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Original Article

Bone microarchitectural alterations in boys with Duchenne muscular dystrophy on long-term glucocorticoid treatment

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Abstract

Introduction

Osteoporosis is a major health issue in boys with Duchenne muscular dystrophy (DMD). Data on the specific bone deficits and microarchitectural alterations in children with DMD were limited. This study aimed to assess the bone microarchitectural alterations in boys with DMD on long-term glucocorticoid using high-resolution peripheral quantitative computed tomography (HR-pQCT).

Materials and methods

This was a cross-sectional, case–control study. Boys with DMD older than 5 years with no prior history of symptomatic fracture and had been on long-term glucocorticoid treatment were recruited from a single tertiary centre. For each participant, three gender- and age-matched controls were selected randomly from an existing HR-pQCT database of healthy individuals.

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Results

Nine boys with DMD at a median age of 9.3 years were included. Three were found to have asymptomatic vertebral compression fracture. The HR-pQCT findings of these nine boys were compared with 27 healthy controls. Trabecular microstructure indices at the distal radius were significantly lower but the cortical vBMD was significantly higher in the DMD boys when compared with healthy controls.

Conclusion

Lower microarchitectural measurement of trabecular bones, but higher cortical vBMD, was observed in DMD boys on long-term oral glucocorticoid. The results from this study provide preliminary, yet important insights into the bone microarchitecture of this group of patients.

Keywords

Duchenne muscular dystrophy
Osteoporosis
HR-pQCT
Glucocorticoid
Bone microarchitecture index
children

Introduction

Duchenne muscular dystrophy (DMD) is an X-linked recessive muscle disease, and is the most common paediatric-onset hereditary muscle disease affecting 1 in 3600–6000 live male births [1]. Due to lack of dystrophin protein, affected patients have progressive muscle weakness. Chronic glucocorticoid therapy, best to start as early as the age of 4, is currently the recommended standard of care to prolong ambulation, maintain pulmonary function and decrease the severity of scoliosis [2]. Osteoporosis is a well-known problem in this group of individuals—it is related to its intrinsic problem of progressive muscle weakness with immobility, and the long-term glucocorticoid use. In addition, the effects of cytokine release and activation of osteoclastogenesis in dystrophin-deficient muscles with altered muscle metabolism also contributed to reduced bone mineral density [3]. Hence, they are at increased risk of fractures. Vertebral compression fractures (VF) and long bone fractures were reported to be observed in up to 44 and 24% of glucocorticoid-treated DMD patients, respectively [4][5]. It is associated with significant morbidity including acute and chronic pain, spinal deformity with further compromised pulmonary function, and may also lead to premature loss of ambulation or even severe and life-threatening complications like fat embolism syndrome [6]. Unfortunately, data on the specific bone deficits and microarchitectural alterations in children with DMD were limited, and there is inadequate evidence to guide use of treatment to prevent osteoporosis in this group of individuals [7].

Dual-energy X-ray densitometry (DXA) is the current recommended modality for assessing areal bone mineral density (aBMD) in children because of its widespread availability, reproducibility and low ionizing radiation and robust paediatric reference data [8]. However, one important limitation with this technique in the paediatric group is the correction for body size, making its use and interpretation particularly in young children complex. DXA measures only the ‘areal density’ (mg/cm^2, i.e. the ratio of bone mineral content to the projected area), instead of true

volumetric density (mg/cm^3). This often underestimates the aBMD value for a smaller bone. Since patients with DMD boys are on long-term glucocorticoid treatment, which would inevitably affect growth, they are always much shorter than the other healthy children; this can be a significant confounding factor in the interpretation of the DXA measurements. In addition, DXA is incapable of providing separate measurement on trabecular and cortical bone density, nor bone geometry and bone strength, and hence, it is limited in its ability to identify specific bone deficits.

Tian et al. clearly demonstrated the limitations with aBMD assessment with DXA in the DMD group. The study looked at the bone health status in 292 ambulant glucocorticoid-treated boys with DMD and found that lumbar spine (LS) aBMD *Z* scores contradictorily improved while VF increased [2]. One caveat to this study is that it was unclear whether collapsed vertebrae were included in the aBMD assessment, which could falsely increase the measurement. Similarly, we had also looked at our DMD boys before and after initiation of glucocorticoid treatment, and we also found contradictory improvement in LS aBMD *Z* score post-glucocorticoid treatment with significant difference of greater than 2 standard deviations (SD) between LS and total body less head (TBLH) (all patients were screened for vertebral fracture with lateral spine radiographs from T4 to L5; collapsed vertebrae, if any, were excluded from aBMD assessment) [9]. These highlighted the complexity and limitations in using DXA findings for bone health assessment in children with chronic illnesses.

