

Bone Mineral Density and Body Composition in Males with Motor Neuron Disease: A Study from Teaching Hospital in Southern Part of India

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Abstract

Background: Osteoporosis and sarcopenia are important aspects of motor neuron disease (MND). Individuals with amyotrophic lateral sclerosis (MND-ALS) have an increased risk of falls and fractures. Currently, the standard of care does not involve a routine assessment of bone mineral density (BMD) and body composition in these patients. We aimed to assess BMD, bone mineral parameters and body composition in men with MND and compared them with healthy controls. **Methods:** Consecutive males between 50 and 80 years of age diagnosed as MND-ALS by revised El Escorial criteria and able to walk unassisted attending Neurology outpatient clinic were recruited into the study. Age, gender and body mass index (BMI) matched healthy controls were recruited from the local community. BMD and body composition were assessed by dual-energy x-ray absorptiometry (DXA). Bone mineral parameters and bone turnover markers (BTMs) were also assessed in them. **Results:** A total of 30 subjects with MND-ALS and 33 controls were recruited. The mean age (years) was 59.2 in cases and 61.2 in controls. The mean BMD (g/cm²) between the two groups was similar; however, BTMs were significantly higher in the MND group ($P < 0.05$). Subjects with MND-ALS had significantly lower mean appendicular lean mass (ALM) (19.9 versus 22.4 kg; $P = 0.007$) and ALM corrected for BMI than the healthy control group (0.858 versus 0.934 kg/kg/m²; $P = 0.034$). Sarcopenic obesity (Percentage fat mass $>27\%$ + ALM/BMI <0.786 kg/kg/m²) was more prevalent in MND-ALS compared to controls (44.5% versus 16.7%; $P = 0.03$). **Conclusion:** Although BMD was not significantly different between subjects with MND-ALS and healthy controls, BTMs were significantly higher in the MND group indicating a high bone turnover state. Sarcopenia and sarcopenic obesity were also more in MND-ALS group than controls. Routine assessment for bone health parameters and body composition indices may be included in management of the patients with MND.

Keywords: Body composition, bone mineral density, motor neuron disease, sarcopenic obesity, Southern India

INTRODUCTION

Amyotrophic lateral sclerosis (ALS), a type of motor neuron disease (MND), is a relentlessly progressive, presently incurable, neurodegenerative disorder that causes muscle weakness, disability, and eventually death. Disease progression may cause difficulty in walking which predisposes them to an increased frequency of falls and subsequent fractures.^[1-4] MND may also be associated with malnutrition and changes in energy expenditure.^[5] There are studies^[6,7] demonstrating the role of motor neuron loss and sarcopenia. However, there is a paucity of data on sarcopenia in MND in the Indian context.

Currently, the standards of care for managing ALS do not focus on bone health. Although similar abnormalities in bone density have been observed in muscular dystrophy, Parkinson's and Alzheimer's diseases, this pilot study intended to quantify the severity of the deterioration of bone health (bone density and bone turnover markers [BTMs]) in the patients with ALS compared to age, gender and BMI-matched healthy controls, in order to determine whether bone health assessment should be a part of the routine assessment in the patients with ALS. We also intended to assess body composition including muscle

mass and sarcopenia, in the patients with MND-ALS and compare them with a matched healthy cohort.

MATERIALS AND METHODS

This cross-sectional study was done over 18 months between September 2017 and February 2019 at a tertiary care teaching hospital in Southern India. Male subjects aged 50-80 years were recruited in the study from the Department of Neurology. Group-1 included the patients with MND-ALS fulfilling the revised El Escorial diagnostic criteria and able to walk

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unassisted. Group-2 included age and BMI-matched healthy male controls from the local community. Subjects who were bed-bound, those with chronic kidney disease, chronic steroid use, HIV disease, dementia, chronic liver disease and those on bisphosphonates, calcitonin or osteoanabolics were excluded from the study. The institutional ethics committee approved the study (as per IRB min no. 10887) and written informed consent was obtained from all participants.

