Turk J Phys Med Rehab 2019;65(3):216-221
DOI: 10.5606/tftrd.2019.3565

Original Article

The relationship of bone mineral density and vitamin D levels with steroid use and ambulation in patients with Duchenne muscular dystrophy

Filiz Meryem Sertpoyraz[1], Bedile İrem Tiftikçioğlu[2]

[1]Department of Physical Medicine and Rehabilitation, Health Science University, Izmir Tepecik Training and Research Hospital, Izmir, Turkey
[2]Department of Neurology, Health Science University, Izmir Tepecik Training and Research Hospital, Izmir, Turkey

Received: September 02, 2018 **Accepted:** February 27, 2019 **Published online:** August 20, 2019

ABSTRACT

Objectives: This study aims to assess the bone mineral density (BMD) and serum levels of 25(OH)-vitamin D and their relationship with steroid use and ambulation in patients with Duchenne muscular dystrophy (DMD).

Patients and methods: Between January 2017 and May 2018, medical records of a total of 67 male patients (mean age, 13.9±4.3 years; range, 8 to 25 years) who were diagnosed with definite DMD were retrospectively analyzed. Demographic data, functional activity level, steroid use, fracture history and location, serum levels vitamin D, and lumbar and hip Z-scores in BMD at the time of the initial admission were recorded.

Results: The mean level of vitamin D was 13.4±7.5 ng/mL. In terms of serum levels of vitamin D, 28 patients (41.8%) had severe deficiency, 31 (46.3%) had insufficiency, and five patients (7.5%) had deficiency. Only three (4.5%) of the patients had sufficient levels of vitamin D. The hip Z-scores were significantly lower than lumbar Z scores. There was no significant difference in the lumbar and hip BMD measurements between the patients with and without steroid use. Lumbar Z-scores were significantly lower in non-ambulatory patients than ambulatory patients.

Conclusion: It is of utmost importance to evaluate the initial serum vitamin D levels in terms of bone health and prescribe replacement in case of deficiency/insufficiency in DMD patients. Since the decrease in the BMD is evident in this patient population, maintaining the mobilization as long as possible, providing loading on the bone for a long time, may be beneficial.

Keywords: Ambulation, bone mineral density, Duchenne muscular dystrophy, steroid, vitamin D.

Duchenne muscular dystrophy (DMD) is an X-linked recessive genetic disorder which accounts for every 3,600 to 6,000 live births. The absent or defective dystrophin protein caused by a mutation in Xp21 dystrophin gene leads to instability and degeneration in muscle fibers. Progressive muscular weakness causes loss of independent ambulation around age of 13 years and premature death.[1]

Corticosteroids are highly effective in maintaining muscular strength, improving walking and survival, decreasing scoliosis, and stabilizing respiratory functions, thus, regarded as the gold standard in treatment. However, corticosteroid therapy increases the risk of osteoporosis and bone fragility.[2,3] Steroid therapy affects bone destruction both directly and indirectly. It suppresses release of growth hormone and inhibits synthesis of insulin-like growth factor I. As a result, osteoblast activity decreases, while osteoclasts are activated directly. On the other hand, calcium absorption from the intestinal mucosa decreases and calcium and phosphate excretion from the kidneys increases, leading to hypocalcemia and secondary hyperparathyroidism. Although the turnover rate of the bone increases, bone formation is inadequate due to suppressed activity of osteoblasts. Eventually, bone resorption and osteoporosis begin.[2-4] Patients with DMD have been reported to have progressive deficiency of bone mineral density (BMD) due to steroid use beginning from the early stages of their disease course.[3] Mechanical loading on bones is as

Corresponding author: Filiz Meryem Sertpoyraz, MD. SBÜ İzmir Tepecik Eğitim ve Araştırma Hastanesi Fiziksel Tıp ve Rehabilitasyon Kliniği, 35170 Yenişehir, Konak, İzmir, Turkey. e-mail: dr.fms70@gmail.com

Cite this article as:
Sertpoyraz FM, İrem Tiftikçioğlu B. The relationship of bone mineral density and vitamin D levels with steroid use and ambulation in patients with Duchenne muscular dystrophy. Turk J Phys Med Rehab 2019;65(3):216-221.