Peripheral quantitative computed tomography (pQCT) is a three-dimensional densitometry tool for appendicular areas, most commonly radius or tibia. In contrast to DXA, pQCT allows clear distinction between the effects of bone size by measuring volumetric bone mineral density (vBMD) [10]. It also provides separate assessment on both cortical and trabecular components [10]. In the recent decade, a newer version, the high-resolution pQCT (HR-pQCT) is developed and has been increasingly being used in research settings. In addition to measurement of global and compartmental vBMD, it can also assess bone microarchitecture in vivo. The scan only involves a 9-mm CT slice and hence the effective radiation dose is low at < 3 mSv per measurement [11].

This study aims to assess the bone microarchitectural alterations in boys with DMD on long-term glucocorticoid using HR-pQCT in comparison to historical healthy controls.

Materials and methods

This is a cross-sectional, case–control study. Boys with DMD older than 5 years, who were seen in the Neuromuscular Disorder Clinic from 2017 to 2018 in Duchess of Kent Children's Hospital, Hong Kong, and had been started on long-term glucocorticoid treatment for at least 1 year were recruited. Patients were excluded if (i) they required daytime ventilatory support, (ii) they were on bisphosphonate therapy or (iii) they had history of symptomatic fracture. For each participant, three gender-, age- (within 1 year of age) and race-matched controls were selected randomly from a pre-existing HR-pQCT database of healthy individuals recruited from local communities of Hong Kong through a combination of private solicitation and advertisements from May 2015 to April 2017. Relevant clinical information was retrieved from patients' records. The study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster and the Joint Chinese University of Hong Kong–New Territories East Cluster Clinical Research Ethics Committee. Written informed consents were obtained from their parents/guardians.

Clinical and biochemical assessment

The diagnosis of DMD was confirmed by either multiplex ligation-dependent probe amplification (MLPA) for those with DMD gene exon deletions or duplications, or direct Sanger sequencing for those with small mutations, performed in the accredited genetic diagnostic laboratory. All patients had a classical DMD phenotype with verification by the attending paediatric neurologist (SHC). For those with negative MLPA and Sanger sequencing but classical DMD clinical phenotype, their diagnoses were confirmed by muscle biopsy. Ambulatory status was categorized as ambulatory, late ambulatory and non-ambulatory. Overall muscle strength was measured by manual muscle testing (MMT) score by experienced physiotherapists. As part of the standard care, serum 25-hydroxyvitamin D is measured on an annual basis and vitamin D supplementation would be given to maintain a 25-hydroxyvitamin D level of > 50 nmol/L. They also had annual screening for VF with either vertebral fracture assessment (VFA) or lateral spine radiographs.

Bone health assessment

Volumetric BMD (vBMD) and trabecular bone microarchitecture were measured at the non-dominant distal radius using the HR-pQCT (XtremeCT I, Scanco Medical, Brüttisellen, Switzerland) according to the relative off-set protocol on a segment

spanning 4.0% of the ulna length proximal to the reference line, where the reference line was placed at the proximal margin of the radial head [12].

Areal bone mineral density (aBMD) was determined by DXA using the Hologic Horizon A system (Hologic, Bedford, MA, USA) at the TBLH and postero-anterior LS. Calculation of *Z* score and height-adjusted *Z* score was based on published reference data [13]. Vertebral fracture assessment (VFA) by DXA was used to evaluate for new and asymptomatic VF. VF is defined based on the Genant semi-quantitative methods [14]. Collapsed vertebrae, if any, were excluded from aBMD measurement by DXA.

Statistical analysis

Descriptive statistics including mean, median, standard error and interquartile range (IQR) were used according to the normality of the data. Wilcoxon rank-sum tests were used to examine statistical significance since data were not normally distributed. Statistical analyses were performed using Stata (StataCorp. 2015. Stata Statistical Software: Release 15.1 College Station, TX: StataCorp).

