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Rev Chil Pediatr. 2020;91(2):209-215
DOI: 10.32641/rchped.v91i2.1269

ORIGINAL ARTICLE

Functional capacity, bone mineral density and neoformation-resorption bone markers in patients under 18 years of age with reduced mobility

Capacidad funcional, densidad mineral ósea y marcadores de neoformación-reabsorción ósea en pacientes menores de 18 años con movilidad reducida

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Received: 27-5-2019; Approved: 18-11-2019

What do we know about the subject matter of this study?

The reduced mobility associated with different neuromuscular disorders causes from the first years of life an increase in bone resorption which leads to a decreased bone mineral density and an increased risk of fractures.

What does this study contribute to what is already known?

This study provides information on pediatric patients with different neuromuscular disorders, evaluating the relationship between bone mineral density, biochemical parameters of bone neoformation and bone resorption, and the decrease in locomotor capacity quantified through a validated functional scale.

Abstract

Introduction: Prolonged immobilization associated with several neurological disorders causes secondary osteoporosis with pathological fractures and persistent bone pain. **Objectives:** To establish the association between bone mineral density (BMD), neoformation and bone resorption markers and the degree of functional capacity in children under 18 years of age with reduced mobility. **Patients and Method:** Cross-sectional study conducted in Ciudad Real, Spain between January 1, 2016, and December 31, 2017 with patients aged between 6 and 18 years diagnosed with different neurological disorders. The following variables were analyzed: age, sex, pubertal stage, functional capacity according to the Functional Mobility Scale (FMS), which assesses the ability to walk from 5, 50 to 500 meters, BMD, 25-hydroxy-vitamin D, alkaline phosphatase and osteocalcin in blood, and N-terminal telopeptide crosslinks in collagen type I (NTX-I) in urine. BMD, alkaline phosphatase, osteocalcin,

Keywords:
Bone mineral density;
vitamin D;
densitometry;
mobility limitation;
bone resorption

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How to cite this article: Rev Chil Pediatr. 2020;91(2):209-215. DOI: 10.32641/rchped.v91i2.1269

and NTX-I values are expressed in Z score according to reference values for age and sex. The Pearson and Spearman correlations were used for data analysis. **Results:** 36 patients (52.7% girls) with an average age of 8.6±4.7 years. Mean FMS value: 5.3 out of 18. Mean BMD: -1.99 ± 1.7 standard deviations (SD), mean alkaline phosphatase: -2.64 ± 1.08, mean osteocalcin: -2.15 ± 1.39, and mean NTX-I: +3 ± 1.72. There was a significant association between BMD and FMS for 5 meters (r = 0.395; p = 0.017) and for total score (r = 0.365; p = 0.029). There were no significant differences according to the stages of pubertal development. **Conclusions**: In this population, there was a decrease in BMD and bone neoformation markers, and an increase of bone resorption markers with no association with pubertal development. Patients with a lower degree of mobility present a lower BMD.

Introduction

The mechanical stress applied to the bone through movement is essential to achieve adequate bone mineralization[1]. Therefore, prolonged immobilization associated with different neurological disorders leads to secondary osteoporosis with pathologic fractures and persistent bone pain[2], which at the same time can worsen the functional condition of these patients.

The muscular force exercised during movement produces bone remodeling as a response that influences the shape and geometry of the bones[3], unlike physical inactivity which affects this bone-muscle functional unit producing a decrease in periosteal apposition, especially in long bones such as the femur and tibia[4]. The physiopathological explanation for this phenomenon lies in the balance alteration between bone neoformation and resorption, favoring the latter thus immobilization would produce an increase in osteoclastic activity mediated by the receptor activator for nuclear factor-kappa b ligand (RANKL)[5]. All this would result in a decreased cortical bone thickness, and thus, a higher risk of fracture[6].

On the other hand, the increase in life expectancy in patients diagnosed with neuromuscular diseases over the last few years means that problems related to bone health in these cases are becoming increasingly prevalent[7].

Since bone mass acquisition occurs during childhood and adolescence, the study in this period is crucial for developing diagnostic and therapeutic strategies to avoid more serious consequences in later years.

Despite knowing the relationship between reduced mobility and the appearance of clinical and biochemical alterations associated with increased bone resorption, the relationship between bone mineral density, bone neoformation, and resorption parameters, and decreased motor capacity qualified according to an internationally validated functional scale has not been studied so far in pediatric patients with different neuromuscular diseases. Therefore, the main objective of this study is to establish the association between bone mineral density, markers of bone neoformation and resorption, and the functional capacity degree in a group of patients aged under 18 years with reduced mobility, and, as secondary objectives, to know if there are differences in bone mineral density and markers of bone neoformation and resorption according to pubertal development, as well as to study the relationship between bone mineral density and body mass index (BMI) in our sample.