This manuscript was presented in 15th International Congress on Neuromuscular Disease, July 6-8, 2018, Vienna Austria.

important as the BMD and structural content for bone strength. Factors negatively affecting the bone health in DMD patients include progressive muscular weakness, immobility, and adverse effects of the corticosteroids on the muscles.[4]

Another factor affecting the bone health is Vitamin D. Deficiency of vitamin D causes abnormalities in osteoblast function and imbalance in calcium metabolism, and increases the risk of fall.[5]

Osteoporosis is highly prevalent in patients with DMD due to the impaired micro-architecture of bone tissue and decreased bone mass. This increases fragility and fracture risk. Fractures are common in long bones of lower limbs and in spine, mainly.[6,7] Poor bone health, characterized by pain, increased fracture risk due to osteopenia and osteoporosis, and fractures, adversely affects the quality of life in DMD patients.[4,8] Thus, it is of importance to monitor BMD regularly, consider the risk factors and schedule treatment, accordingly.

In the present study, we aimed to assess the BMD and serum levels of 25(OH)-vitamin D and to examine the relation to steroid use and ambulation in patients with DMD.

PATIENTS AND METHODS

This cross-sectional, descriptive study was conducted at Izmir Tepecik Training and Research Hospital, Neuromuscular Diseases Unit between January 2017 and May 2018. Medical records of a total of 67 male patients (mean age: 13.9±4.3 years; range, 8 to 25 years) who were diagnosed with definite DMD were retrospectively analyzed. Diagnosis of DMD was based on clinical findings, blood biochemistry, genetic testing, and muscle biopsy, when necessary. Patients under eight years old were excluded, as BMD could not be measured in this age group. Considering life span of the patients, the upper limit of age was set at 25 years. Those with endocrinologic, malignant, or rheumatic conditions which might lead to osteoporosis, and those using medications which might alter the bone metabolism were excluded. A written informed consent was obtained from each participant or caregiver. The study protocol was approved by the institutional Ethics Committee (No. 2018/71). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Demographic data (age, sex, education status, and duration of disease), history of steroid use, duration and dose of steroid use (if present), history of fracture and location (if present), functional activity scores (ambulation status), and laboratory parameters of serum level of Vitamin D, calcium, parathyroid hormone, phosphorus, creatinine kinase, and lactate dehydrogenase were recorded. The patients were using either 0.75 mg/kg/day of prednisolone or 0.9 mg/kg/day of deflazacort as steroid therapy, if present. Duration of steroid use was classified as 0-12 months, 13-60 months, and ≥61 months.

All patients were classified in five stages, based on their functional activity level, as defined by the Neuromuscular Network (NMD).[9] Those in Stages 1 to 3 were considered ambulatory, otherwise non-ambulatory.

Serum levels of vitamin D

Serum levels of vitamin D were measured as 25(OH) vitamin D using the COBAS 411 device (Roche Diagnostics GmbH, Mannheim, Germany) via electrochemiluminescence (ECLIA) and the results were expressed in ng/mL. Vitamin D status was defined as deficient for 0-20 ng/mL, insufficiency for 21-29 ng/mL, and sufficient for ≥30 ng/mL.[10]

Bone mineral density measurement

Dual energy X-ray absorptiometry (DXA), which is the most frequently used and most reliable method, is used to evaluate BMD.[10] It yields the relationship between the BMD and risk of fracture. The fact that it uses low levels of X-ray is considered as its major advantage.[11] The DXA measures the BMD in the real-setting and express the amount of mineral per square centimeter in g/cm^2. For the assessment, T and Z scores are used. For the measurement of BMD, Z score means how many standard deviations higher or lower the measurement is than those of the age- and sex-matched individuals. It is used to assess osteoporosis in children and premenopausal women. The bone mass less than excepted for chronological age is considered, if Z score equals to or is lower than -2 SD, and normal bone mass excepted for chronological age is considered, if it is greater than -2.[12] In the present study, the BMD was assessed measuring by Hologic DXA device (Hologic Inc., Bedford, MA. USA). Lumbar (L1-L4) and hip total Z scores and BMD of all patients were expressed in g/cm^2.