Results

Nine boys with DMD were included. Their median age was 9.3 years (IQR 8.5–10.8 years). They were started on prednisolone at a median age of 5.5 years (IQR 4.8–7.8 years) for a median duration of 4.2 years (IQR 2.5–4.8 years). All of them are pre-pubertal and none of them had been put on testosterone for pubertal induction. The median prednisolone dose was at 0.66 mg/kg/day (IQR 0.54–0.71 mg/kg/day). Three were non-ambulatory, while four and two were at ambulatory and late ambulatory state, respectively. Their vitamin D statuses were kept at optimal level (median 25-OH vitamin D = 70 nmol/L, IQR 65 to 73 nmol/L) with mean daily vitamin D supplementation dose of 2800 iu (IQR 1900–2800 iu). Their median DXA aBMD *Z* scores at LS and TBLH were −1.3 SD (IQR − 2.2 to − 1.2 SD) and −4.4 SD (IQR − 5 to − 3.1 SD), respectively, while the corresponding height-adjusted *Z* scores were −0.3 SD (IQR − 0.6 to 0.2 SD) and − 1.7 SD (− 2.9 to − 1.2 SD), respectively. None of them were known to have a history of long bone or VF at the time of recruitment. As part of the workup, three were found to have asymptomatic vertebral compression fracture by VFA (one had grade 3 VFs at T7 to T9, and two had grade 1 VF, one at L1 and one at L4, according to Genant semi-quantitative methods; all VF were confirmed by lateral spine radiographs from T4 to L5). One of the three also developed a long bone fracture at the tibia after a fall on level ground within 6 months after the assessment.

The HR-pQCT findings of these 9 boys were compared with 27 gender-, age- and race-matched healthy controls. There was no significant difference in the body mass index between the two groups, but the DMD group was significantly shorter. Trabecular microstructure indices, including trabecular vBMD, trabecular bone volume to tissue volume, number of trabeculae, trabecular thickness and trabecular separation at the distal radius, were significantly inferior in the DMD boys when compared to the healthy boys ($p < 0.05$). Conversely, cortical vBMD was significantly higher in the DMD group (Table 1). Figure 1 shows the HR-pQCT images of a boy with DMD and his age-matched control.

Table 1

Clinical characteristics and distal radius HR-pQCT findings between boys with DMD and healthy controls

	DMD boys (*n* = 9) Median (IQR)	**Age-matched healthy controls (*n* = 27) Median (IQR)**	***p* value**
Age (years) [a]	9.3 (8.5, 10.8)	10.2 (8.5, 11.3)	0.712
Body weight (kg) [a]	30.6 (21.5, 38.1)	34.0 (24.4, 44.8)	0.411
Body weight (SD) [a]	− 1.59 (− 2.22, − 0.34)	− 0.49 (− 1.17, 0.30)	0.050
Body height (cm) [a]	125.0 (117.0, 131.8)	138.5 (128.9, 150.2)	0.094
Body height (SD) [a]	− 2.22 (− 2.87, − 2.12)	0.09 (− 1.11, 0.58)	**0.0001**
BMI (kg/cm^2) [a]	18.1 (15.7, 18.1)	17.3 (14.3, 19.1)	0.201
BMI (SD) [a]	− 0.09 (− 7.17, 0.98)	− 0.8 (− 1.29, 0.61)	0.832
Duration of prednisolone treatment (years)	4.2 (2.5, 4.8)	Not applicable	–
Prednisolone dose (mg/kg/day)	0.66 (0.54, 0.71)	Not applicable	–

DXA dual-energy X-ray absorptiometry, *aBMD* areal bone mineral density, *LS* lumbar spine, *TBLH* total body less head, *vBMD* volumetric bone mineral density, *BV/TV* bone volume fraction, *Tb.N* trabecular number, *Tb.Th* trabecular thickness, *Tb.Sp* trabecular separation