Clinical examination and anthropometric measures

A detailed physical examination was performed in all subjects. Bodyweight was measured in kg using an electronic scale and height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. BMI was calculated as the ratio of weight/height in metres² (kg/m²). Waist circumference (WC) was measured midway between the lower margin of the last palpable rib and the highest point of the iliac crest, using a stretch-resistant tape. Hip circumference (HC) was measured around the widest portion of the buttocks.

Assessment of bone mineral parameters and body composition

Bone mineral density (BMD) was assessed by Dual-energy X-ray absorptiometry (DXA) machine (Hologic-QDR 4500-W Discovery-A; Hologic Inc; Bedford, MA, USA) at the lumbar spine (LS), femoral neck (FN), total hip (TH) and forearm. The coefficient of variation for assessment of BMD at all these sites was <3% at our institute. Based on the International society of clinical densitometry (ISCD) criteria, osteoporosis was defined as a T-score at the FN, TH or LS ≤ -2.5 , osteopenia was diagnosed as a T-score between -2.5 and -1.0 , and normal BMD was defined as a T-score ≥ -1 .^[8] Body composition was also assessed by DXA scan. Sarcopenia was diagnosed when either appendicular lean mass (ALM) was <19.75 kg or ALM/BMI was <0.786 kg/kg/m². Sarcopenic obesity was defined as a combination of total body fat >27% and ALM/BMI <0.786 kg/kg/m².^[7]

Biochemical parameters

Fasting serum calcium, phosphorus, albumin, alkaline phosphatase and creatinine were estimated using standard colorimetric methods. Intact parathyroid hormone (iPTH) and testosterone were estimated using automated chemiluminescent immunoassays. 25-hydroxy vitamin D, fasting plasma BTMs such as serum N-terminal pro-peptide of type 1 procollagen (PINP) and C terminal telopeptide of type 1 collagen (CTx) were assayed using automated electro-chemiluminescence assay (Roche).

Statistical analysis

Descriptive variables were expressed as mean and standard deviation while categorical variables were expressed as frequencies and percentages. Student's *t*-test was used to compare the means between two groups. The categorical variables were compared using Chi-square or Fisher's exact test as appropriate. Pearson's correlation coefficient was used to assess correlation between two variables. A two-tailed *P* value <0.05 was considered to be statistically significant. Statistical analysis was performed using statistical package for social sciences (SPSS version 25.0 Chicago, IL, USA)

RESULTS

A total of 30 males with MND-ALS (Group-1), and 33 years of age and BMI-matched controls (Group-2) were recruited in this study. At recruitment, all patients diagnosed to have MND-ALS were on treatment with Riluzole. The mean (SD) age of the study participants was 59.2 (7.1) years in cases and 61.2 (5.9) among controls. The mean duration of MND was 16.8 (13.0) months. Other baseline characteristics are described in Table 1. The mean 25-hydroxy vitamin D level trended lower in cases compared to controls (28.5 vs 33.6 ng/mL; *P* = 0.075). However, the proportion of subjects with vitamin D deficiency was significantly higher among cases compared to controls (20% vs 15%). The mean CTx levels (698.9 vs

Table 1: Comparison of baseline clinical and biochemical characteristics

Variables	Mean (SD)		<i>P</i>
	Group-1 (MND-ALS) (<i>n</i> =30)	Group-2 (Controls) (<i>n</i> =33)	
Age (years)	59.2 (7.1)	61.2 (5.9)	0.125
BMI (kg/m ²)	23.5 (3.7)	24.1 (2.1)	0.531
Waist hip ratio	0.98 (0.04)	0.98 (0.05)	0.982
Albumin corrected Calcium (mg/dL)	9.4 (0.5)	9.6 (0.4)	0.258
Serum phosphorus (mg/dL)	3.7 (0.5)	3.5 (0.4)	0.409
Serum albumin (g/dL)	4.5 (0.3)	4.6 (0.3)	0.126
Serum alkaline phosphatase (U/L)	75.3 (22.8)	89.5 (22.8)	0.052
Creatinine (mg/dL)	0.71 (0.1)	0.83 (0.1)	0.058
25 hydroxy Vitamin-D (ng/mL)	28.5 (11.6)	33.6 (11.6)	0.075
Parathyroid Hormone (pg/mL)	47.7 (16.6)	53.1 (22.0)	0.417
CTx* (pg/mL)	698.9 (309.5)	401.1 (158.7)	<0.001
PINP* (ng/mL)	64.3 (40.0)	47.1 (16.0)	0.030
Testosterone (ng/dL)	402.5 (192)	390.1 (99)	0.755

*CTx – C-terminal telopeptide of type 1 collagen; PINP – Procollagen type 1 N-terminal propeptide

401.1 pg/mL) and P1NP levels (64.3 vs 47.1 ng/mL) were significantly higher in the MND-ALS group compared to controls ($P < 0.05$).