Statistical analysis

Statistical analysis was performed using the IBM SPSS version 23.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in mean ± standard deviation (SD) and median (min-max) values

Table 1. Baseline demographic and clinical data of patients with DMD (n=67)

	n	%
Sex		
Male	67	100
Educational level		
Illiterate	1	1.5
Primary school	40	59.7
Secondary school	25	37.3
Higher education	1	1.5
Ambulation		
Ambulatory	22	32.8
Non-ambulatory	45	67.2
Steroid use		
Yes	55	82.1
No	12	17.9
Duration of steroid use		
0-12 month	4	7.3
13-60 month	13	23.6
61+ month or more	38	69.1
Type of steroid		
Prednisolone	52	95
Deflazacort	3	5
History of fracture		
Yes	65	97
No	2	3
Fracture of long bones	2	
Humerus	1	
Femur	1	

DMD: Duchenne muscular dystrophy.

and number and frequency. Categorical variables were compared using the chi-square test (or Fisher's exact test, where appropriate). Continuous variables were compared using the Mann-Whitney U test between the patient groups. Linear correlations between the Z-scores and vitamin D levels were tested using the Spearman rho correlation analysis with a cut-off value of >0.600 for strong correlation. A *p* value of <0.05 was considered statistically significant.

Table 2. Laboratory data of the patients with DMD expressed

	Mean±SD	Min-Max
25(OH)-Vitamin D (ng/dL)	13.4±7.5	3-36
Calcium (mg/dL)	9.8±0.4	8.5-11
Parathyroid hormone (pg/mL)	44.9±20.2	13-109
Creatinine kinase (U/L)	4594±4179	84-19570
Lactate dehydrogenase (U/L)	512.4±277	191-1496

DMD: Duchenne muscular dystrophy; SD: Standard deviation; Min: Minimum; Max: Maximum.

RESULTS

Baseline demographic and clinical characteristics of the patients are summarized in Table 1. The mean duration of diagnosis was 9.3±4.3 years.

Laboratory data of patients are summarized in Table 2. The mean value of vitamin D levels was in the range of deficiency. The majority of patients (n=59, 88.0%) had vitamin D deficiency, five patients (7.5%) had insufficiency, and three patients (4.5%) had sufficient levels of serum vitamin D.

Two patients had a history of fracture in long bones (one humerus and one femur). Both patients were using steroids for more than five years. They had severely deficient serum levels of vitamin D (<10 ng/mL).

There was a significant difference between the lumbar and hip Z scores of the patients ($p<0.05$). However, no significant difference was found between the lumbar and hip BMD of the patients ($p>0.05$).

Table 3 shows the comparison between the patients with and without steroid use and ambulatory and non-ambulatory patients for serum Vitamin D levels and BMD measurements. The patients with and without steroid use had both deficient levels of Vitamin D. According to hip Z scores, the scores were significantly lower in the non-ambulatory patients, compared to the ambulatory ones.

Table 3. Lumbar and hip Z scores, BMD, vitamin D according to steroid use and ambulation in patients with DMD

	History of steroid use	No history of steroid use		Ambulatory	Non-ambulatory	
	Mean±SD	Mean±SD	*p*	Mean±SD	Mean±SD	*p*
25(OH) vitamin D	13.2±7.3	14.2± 8.7	0.695	11.6±6.3	13.6±7.6	0.492
Lumbar Z score	-2.0±1.4	-1.4±1.5	0.144	-1.2±1.5	-2.0±1.4	0.447
Hip Z score	-3.2±1.1	-2.6±1.2	0.144	-2.5±0.8	-3.2±1.2	0.030
Lumbar BMD	0.590±0.154	0.579±0.165	0.664	0.543±0.124	0.594± 0.157	0.447
Hip BMD	0.539±0.169	0.481±0.224	0.579	0.556±0.099	0.527±0.185	0.455

DMD: Duchenne muscular dystrophy; SD: Standard deviation; 25(OH) vitamin D: 25-hidroksi vitamin D; BMD: Bone mineral density; p<0.05 statistically significant.