Patients and Method

Transversal study. Data collection was carried out from 1 January 2016 to 31 December 2017. Those patients seen at the health area of the *Hospital General Universitario de Ciudad Real* (Spain) and that were being monitored by the Pediatric Endocrinology and Physical Medicine and Rehabilitation Units within the established period were considered for participating in the study. The following were the inclusion criteria: age between 6 and 18 years and diagnosed with different neurological diseases with associated reduced mobility according to the Functional Mobility Scale (FMS) score[9].

Patients who received maintenance treatment with corticosteroids, anticonvulsants, calcium or vitamin D supplements, and bisphosphonates were excluded. After considered all these inclusion and exclusion criteria and given the possibility of having only a limited number of patients that would prevent achieving an ideal sample, the participants of the study were selected through consecutive non-probability sampling.

The following variables were analyzed: chronological age, sex, weight, height, BMI, pubertal development according to Tanner's stages, bone age according to the Greulich & Pyle atlas, bone mineral density determined by dual-energy x-ray absorptiometry (DXA) of the lumbar spine (L2-L4), functional capacity according to FMS score, blood level of 25-hydroxyvitamin D3, parathyroid hormone (PTH), total alkaline phosphatase and osteocalcin, and urine levels of the amino-

terminal telopeptide of type I collagen (NTx-I). Total alkaline phosphatase and osteocalcin were established as parameters of bone neoformation and NTx-I as a parameter of bone resorption.

The variables age, sex, weight, height, BMI, and pubertal development were directly collected by the same researcher through the anamnesis and physical examination of the patients, recording weight and height from the same scale and the same stadiometer respectively. BMI data were expressed in Z score according to reference values for the Spanish pediatric population[10].

The Functional Mobility Scale (FMS)[9] is a validated scale for patients aged 6 to 18 years with neuromuscular disease, which assesses the ability to move around in three areas of daily life: at home (distances up to 5 meters), at school (distances up to 50 meters), and in the community area (distances up to 500 meters).

The FMS data were collected through the researcher's direct observation for the distance up to 5 meters (at home) and by interviewing the patient or caregiver for the distances up to 50 and 500 meters (at school and community area respectively). Each distance was scored as 6 points if the patient could move without any limitation, 5 points if she or he could walk independently but could not run, 4 points if the patient needed walking canes, 3 points if she or he required forearm crutches, 2 points if she or he required a walker, 1 point if the patient could move in a wheelchair, and 0 points if she or he could not move independently in any case.

Bone mineral density was assessed using the Hologic® system and the results were expressed as Z score for sex and bone age according to the reference values of our population[11] after applying the corresponding correction factor.

The total alkaline phosphatase, osteocalcin, and NTx-I levels were expressed as Z score for age, sex, and pubertal stage according to the reference values for the pediatric age[12-14]. Regarding vitamin D, it was classified as deficit if the 25-hydroxy vitamin D3 levels were < 12 ng/ml and as insufficient if the levels were ≥ 12 ng/ml and < 20 ng/ml[15].

All the analytical variables were recorded early in the morning with patients fasting following the protocols for collecting and processing samples of the Clinical Analysis Laboratory of the *Hospital General Universitario de Ciudad Real*. The total alkaline phosphatase was determined through kinetic spectrophotometry method (optimized Bessey-Lowry-Brock method), for measuring the osteocalcin we used the N-MID® Osteocalcin ELISA kit (Abbexa), for quantifying NTx-I, the Elecsys® ß-CrossLaps kit (Roche) and, finally, for determining 25-hydroxy-vitamin D3, the chemiluminescent immunoassay Immulite® 2000.

Statistical analysis

Data analysis was performed with the SPSS v.19.0 software. The results of the descriptive statistical study were defined using central tendency and statistical dispersion, presented as percentages and histograms.

Regarding inferential statistical analysis, the goodness of fit was tested with the normal distribution of quantitative variables through the Shapiro-Wilk test. To establish correlations between variables, in cases where there was a good fit with normal distribution, we used the Pearson correlation coefficient, and in the remaining cases, the Spearman's correlation coefficient. Data were stratified according to different stages of pubertal development. The results were expressed as mean and median values and 95% confidence intervals (95% CI). In all cases, a $p < 0.05$ value was considered statistically significant.

This study was carried out under the principles of the Declaration of Helsinki and modifications concerning human research. Parents or legal guardians of each participant signed an informed consent form. The study was approved by the Clinical Research Ethics Committee of the hospital where it was conducted.

Results

Considering the reference population and the inclusion and exclusion criteria, 39 patients were included during the established period, out of which 3 did not accept to participate or did not complete the study protocol, resulting in a final sample of 36 patients. Regarding the causes of reduced mobility in our sample, the most frequent group (41.6%) was secondary to encephalopathies of different etiologies (associated with chromosomal diseases and malformation, metabolic, and idiopathic syndromes), followed by cerebral palsy (33.3%), neuromuscular diseases (13.8%), and finally those of vascular origin (11.1%).