Bone mineral density and body composition assessment

The mean (SD) BMD of the two groups for all skeletal sites is shown in Table 2. There was no statistically significant difference in BMD between the two groups. The various components of body composition assessment are shown in Table 3. Serum testosterone levels that have an influence on BMD in men were not significantly different between cases and controls (402.5 vs 390.1 ng/dL). The indicators of sarcopenia namely, the appendicular lean mass (19.92 vs 22.49 kg) and appendicular lean mass adjusted for BMI (0.858 vs 0.934 kg/kg/m²) were significantly lower in the MND-ALS group compared to controls ($P < 0.05$). The total body fat percentage and other body fat parameters, that is, fat mass adjusted for height and estimated visceral adipose tissue also did not show significant difference between the groups. The proportion of subjects with sarcopenia was significantly higher among cases compared to controls (58.6% vs 18.2%; $P = 0.001$). This difference remained significant even after correcting for BMI (27.6% vs 6.1%; $P = 0.036$). It was also noted that the prevalence of sarcopenic obesity was significantly higher in the MND group as compared with controls (44.5% in cases vs 16.7% in controls; $P = 0.03$).

Table 2: Comparison of BMD between the two groups

Variables	Mean (SD)		P
	Group-1 (MND-ALS) (n=30)	Group-2 (Controls) (n=33)	
Femoral neck BMD (g/cm ²)	0.768 (0.149)	0.750 (0.092)	0.515
Total hip BMD (g/cm ²)	0.919 (0.143)	0.929 (0.095)	0.940
Lumbar spine BMD (g/cm ²)	0.922 (0.148)	0.982 (0.130)	0.172
Forearm BMD (g/cm ²)	0.764 (0.055)	0.763 (0.572)	0.823
Total bone mineral content (BMC) g	2415.4(362.7)	2351.18(280.7)	0.443

Table 3: Comparison of body composition parameters between three groups

Variables	Mean (SD)		P Independent T-test
	Group-1 (n=30)	Group-2 (n=33)	
Total body fat (%)	29.01 (5.08)	29.71 (2.61)	0.503
Fat mass/height ² (kg/m ²)	7.06 (1.94)	7.29 (1.35)	0.597
Est.VAT volume (cm ³)	615.6 (191.4)	645 (180.0)	0.531
Lean/height ² (kg/m ²)	16.02 (2.10)	16.27 (1.70)	0.615
Appen.Lean mass/height ² (kg/m ²)	6.97 (1.01)	7.21 (0.75)	0.302
Appendicular lean mass -ALM (kg)	19.92 (3.60)	22.49 (3.66)	0.007
ALM/BMI (kg/kg/m ²)	0.858 (0.146)	0.934 (0.124)	0.034

Bone resorption marker CTx showed a significant negative correlation with the TH BMD ($r = -0.414$; $P = 0.025$) and the bone formation marker P1NP had a significant negative correlation with lumbar spine BMD ($r = -0.477$; $P = 0.009$) in the MND-ALS group.

DISCUSSION

This cross-sectional study evaluating bone health and body composition in male subjects with ALS is the first of its kind from India. The BMD at the LS and TH trended lower in cases than in controls. The BMD at the FN did not differ significantly between cases and controls, probably due to a well-preserved BMI, relatively shorter duration of the disease and preserved mobility in the MND-ALS group. BTMs namely CTx and P1NP were significantly higher in the MND-ALS group compared to controls. Also, the prevalence of sarcopenic obesity was significantly higher in subjects with MND compared to healthy controls.