DISCUSSION

In the present study, according to lumbar and hip total Z scores and BMD, hip Z scores of DMD patients were low in consistent with the previous studies. Similarly, Larson and Henderson[13] evaluated BMD, mobility, and fracture in 41 men with DMD. They reported that total BMD in the hip was lower than in the lumbar region even in the ambulatory period. In the recentyl studies also reported that decrease in BMD accelerated even more with loss of ambulation.[14] Aparicio et al.[15] reported proximal femoral osteopenia in 20% and osteoporosis in 80% of 10 ambulatory children who were not using any steroids. They found osteopenia in lumbar region in 30% of the children. It has been shown that, in DMD patients, progressive proximal muscle weakness in the lower limbs, decreased loading on bones, and poor mobility lead to decrease in BMD beginning during early stages of the disease, influenced by long-term steroid use and its cumulative dose in the vertebra.[16]

When we compared the patients according to total hip Z scores, the scores of non-ambulatory patients were significantly lower than ambulatory patients. Tian et al.[17] evaluated BMD in 292 ambulatory patients with DMD and found lower femoral and whole-body Z scores. They concluded that assessment of lumbar region alone would not sufficient and emphasized that vertebral fractures should be also evaluated for the assessment of osteoporosis.

Two patients in this study had a fracture in long bones after falling from height. Both patients were using steroids for more than five years and had severe deficiency of vitamin D (<10 ng/mL). No significant difference in BMD was found, compared to other patients. They both had lost ambulation after the fracture. The risk of fracture in DMD was reported to be 21 to 40% in male pediatric population relative to the age-matched healthy children.[15] In a retrospective study of 378 children with DMD, McDonald et al.[18] reported that 20.9% of patients had fractures, occurred mainly after a falling. Ambulatory patients or the ones using wheelchair had fractures of the lower limbs, mostly; however, fractures of the upper limbs were more common in patients using orthoses. They also reported that 27% of patients with fractures had lost mobility.

In this study, 82% of the patients were using steroids. No significant difference was found in BMD between the patients on steroid treatment (n=55) and the ones not using any steroids (n=12). Steroids are used to maintain the muscle strength and mobilization by decreasing muscle destruction, focal inflammation, and necrosis and protect the cardiac and respiratory functions in early stages of the disease. Nevertheless, they adversely affect the bone health and increase the risk of fracture due to osteopenia and osteoporosis.[2,9] Many studies have reported that steroids increase vertebral fracture risk and pain. Continuing steroid treatment, despite its adverse effects on vitamin D and advanced age, further decreases the BMD in patients with DMD.[18-20] Pradhan et al.[21] started prednisolone treatment in 44 patients with DMD prior to loss of ambulation and followed 23 patients with DMD as the control group for two years. Loss in motor performance and muscle strength occurred much later in patients who were using prednisolone and steroids delayed transition to wheelchair for about three years.[21] There are other studies reporting that loss of bone is more rapid, decrease in lumbar vertebral Z scores is higher, and fragility fractures are more common in the patients with DMD using steroids.[22,23]

Serum levels of vitamin D were low in our patients and deficiency was found in 96% of the group. Deficiency of vitamin D is common in patients with DMD.[24-26] We found no significant difference in serum levels of vitamin D between the patients with or without the steroid treatment. Deficiency was common in both groups. Apparently, the importance of diagnosing the deficiency is essential, considering the necessity of optimizing the levels of vitamin D, particularly during the course of steroid treatment. Several studies have reported beneficial effects of vitamin D on bone health in DMD patients and replacement therapy has increased the BMD.[19,24,27,28] Moreover, Alshaikh et al.[19] assessed Vitamin D levels in DMD patients who were using steroid treatment and reported deficiency or insufficiency in 57% of patients who were previously treated with vitamin D replacement therapy and in 70% of the patients who were not given. They suggested that maintenance therapy was required with daily recommended dose of 1500 IU, following the replacement therapy.