Table 1 shows the main descriptive results of the sample studied. There is a decreased average value in bone mineral density compared with the reference values for age and sex. Therefore, 47.2% of the population studied had a Z score below -2, and only 33.3% of the patients had a Z score above -1, and in less than half of them (13.8% of the total sample) the Z score was above 0. Even so, none of the patients in the sample had had previous bone fractures.

There were low average values in the parameters of bone neoformation, such as total alkaline phosphatase and osteocalcin, and increased parameters of bone resorption, such as NTx-I. In the remaining parameters, 30.5% of the patients in the sample presented values of 25-hydroxy vitamin D3 < 20 ng/ml and 16.6% presented values < 12 ng/ml.

Table 1. Descriptive results of the

Variables	Results
Age (years) (mean, SD)	8.6 ± 4.7
Sex (%)	52.7% mujeres, 47.3% varones
Pubertal development (Tanner's stages) (%)	1 (67%), 2 (11%), 3 (17%), 4 (5%)
BMI (Z score) (mean, SD)	-0.5 ± 1.8
Bone Mineral Density (Z score) (mean, SD)	-1.99 ± 1.7
Total Alkaline Phosphatase (Z score) (mean, SD)	-2.64 ± 1.08
PTH (pg/ml) (mean, SD)	38.4 ± 21.05
Osteocalcin (Z score) (mean, SD)	-2.15 ± 1.39
NTX-I (Z score) (mean, SD)	3 ± 1.72
25-hydroxy-vitamin D3 (ng/ml) (mean SD)	28.97 ± 13.38

SD: Standard Deviation. BMI: Body Mass Index. PTH: Parathyroid hormone. NTX-I: Amino-terminal telopeptide of type I collagen.

Table 2. Functional Mobility Scale (FMS) score distribution in the study group.

Score	FMS 5 meters	FMS 50 meters	FMS 500 meters
0	12 (33.3%)	16 (44.3%)	21 (58.3%)
1	7 (19.4%)	6 (16.6%)	6 (16.6%)
2	5 (13.8%)	1 (2.7%)	1 (2.7%)
3	0	0	0
4	2 (5.5%)	4 (11.1%)	3 (8.3%)
5	8 (22.2%)	8 (22.2%)	5 (13.8%)
6	2 (5.5%)	1 (2.7%)	0

Table 3. Relationship between functional capacity (FMS) and bone mineral density (BMD)

	Correlation coefficient*	p value
FMS 5 meters - BMD	0.395	0.017
FMS 50 meters - BMD	0.385	0.071
FMS 500 meters - BMD	0.116	0.501
FMS total - BMD	0.365	0.029

*Analysis was performed using Spearman's correlation coefficient.

Table 2 shows the FMS score distribution in the study group. There was a significant motor impairment in many cases, where 33.3%, 44.3% and 58.3% of the patients had score 0 at distances of 5, 50 and 500 meters respectively.

Regarding the relationship between functional capacity and bone mineral density (table 3), there was a statistically significant association between the FMS score at 5 meters and bone mineral density ($r = 0.395$; $p = 0.017$), as well as between the total FMS score and bone mineral density ($r = 0.365$; $p = 0.029$).

In addition, there was no significant correlation between the bone neoformation and resorption parameters with functional capacity (table 4) or with bone mineral density (table 5), and also, there was no relationship between BMI and bone mineral density values ($r = 0.268$; $p = 0.115$).

Finally, no significant differences were observed when stratifying the results between those patients without the onset of puberty (Tanner stage 1) and those with some degree of development (Tanner stages 2 onwards).

Table 4. Relationship between functional capacity (FMS) and bone neoformation and resorption markers

	FMS 5 meters		FMS 50 meters		FMS 500 meters		FMS total	
	Correlation coefficient*	p value	Correlation coefficient*	p value	Correlation coefficient*	p value	Correlation coefficient*	p value
Total Alkaline Phosphatase	0.209	0.222	0.127	0.461	0.132	0.443	0.174	0.310
PTH	0.343	0.401	0.357	0.33	0.334	0.46	0.302	0.415
Osteocalcin	0.201	0.239	0.083	0.630	0.136	0.428	0.179	0.297
NTX-I	-0.231	0.175	-0.087	0.616	0.080	0.641	-0.177	0.302
25-OH- D3	-0.151	0.379	-0.044	0.800	0.018	0.915	-0.138	0.422

PTH: Parathyroid hormone. NTX-I: Amino-terminal telopeptide of type I collagen. 25-OH-D3: 25-hydroxy-vitamin D3. *Analysis was performed using Spearman's correlation coefficient

Discussion

Considering the predominance of bone resorption in cases of reduced mobility, it has been reported that the risk of fracture has increased by 4% annually in patients with cerebral palsy, as well as the prevalence of fractures by 11 to 30% in patients with spina bifida, and by 20.9 to 43% in patients with Duchenne muscular dystrophy[18]. In the sample analyzed, although there was no history of pathologic fractures, there was a decrease in bone mineral density compared with the reference values for age and sex, as well as a significant relationship between bone mineral density and functional capacity, especially at home (FMS at 5 meters) and in general assessment (total FMS).

