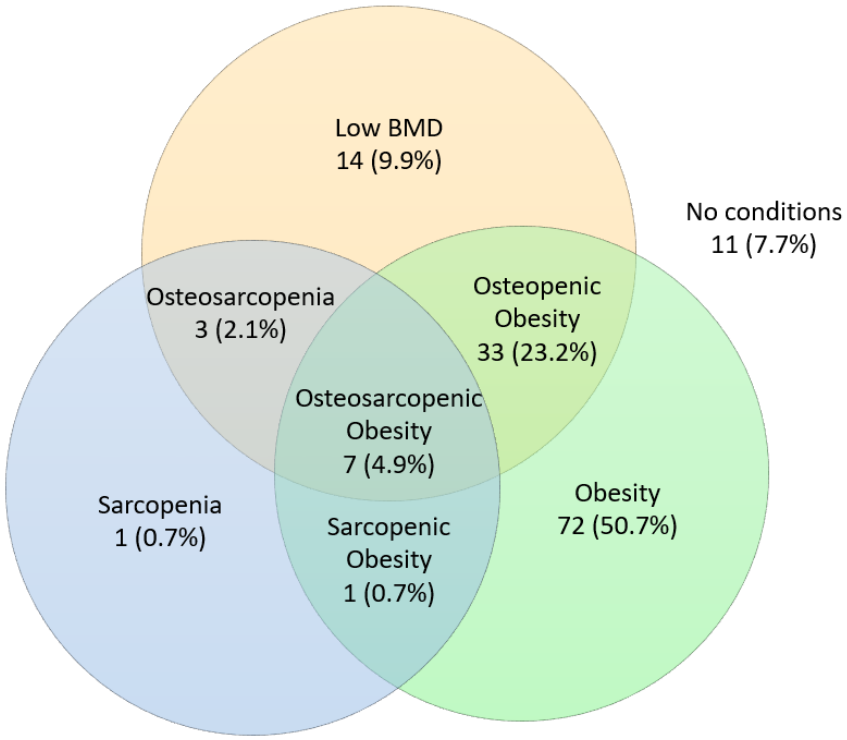
Body composition parameters	**Two-way ANOVA[#]**			
	Factors	Mean Square	F-value	P-value
Total body fat (%)	BMD status	156.1	2.9	0.056
	Sex	3834.9	71.5	<0.0001
	BMD status*Sex	34.0	0.63	0.531
Load Capacity Index (kg/kg)*	BMD status	0.08	2.7	0.067
	Sex	2.1	71.6	<0.0001
	BMD status*Sex	0.002	0.73	0.929
Lean Soft Tissue (LST)(kg)*	BMD status	1337.5	35.5	<0.0001
	Sex	2750.2	73.1	<0.0001
	BMD status*Sex	146.5	3.89	0.052
Lean Soft Tissue Index (LSTI) (kg/m^2)*	BMD status	82.2	21.5	<0.0001
	Sex	66.9	17.5	<0.0001
	BMD status*Sex	10.8	2.81	0.062
Appendicular Skeletal Muscle Mass (ASM) (kg)*	BMD status	425.8	25.2	<0.0001
	Sex	612.0	36.2	<0.0001
	BMD status*Sex	42.2	2.50	0.084
Appendicular Skeletal Muscle Index (ASMI) (kg/m^2)*	BMD status	130.7	17.0	<0.0001
	Sex	7.7	7.7	0.006
	BMD status*Sex	2.11	2.11	0.123

[#]2-way ANOVA: F values and significance with p-values
Abbreviation: BMD (bone mineral density)

Osteosarcopenic obesity

Obesity was considered as the presence of high percentage body fat. Prevalence of obesity was similar between groups with normal (n=72, 86.7%), low BMD (n=33, 70.2%) and osteosarcopenia (n=7, 70%). The intersection of obesity with other conditions (low BMD, and sarcopenia) can be seen in Figure 3. Osteopenic obesity was present in 33 participants (23.2%), and sarcopenic obesity in 1 participant (0.7%).

Figure 3. Intersections between low BMD, sarcopenia, and obesity.