Our two patients with fracture had severe deficiency of vitamin D, but there was no difference in levels of vitamin D between the patients with and without a fracture. This could be due to the deficiency of vitamin D levels being at high rates in the entire group of patients with DMD. Previously, in their study assessing the relationship between levels of vitamin D and fractures in 48 patients with DMD, Perera et al.[20] found vitamin D deficiency in 84% of the patients and noted that risk of fracture increased by advanced age, loss of mobilization, and steroid use. They reported

fracture in 43% of the patients. This risk can be reduced by supplementation therapy.[14]

Nonetheless, there are some limitations to this study. The main limitation of the study is the lack of a control group. There were no healthy control children in the study, as BMD was unable to be measured in a healthy pediatric patient group. Second, this was a cross-sectional study; however, longitudinal follow-up of these patients would reveal the impact of steroid therapy on bone health and vitamin D metabolism in disease course. In addition, a prospective study would enable us to monitor the effects of vitamin D replacement therapy.

In conclusion, it is of importance to measure the serum levels of vitamin D early in the disease course in terms of the bone health and adequate replacement and maintenance therapies should be given in case of deficiency in DMD patients. Controlled, multi-center, prospective clinical studies with a higher number of patients are warranted both to better understand the roles of vitamin D levels and BMD in the disease course and to clarify the practice algorithms and treatment regimens in patients with DMD.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The authors received no financial support for the research and/or authorship of this article.