Despite the benefits of physical activity on bone mass, there is currently no evidence on the effects of exercise in patients with reduced mobility[1,19]. However, there has been found an increase in bone mineral density in patients with cerebral palsy after prolonged standing[20], as well as after whole-body vibration exercises[21,22]. These could be promising interventions that could be used as preventive measures or as a complement to other treatments, although there is a need for studies with larger numbers of patients and longer follow-up periods[1].

In relation to the increase in osteoclastic activity in patients with reduced mobility, in our study group, there was an increase in NTx-I compared with reference values, as well as a decrease in bone neoformation parameters such as osteocalcin or total alkaline phosphatase. These indicators are considered as predictors of the bone mass loss and the risk of presenting fractures[23]. Thus, in adult patients, a relationship has been observed between bone mineral density and osteocalcin, alkaline phosphatase and NTx-I[24,25], which has not been verified in our work.

In one of the few studies carried out in pediatric patients, there was no relationship between osteocalcin and the functional state of a group of children diagnosed with myelomeningocele[26], which is in line with the results of our study. However, it should be noted that these markers of bone neoformation-reabsorption have difficult interpretation, as they show great variability depending on different factors (age, sex, and BMI)[27]. This is why their determination should only be requested from patients at high risk of low bone mineral density and their values should be adjusted to these factors[28].

Regarding vitamin D levels, in our study group, 30.5% of the patients presented levels of 25-hydroxy-vitamin D3 < 20 ng/ml, a percentage that is within the range of other studies carried out in our country in the pediatric population, which varies between 8.3%[28] and 52.7%[29]. None of these patients presented hyperparathyroidism secondary to vitamin D insufficiency or deficit. With respect to the influence of vitamin D on bone mineralization in patients with neuromuscular diseases, a positive correlation has been found between

Table 5. Relationship between bone mineral density and bone neoformation and resorption markers

	Bone mineral density	
	Correlation coefficient*	p value
Fosfatasa alcalina total	0.029	0.868
PTH	0.215	0.203
Osteocalcina	0.313	0.063
NTX-I	-0.123	0.474
25-hidroxi-vitamina D3	-0.201	0.239

PTH: Parathyroid hormone. NTX-I: Amino-terminal telopeptide of type I collagen. *Analysis was performed using Pearson correlation coefficient.

vitamin D levels and cortical bone mineral density[1]. It is worth to mention that the group of patients with neurological diseases and altered functional capacity are at high risk of vitamin D insufficiency since it is very difficult for them to go outdoors and properly expose to sunlight, as well as due to the nutritional problems they frequently present and the use of medications that can interfere with vitamin D metabolism, such as corticosteroids or anticonvulsants[7]. For this reason, it is recommended to determine the levels of 25-hydroxy vitamin D3 in those cases at risk of having decreased bone mineral density, such as prolonged immobilization[30].

Therefore, reduced mobility is considered as a risk situation for reduced bone mineral density and increased risk of fracture[1,2,7,31]. In this group of patients, it is recommended to perform bone densitometry when they can benefit from therapeutic intervention and when the results can modify the clinical approach[31]. However, we must make decisions according to each individual, since bone fragility is influenced by other factors such as genetics, age, sex or certain drugs[7,31].

As limitations of our study, it would be necessary to check our results on the bone mass in a larger number of patients, as well as to extend them to other parameters of bone neoformation-reabsorption that it has not been possible to determine in this work, such as bone alkaline phosphatase, amino-terminal propeptide of type I procollagen, C-terminal telopeptide of type I collagen, and pyridinoline. New bone densitometry techniques could show the effects that the lack of mobility produces in both cortical and trabecular bone.

As conclusions of our series of patients, it should be noted that a decrease in bone mineral density has been observed depending on the degree of mobility, as well as a predominance of resorption markers over those of bone neoformation. Since bone mass acquisition takes place during the first two decades of life, strategies could be developed to make an early diagnosis of bone health problems in these patients with reduced mobility in order to prevent complications in later years.

Ethical Responsibilities

Human Beings and animals protection: Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

Data confidentiality: The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

Rights to privacy and informed consent: The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the correspondence author.

Conflicts of Interest

Authors declare no conflict of interest regarding the present study.

Financial Disclosure

Authors state that no economic support has been associated with the present study.

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