[a]Comparison between groups made using Wilcoxon rank-sum tests

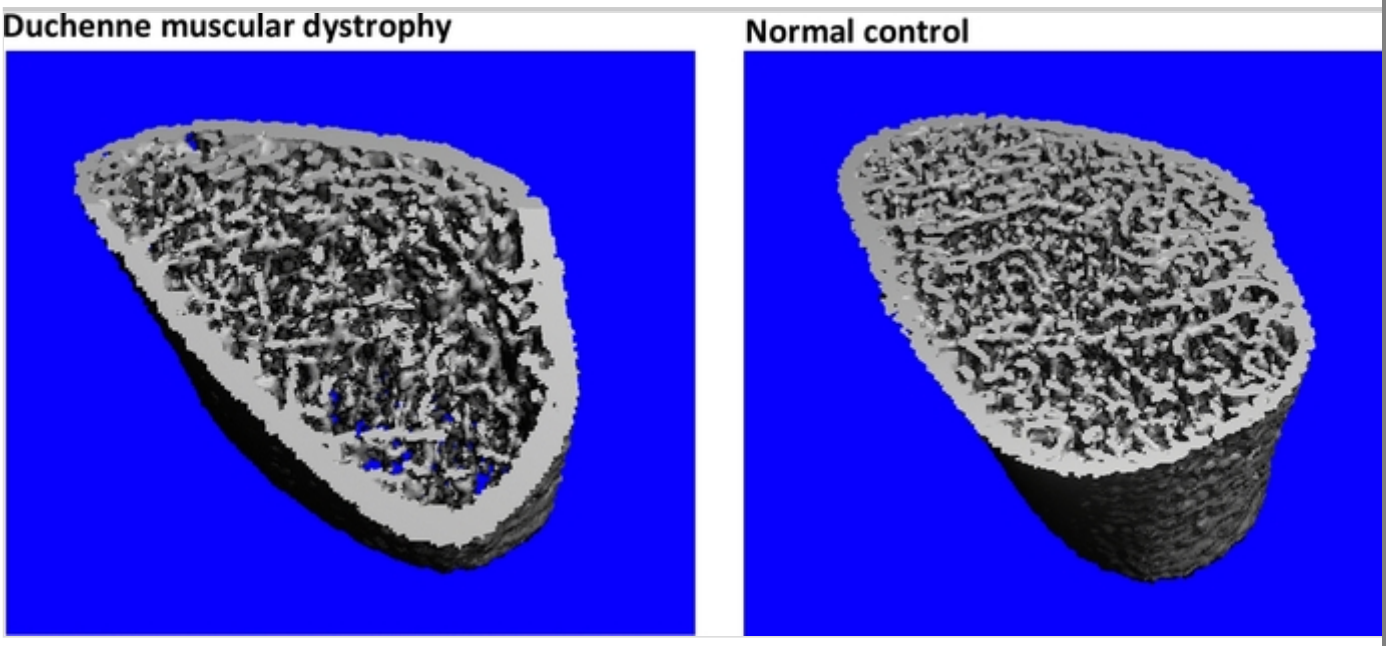
	DMD boys (*n* = 9) Median (IQR)	**Age-matched healthy controls (*n* = 27) Median (IQR)**	***p* value**
25-OH vitamin D levels (nmol/L)	70 (65, 73)	Not available	–
DXA aBMD *Z* score (LS)	− 1.3 (− 2.2, − 1.2)	Not available	–
DXA aBMD *Z* score (TBLH)	− 4.4 (− 5, − 3.1)	Not available	–
Ambulatory status	Ambulatory: 4 Late ambulatory: 2 Non-ambulatory: 3	All ambulatory	–
Total vBMD (mg/cm^3)[a]	268.3 (256.7, 295.4)	292.4 (272.1, 316.3)	0.501
Cortical vBMD (mg/cm^3)[a]	762.7 (719.9, 798.5)	701.2 (653.1, 723.0)	**0.034**
Trabecular vBMD (mg/cm^3)[a]	111.8 (89.1, 133.7)	175.7 (156.3, 194.4)	**0.003**
Total area (mm^2)[a]	103.3 (85.1, 136.6)	142.8 (111.8, 185.4)	0.062
Cortical area (mm^2)[a]	23.1 (19, 25.3)	20.9 (16.2, 33.9)	0.811
Trabecular area (mm^2)[a]	78.7 (59.4, 109.5)	115.3 (88.7, 138.2)	0.061
Cortical thickness (mm)[a]	0.53 (0.48, 0.7)	0.50 (0.34, 0.65)	0.452
BV/TV[a]	0.093 (0.074, 0.111)	0.146 (0.130, 0.162)	< **0.001**
Tb.N (/mm)[a]	1.35 (0.84, 1.9)	1.96 (1.70, 2.04)	**0.009**
Tb.Th (mm)[a]	0.069 (0.066, 0.07)	0.076 (0.071, 0.082)	**0.035**
Tb.Sp (mm)[a]	0.673 (0.467, 1.107)	0.437 (0.412, 0.510)	**0.005**

DXA dual-energy X-ray absorptiometry, *aBMD* areal bone mineral density, *LS* lumbar spine, *TBLH* total body less head, *vBMD* volumetric bone mineral density, *BV/TV* bone volume fraction, *Tb.N* trabecular number, *Tb.Th* trabecular thickness, *Tb.Sp* trabecular separation

[a]Comparison between groups made using Wilcoxon rank-sum tests

Fig. 1

HR-pQCT images of a 9-year-old boy with Duchenne muscular dystrophy (DMD) vs. age- and gender-matched healthy control showing lower microarchitectural measurement of trabecular bones [including lower trabecular vBMD (111.8 vs. 118.2 mg/cm^3, lower trabecular number (1.35 vs. 1.51/mm) and increased trabecular separation (0.673 vs. 0.595 mm)] but higher cortical vBMD (719.5 vs. 657.6 mg/cm^3) in the boy with DMD on long-term oral glucocorticoid



Discussion

To our knowledge, this is the first study describing the bone microarchitectural indices in DMD boys on long-term glucocorticoid based on HR-pQCT findings. Overall, HR-pQCT revealed lower microarchitectural measurement of trabecular bones in this group of individuals. Conversely, they had significantly higher cortical vBMD.