REFERENCES

1. Chaturvedi LS, Mukherjee M, Srivastava S, Mittal RD, Mittal B. Point mutation and polymorphism in Duchenne/Becker muscular dystrophy (D/BMD) patients. Exp Mol Med 2001;33:251-6.
2. Quinlivan R, Roper H, Davie M, Shaw NJ, McDonagh J, Bushby K. Report of a Muscular Dystrophy Campaign funded workshop Birmingham, UK, January 16th 2004. Osteoporosis in Duchenne muscular dystrophy; its prevalence, treatment and prevention. Neuromuscul Disord 2005;15:72-9.
3. Biggar WD, Bachrach LK, Henderson RC, Kalkwarf H, Plotkin H, Wong BL. Bone health in Duchenne muscular dystrophy: a workshop report from the meeting in Cincinnati, Ohio, July 8, 2004. Neuromuscul Disord 2005;15:80-5.
4. Joyce NC, Hache LP, Clemens PR. Bone health and associated metabolic complications in neuromuscular diseases. Phys Med Rehabil Clin N Am 2012;23:773-99.
5. Fidan F, Alkan BM, Tosun A. Çağın Pandemisi: D vitamini eksikliği ve yetersizliği. Turk J Osteoporos 2014;20:71-4.
6. NICE (National Institute for Health and Clinical Excellence) Osteoporosis: assessing the risk of fragility fracture. NICE guideline (CG146). 2012. Available from: https://www.nice.org.uk/guidance/cg146
7. Ness K, Apkon SD. Bone health in children with neuromuscular disorders. J Pediatr Rehabil Med 2014;7:133-42.
8. Morgenroth VH, Hache LP, Clemens PR. Insights into bone health in Duchenne muscular dystrophy. Bonekey Rep 2012;1:9.
9. Bushby K, Finkel R, Birnkrant DJ, Case LE, Clemens PR, Cripe L, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. Lancet Neurol 2010;9:77-93.
10. Osteoporoz ve Metabolik Kemik Hastalıkları Tanı ve Tedavi Kılavuzu. Türkiye Endokrinoloji ve Metabolizma Derneği 2017. Available at: http://www.temd.org.tr/admin/uploads/tbl_gruplar/20180507224049-2018-05-07tbl_gruplar224043.pdf
11. Sindel D. Osteoporosis Treatment at Present and in the Future. Turk J Phys Med Rehab 2013;59:330-7.
12. Mølgaard C, Thomsen BL, Prentice A, Cole TJ, Michaelsen KF. Whole body bone mineral content in healthy children and adolescents. Arch Dis Child 1997;76:9-15.
13. Larson CM, Henderson RC. Bone mineral density and fractures in boys with Duchenne muscular dystrophy. J Pediatr Orthop 2000;20:71-4.
14. Vestergaard P, Glerup H, Steffensen BF, Rejnmark L, Rahbek J, Moseklide L. Fracture risk in patients with muscular dystrophy and spinal muscular atrophy. J Rehabil Med 2001;33:150-5.
15. Aparicio LF, Jurkovic M, DeLullo J. Decreased bone density in ambulatory patients with duchenne muscular dystrophy. J Pediatr Orthop 2002;22:179-81.
16. Söderpalm AC, Magnusson P, Åhlander AC, Karlsson J, Kroksmark AK, Tulinius M, et al. Bone mass development in patients with Duchenne and Becker muscular dystrophies: a 4-year clinical follow-up. Acta Paediatr 2012;101:424-32.
17. Tian C, Wong BL, Hornung L, Khoury JC, Miller L, Bange J, et al. Bone health measures in glucocorticoid-treated ambulatory boys with Duchenne muscular dystrophy. Neuromuscul Disord 2016;26:760-7.
18. McDonald DG, Kinali M, Gallagher AC, Mercuri E, Muntoni F, Roper H, et al. Fracture prevalence in Duchenne muscular dystrophy. Dev Med Child Neurol 2002;44:695-8.
19. Alshaikh N, Brunklaus A, Davis T, Robb SA, Quinlivan R, Munot P, et al. Vitamin D in corticosteroid-naïve and corticosteroid-treated Duchenne muscular dystrophy: what dose achieves optimal 25(OH) vitamin D levels? Arch Dis Child 2016;101:957-61.
20. Perera N, Sampaio H, Woodhead H, Farrar M. Fracture in duchenne muscular dystrophy: Natural history and vitamin d deficiency. J Child Neurol 2016;31:1181-7.
21. Pradhan S, Ghosh D, Srivastava NK, Kumar A, Mittal B, Pandey CM, et al. Prednisolone in Duchenne muscular dystrophy with imminent loss of ambulation. J Neurol 2006;253:1309-16.
22. Quinlivan R, Shaw N, Bushby K. 170th ENMC International Workshop: bone protection for corticosteroid treated Duchenne muscular dystrophy. 27-29 November 2009, Naarden, The Netherlands. Neuromuscul Disord 2010;20:761-9.

23. Mayo AL, Craven BC, McAdam LC, Biggar WD. Bone health in boys with Duchenne Muscular Dystrophy on long-term daily deflazacort therapy. Neuromuscul Disord 2012;22:1040-5.
24. Bianchi ML, Morandi L, Andreucci E, Vai S, Frasunkiewicz J, Cottafava R. Low bone density and bone metabolism alterations in Duchenne muscular dystrophy: response to calcium and vitamin D treatment. Osteoporos Int 2011;22:529-39.
25. James KA, Cunniff C, Apkon SD, Mathews K, Lu Z, Holtzer C, et al. Risk factors for first fractures among males with Duchenne or becker muscular dystrophy. J Pediatr Orthop 2015;35:640-4.
26. McMillan HJ, Campbell C, Mah JK. Duchenne muscular dystrophy: Canadian paediatric neuromuscular physicians survey. Can J Neurol Sci 2010;37:195-205.
27. Bianchi ML. How to manage osteoporosis in children. Best Pract Res Clin Rheumatol 2005;19:991-1005.
28. Boyce AM, Tosi LL, Paul SM. Bisphosphonate treatment for children with disabling conditions. PM R 2014;6:427-36.