Among the bone biochemical parameters, the mean 25-hydroxy vitamin D level was lower in the MND group as compared with healthy controls, albeit not statistically significant; but 20% of cases had vitamin D deficiency compared to 15% among controls. As nutritional calcium deficiency is common in subjects from South India,^[9,10] this was not objectively assessed in the present study. Vitamin D deficiency is also a common problem in our population. Vitamin D is involved in the reduction of oxidative stress in the nervous system, and vitamin D deficiency is implicated in the pathogenesis of various neurodegenerative conditions such as multiple sclerosis, Parkinson's disease (PD) and Alzheimer's disease (AD). Although previous studies have evaluated the neuroprotective role of vitamin D supplementation in ALS, many of these have yielded controversial results.^[11,12] Previous studies have shown that the levels of vitamin D in subjects with ALS were lower than in healthy controls, but vitamin D supplementation did not have a significant effect on the progression of the disease. However, in this study the authors wish to draw attention to the fact that the mean vitamin D trended lower in the diseased cohort. Although it seems to be commonplace, it is deemed to be a potentially correctable risk factor in this neurodegenerative illness which is known for its otherwise relentless progression. Sub-optimal sun exposure in subjects with MND may be a reason for low vitamin D levels, but this was not assessed in the current study. In literature, some studies have shown the protective effects of vitamin D on falls in neurodegenerative diseases^[13-15]; however, the design of the present study is cross-sectional, and therefore, will not suffice to make a definitive conclusion on the effect of vitamin D supplementation on falls. This aspect will be prospectively assessed in this cohort.

BTMs CTx and P1NP were significantly higher in cases than healthy controls although BMD did not significantly differ between the groups. Elevated bone resorption markers in MND-ALS have also been reported by previous studies.^[16,17]

BTMs reflect “real time” bone remodelling. This may not be reflected in the BMD measured at a single point in time. Rather, significant changes in BMD may take time to manifest, which can be ascertained only on serial follow-up of these patients. Identification of a high bone turnover warrants attention as this may herald bone loss and plausible fragility fractures in the future. The assays for BTMs are standardized if samples are collected and transported with suitable precautions to avoid pre-analytical errors. In our previous study, we have demonstrated that BTMs have good sensitivity to predict osteoporosis at the FN and LS. β Cross laps-CTx, a marker of bone resorption has a sensitivity of 86% at a cut-off value of 405.4 pg/mL for prediction of osteoporosis at the FN and procollagen type 1 N-terminal propeptide (PINP), a marker of bone formation at a cut-off value of 49.85 μ g/mL has a sensitivity of 84%.^[18] BTMs have also been studied for prediction of fractures. In a nested case-control study from the EPIDOS cohort of postmenopausal women, which included 115 fracture subjects as cases and 293 controls, CTx had a significant prediction of fracture, with relative hazard of 1.86 (1.01–3.76).^[19] Also, there is a good evidence to support that BTMs are a good tool to assess compliance as well as resistance to bisphosphonate therapy and also resistance to therapy.^[20,21] In this study, the impact of high bone turnover on BMD could not be assessed due to the cross-sectional study design.

In this study, serum testosterone levels were not significantly different between cases and controls. In an epidemiological study in older men, low testosterone was associated with an increased risk of hip fracture (HR, 1.88; 95% CI, 1.24-2.82) and non-vertebral fractures (HR, 1.32; 95% CI, 1.03-1.68) after adjustment for other risk factors.^[22] The Framingham study showed that men with both low estradiol and testosterone levels had the greatest risk for hip fractures (adjusted hazard ratio: 6.5, 95% CI, 2.9-14.3).^[23] The Endocrine society guidelines recommend measurement of serum testosterone in men being evaluated for osteoporosis and those being considered for pharmacological therapy with bone active agents. Estradiol measurement is not recommended in men because of the lack of easily available, accurate assay methods (mass spectrometry) and the absence of validated clinical algorithms that incorporate estradiol measurements into treatment decisions.^[24]

In a cross-sectional study by Fang F, *et al.*^[25] increased bone resorption was postulated to be caused by elevated blood lead levels in MND, thus implicating lead as a plausible factor in the pathogenesis of MND. However, this seems to be a postulate and needs to be validated prospectively. A retrospective study assessing history of heavy metal exposure found no significant association between heavy metal exposure and the pathogenesis of ALS.^[26] Blood lead measurement was not a part of this study, as by protocol, estimation of lead is undertaken by the department of neurology only in diseases with a pure lower motor neuron involvement.