Presented as n (%). High total body fat: men >25% and women >32% (38)

DISCUSSION

This is the first study to evaluate the relationship of BMD with muscle mass parameters in NDD-CKD patients. We found a high prevalence of low BMD, with similar sex distribution, which was associated with low muscle mass. Furthermore, we also analyzed muscle strength and MQI and observed a positive correlation between HGS and MQI with T-score in males and females. Osteosarcopenia was present in ~7% of the studied patients.

Prevalence of low BMD and its association with other body composition parameters, muscle strength and quality

Low BMD was present in 39.3% of patients. A meta-analysis reported a prevalence of osteopenia in CKD patients varying from 33.3% to 81%, with an average of ~46%, including non-dialysis dependent and under dialysis therapy; a higher prevalence of low BMD in women was also reported (8). In a study with 2128 NDD-CKD patients, the prevalence of osteopenia and osteoporosis was 33% and 8% respectively (4).

The osteopenia and osteoporosis groups presented with significantly lower BMI in both sexes. Significant inverse relationship between BMD and anthropometric values (e.g. body weight and BMI) have been reported previously in the general population (15) and in CKD patients (8). This association is explained by gravitational loading that stimulates the maintenance of BMD (15).

Males with low BMD presented with significantly lower total percentage body fat and trunk fat, compared to males with normal BMD, while no statistical difference of adiposity parameters were observed in females among the groups with normal BMD, osteopenia, and osteoporosis. Moreover, positive correlations between T-score and body fat parameters were significant only in men. The association between fat mass and BMD is controversial, with studies showing both positive and negative correlations (11). Android fat in healthy men is

positively associated with BMD, whereas gynoid fat in healthy women is inversely associated with BMD (40).

As expected, patients in the osteopenia and osteoporosis groups also presented with lower muscle mass parameters (LST, LSTI, ASM and ASMI) in both sexes, as skeletal muscle supports the bones (7). Positive correlations between T-score and all these parameters were observed in both sexes, with a small difference in the correlation coefficient value when the indexes (LSTI and ASMI) were considered, showing that height partially mediates this association with BMD. This is corroborated by previous studies reporting positive correlations of LST and ASMI with total BMD and total T-score (24), and between ASMI with lumbar spine BMD (7, 25), femoral neck BMD (7, 25), or distal 1/3 radius BMD (25) in patients undergoing hemodialysis.

Our HGS findings highlighted that patients with normal BMD presented with higher values compared to those with low BMD, and a positive correlation between HGS and T-score in both sexes. A meta-analysis showed a positive correlation between HGS and T-score in the general population (23). The 1/3 radius BMD was a determinant for HGS in CKD patients undergoing hemodialysis, according to multivariate forward linear regression analysis (25).

The prevalence of poor muscle quality was ~45% in males and ~75% in females, which compared to 15% in healthy older adults from Lee and Dierickx (21) was higher. However, the cut-points (21) may not be accurate, as they had a small sample size, specially for females.

Osteosarcopenia

The association of BMD with muscle parameters in the present study corroborates with the idea of a bidirectional relationship between osteopenia and sarcopenia that may lead to osteosarcopenia. In fact, individuals with osteosarcopenia had lower muscle strength and functional performance compared to those with osteoporosis or sarcopenia alone (15). Low

muscle mass, strength, and quality are important factors to be monitored and treated in NDD-CKD patients, an approach that may decrease adverse outcomes (41).

The proposed diagnostic criteria for osteosarcopenia is not a consensus (23). The prevalence of osteosarcopenia observed in the present study was 7.3% in men and 6.7% in women, which is lower than the prevalence observed in a subset of healthy Iranians aged 60-64 years (42). In the Iranian study, osteosarcopenia was defined as low T-score, low ASMI and low strength, present in 14.3% in men and 20.3% in women (42). Additional studies reporting the prevalence of osteosarcopenia include 14.2% in geriatric inpatients in Austria (43), 10.4% in Chinese men and 15.1% in Chinese women over 80 years old (44), and 56% in Ecuadorians that also included individuals attending at a rheumatology clinic (45).