Our observed divergent effects of glucocorticoid therapy on cortical and trabecular vBMD had also been described in children with steroid-sensitive nephrotic syndrome, in which, higher cortical vBMD *Z* scores and lower trabecular vBMD *Z* scores by pQCT were observed when compared with control [15]. This observation is consistent with the histomorphology seen on transiliac biopsy of DMD boys [16]. Likewise, another study looking at the longitudinal changes in vBMD with glucocorticoid treatment in children with nephrotic syndrome also showed consistent findings. This has been postulated to be related to suppressed bone

formation and greater secondary mineralization with glucocorticoid treatment [17]. Interestingly, this is contrary to the observation in adults with glucocorticoid-induced osteoporosis (GIOP), in which, patients with GIOP and VF were observed to have lower total vBMD and thinner cortical thickness, independent of aBMD [18]. This highlights the difference between growing skeleton and mature skeleton.

Interestingly, among our DMD boys, the median LS *Z* score was higher than the mean TBLD Z-score; this would be suggestive of more cortical bone defects than trabecular defects, which was opposite to what was observed by HR-pQCT. Despite the fact that collapsed vertebrae, if any, would be excluded from aBMD measurement by DXA. However, endplate deformity (rather than frank vertebra compression fracture) secondary to trabecular bone defects, especially if generalised across all vertebral bodies, would not be picked up as 'vertebral compression fracture' and would be included for aBMD measurement. This could lead to spuriously higher LS aBMD. These findings were actually consistent with the observations of contradictory improvement in LS aBMD *Z* score post-glucocorticoid treatment with significant difference of greater than 2 standard deviations (SD) between LS and TBLH in the reported cohorts of DMD boys [2] [9]. The discrepancy of findings across the two modalities (DXA vs. HR-pQCT) underscored the complexity and limitations in using DXA findings for bone health assessment in children with on long-term glucocorticoid.

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More data on the specific bone deficits in cortical and trabecular density, bone geometry, microstructure and bone strength in children with various chronic illnesses at risk of bone fragility are needed. This is especially important in the context of DMD, in which, the pathophysiology of compromised bone health involves a 'perfect storm' of progressive muscle weakness, osteotoxic effect of glucocorticoid and the consequence of cytokine release and activated osteoclastogenesis in the dystrophin-deficient muscles [3]. More detailed and in-depth bone health assessment would increase our understanding of fracture susceptibility and may also allow the identification of modifiable factors that contribute to accelerated bone loss and fracture risk in these high-risk individuals, which might suggest novel strategies to reduce fracture rates and its associated morbidities.

There are several limitations to this study. First, the small sample size limits the generalizability of the findings. Second, the DXA findings, vitamin D status and pubertal staging of our healthy age-matched controls were not available for

comparison and adjustment of potential confounders. On the other hand, our patients represent a relatively young cohort of DMD because of our inclusion criteria from a single centre. This might be a strength of the study as it represents a rather homogeneous group in terms of age, prednisolone regimen and standard of care, but it would limit the generalization of the results to older DMD boys. Despite all the limitations, this study has provided some insights into the bone microarchitecture alterations in this group of children.

Conclusion

Reduced vBMD and inferior microarchitecture alterations of trabecular bones but higher cortical vBMD were observed in DMD boys on long-term oral glucocorticoid. Given the limitations of the study design, the findings should be viewed as preliminary. Further studies with larger sample size, improved methodologies and refined HR-pQCT scanning protocol adjusted for bone length are needed in the future.

Publisher's Note

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Author contributions

JYT contributed in study design, recruitment of subjects, data analysis and manuscript preparation. SHC contributed to the study design, recruitment of subjects and interpretations of data. TPL contributed to the HR-pQCT scanning protocols and interpretations of DXA and HR-pQCT findings. All authors contributed to interpretation of findings and critically revised the manuscript and approved the final version of the manuscript for submission.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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