Despite advances in understanding the genetics of ALS, the cause of approximately a third of familial ALS and most of the sporadic ALS cases remains unknown. The Valosin-containing

protein (VCP) mutations have been associated with 1%-2% of familial ALS^[27] and < 1% of sporadic ALS.^[28] The identification of the remaining genes represents a substantial challenge, for which novel approaches are being explored.^[29] Missense mutations in the gene encoding VCP have been associated with inclusion body myopathy associated with Paget's disease of bone and frontotemporal dementia, a dominant progressive disorder that maps to chromosome 9p21.1–p12.^[30] VCP is associated with a variety of cellular activities, including cell cycle control, membrane fusion and the ubiquitin-proteasome degradation pathway, which may be the common pathogenic mechanism involved in all the associated disorders.

Data regarding the effect of riluzole on bone health is scarce. However, in a biological experiment of riluzole on mesenchymal stem cells and osteoblasts, no cytotoxicity was observed. There was upregulation of alkaline phosphatase activity in the osteoblasts and at high concentrations, riluzole led to a decrease in osteogenic gene expression, including Runx 2 and type 1 collagen.^[31] This being said, whether the increased levels of BTMs in our study can be wholly attributed to riluzole cannot be established with certainty.

There is some evidence to link trauma and fractures with predisposition to ALS from retrospective studies.^[32] However, this needs further validation from well-designed prospective studies to determine causation. A prospective cohort study in women with PD showed that women with PD had 7.3% lower BMD at the TH and 2.6-fold increased age-adjusted risk of hip fracture.^[33] Lorefalt *et al.* reported similar reduction in BMD at TH and FN in patients with PD compared to controls (3.9 vs 1.2%).^[34] A meta-analysis of nine studies showed that patients with AD had higher risk for hip fractures (OR 2.58; 95% CI 2.03-3.14) compared to healthy controls. Low 25 (OH) vitamin D and calcium level, with secondary hyperparathyroidism have been proposed to be the reasons for lower BMD and increased fracture risk in this group of patients.^[35]

Thus, neurodegenerative disorders including MND, PD and AD are all associated with low BMD and increased fracture risk. Whether osteoporosis is the result of decreased load bearing due to the neurodegenerative disorder, or shares common pathophysiological mechanisms with these disorders is yet to be determined.

The proportion of subjects with sarcopenia was higher in the MND-ALS group. This may predispose to insulin resistance, diabetes mellitus, increased cardiovascular disease (CVD) risk and a higher propensity to falls and fractures.^[36,37] Nonetheless, further studies are required to elucidate the underlying putative factors.

The strength of this study is that this is the first study from India to assess bone mineral density, bone mineral parameters and body composition in male subjects with MND and compare them with matched controls. However, this study is limited by its cross-sectional design and small sample size. Other limitations of this study include non-assessment of glucose and

insulin levels to determine insulin resistance, and nutritional calcium intake.

CONCLUSION

Although BMD was not significantly different between subjects with MND-ALS and healthy controls, BTMs (CTx, P1NP) were significantly higher in the MND group indicating a high turnover state. BTMs are earlier indicators of deterioration of bone health, and hence, we recommend testing for serum calcium, 25-hydroxy vitamin D, BTMs and BMD in patients with MND-ALS. MND being a progressive neurological disorder, the risk of fall and fracture further increases with progression of disease, necessitating the assessment and treatment of osteoporosis to prevent fractures. With the results of our study we propose to initiate early treatment with calcium and vitamin D supplementation in those who are deficient, and also to initiate bisphosphonate therapy after vitamin D repletion in those with low bone mass on DXA scan. Moreover, sarcopenia and sarcopenic obesity were also more in MND-ALS group than controls. This calls for a systematic assessment of metabolic parameters such as blood glucose and lipid profile in these patients.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient (s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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