The prevalence of obesity among the osteosarcopenia group was comparable to the normal group and the group with only low BMD. To the best of our knowledge, no study so far has explored osteosarcopenic obesity in people with CKD. In our studied patients the prevalence was ~5%. A study found a 6.8% prevalence of osteosarcopenic obesity in general older adult population (46). Noteworthy, this low prevalence may be partially attributed to the used threshold for ASM, which is not specific for individuals with CKD. Therefore, we emphasize the importance of studies aiming to define cut-points for this population.

Relationship between BMD and biochemical parameters

In general, no differences in laboratory parameters were found among groups with normal BMD, osteopenia, and osteoporosis, including those related to renal function (creatinine, eGFR, urea, and uric acid) and bone mineral metabolism (calcium, phosphorus, PTH and vitamin D). The frequency of hyperphosphatemia and vitamin D deficiency was low (<20%). Hyperparathyroidism was observed in 62% of patients. The mean normal serum concentration of phosphorus hereby observed corroborates with reports that changes in the

bone mineral metabolism pathways predate the development of overt hyperphosphatemia (47-49). Although it is expected that bone mineral metabolism parameters affect bone health, other studies also found no significant association between vitamin D (4, 50-52), calcium and phosphate (50-52) and PTH (52) with BMD and/or T-score in NDD-CKD patients. The lack of differences in laboratorial parameters suggests that body composition, especially markers of muscle mass, is more associated with BMD than the metabolic alterations related to the loss of renal function in NDD-CKD patients. However, a significant difference in variables related to glucose metabolism (glucose, insulin, and HOMA-IR) was observed. These were higher in those with normal BMD, indicating impaired glucose uptake and insulin resistance compared to those with low BMD. The BMI in the normal BMD group (~28 kg/m^2) may explain this difference, as excess body weight increases the risk of insulin resistance through various mechanisms (53).

Additional considerations

Although this is the first study to analyze parameters of osteosarcopenia in patients with NDD-CKD, some limitations should be acknowledged, including the cross-sectional design (thus causality cannot be inferred), and the evaluation of muscle strength and quality only in a subgroup of participants. Study strengths include the evaluation of bone densitometry using a gold-standard method (i.e., DXA).

Unfortunately, not all factors related to BMD were included in this analysis such as diet, ethnicity, socio-economic status and physical activity; as such, these were not included as covariates in adjustment analyses. Although diet was not systematically assessed, patients were following nutritional recommendations based on guidelines for this condition. Physical activity was not evaluated but most of the population is retired. Additionally, ethnicity was not specified

but the studied population comprises a multiethnic group, according to the most recent estimates of the Brazilian Institute of Geography and Statistics (39).

Finally, therapeutic approaches to prevent and treat osteosarcopenia should be considered. These include physical activity, dietary alterations including use of nutritional supplements, and medications to treat CKD-mineral and bone disorders. As the recommended dietary modifications include increase in protein intake, and this is not feasible for these patients, targeted strategies need to be developed for this population, specially resistance and balance exercise may improve bone and muscle status (15).

CONCLUSION

Markers of muscle mass, as well as, muscle strength and quality parameters were consistently positively associated with BMD, while body fat parameters were associated with BMD only in males. Biochemical variables associated with kidney function and bone mineral metabolism were not significantly associated with BMD status. This indicates that muscle mass is potentially mediating the association with bone health in NDD-CKD patients.

The prevalence of osteosarcopenia highlights the importance of monitoring and treating mineral and bone disorder and other alterations in body composition, especially muscle mass and function. Clinical trials are needed to explore whether therapeutic interventions targeting improvements in muscle anabolism and function can optimize body composition in CKD patients without hyperphosphatemia.

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STATEMENT OF AUTHORSHIP

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Rachel Bregman: contributed to manuscript drafting and approval of the final version of the manuscript.

Carla M. Prado: contributed to the study conception and design, data interpretation, manuscript drafting and approval of the final version of the manuscript.

Maria Inês Barreto Silva: contributed to the study conception and design, data analysis and interpretation, manuscript drafting and approval of the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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