



Trabajo Original

Bone mineral density and biochemical and hormonal indicators in children with quadriplegic cerebral palsy

Densidad mineral ósea e indicadores bioquímicos y hormonales en niños con parálisis cerebral cuadripléjica

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Abstract

Introduction: children with cerebral palsy (CP) have multiple risk factors for low bone mineral density or osteoporosis.

Objective: to explore the association between bone mineral density (BMD) and biochemical and hormonal indicators of bone metabolism in children with quadriplegic cerebral palsy (CP).

Methods: a cross-sectional analytical study included 59 participants from six to 18 years of age with quadriplegic CP. Serum concentrations of calcium, phosphorus, 25OHD metabolite, parathyroid hormone (PTH), alkaline phosphatase, and thyroid hormones were determined using standardized methods. The BMD measurement was obtained from the lumbar spine expressed in g/cm² and Z-score. Unpaired Student's t-test, Chi-square test, odds ratio and Pearson's correlation were performed.

Results: participants with CP and malnutrition had lower serum concentrations of calcium, phosphorus and alkaline phosphatase. Those who had low BMD showed lower serum concentrations of calcium, phosphorus and alkaline phosphatase. Most participants with low and normal BMD had vitamin D deficiency (27.1% and 10%) and insufficiency (35.4% and 30%), respectively. There was a significant correlation between BMD and serum concentrations of calcium, phosphorus, alkaline phosphatase, vitamin D and thyroid-stimulating hormone (TSH). There were no differences in the biochemical and hormonal indicators by level of gross motor function, use of anticonvulsants and oral *versus* enteral feeding method.

Conclusion: malnutrition and alteration of vitamin D nutritional status were associated with low BMD and alterations of biochemical indicators of bone metabolism in pediatric patients with quadriplegic CP. The relationship between BMD and biochemical indicators of bone metabolism in children with quadriplegic CP was also demonstrated.

Key words:

Bone mineral density. Quadriplegic cerebral palsy. Bone metabolism. Vitamin D. Phosphorus. Alkaline phosphatase.

Resumen

Introducción: los niños con parálisis cerebral (PC) presentan múltiples factores de riesgo de densidad mineral ósea baja u osteoporosis.

Objetivo: explorar la asociación entre la baja densidad mineral ósea (DMO) e indicadores bioquímicos y hormonales del metabolismo óseo en niños con PC cuadripléjica.

Métodos: un estudio transversal analítico incluyó a 59 participantes de entre seis y 18 años de edad con PC cuadripléjica. Las concentraciones séricas de calcio, fósforo, metabolito 25OHD, hormona paratiroidea (PTH), fosfatasa alcalina y hormonas tiroideas se determinaron utilizando métodos estandarizados. La medición de DMO se obtuvo de la columna lumbar expresada en g/cm² y puntaje Z. Se realizaron pruebas t de Student no pareada, Chi-cuadrado, razón de momios y correlación de Pearson.

Resultados: los participantes con PC y desnutrición tenían concentraciones séricas más bajas de calcio, fósforo y fosfatasa alcalina. Los participantes con DMO baja tuvieron menor concentración sérica de calcio, fósforo y fosfatasa alcalina. Los participantes con DMO baja y normal tenían deficiencia de vitamina D (27.1% y 10%) e insuficiencia (35.4% y 30%), respectivamente. Hubo una correlación significativa entre DMO y las concentraciones séricas de calcio, fósforo, fosfatasa alcalina, vitamina D y hormona estimulante de la tiroides.

Conclusión: la desnutrición y la alteración del estado nutricional de la vitamina D se asociaron con DMO baja y alteraciones de los indicadores bioquímicos del metabolismo óseo. Se demostró una asociación entre DMO e indicadores bioquímicos y hormonales del metabolismo óseo en niños con PC cuadripléjica.

Palabras clave:

Densidad mineral ósea. Parálisis cerebral cuadripléjica. Metabolismo óseo. Vitamina D. Fósforo. Fosfatasa alcalina.

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INTRODUCTION

Cerebral palsy (CP) is a group of disorders of the development of movement and posture, causing limitation of activity, which is attributed to non-progressive damage of the developing brain, in the fetal period or in the first years of life (1). Children with CP have multiple risk factors for low bone mineral density (BMD) or osteoporosis, including the degree of gross motor impairment, protein-energy malnutrition (PEM), the method of feeding and the use of anticonvulsants (2-6).

Anticonvulsant drugs affect BMD due to their adverse effects on the serum concentration of calcium, phosphorus, vitamin D, parathyroid hormone, alkaline phosphatase and thyroid hormones causing hypocalcemia, hypophosphatemia, elevated alkaline phosphatase concentration, decreased vitamin D concentration, alterations in thyroid profile and increase in PTH concentration (4,6,7).

The biochemical markers related to bone metabolism in children and adolescents are involved in bone formation, skeletal growth, and bone resorption. In addition, they are influenced by multiple factors, such as age, pubertal stage, growth rate, hormonal regulation, and nutritional status (8). There is increasing evidence reporting the relationship of BMD with these biochemical markers of bone metabolism in children with CP (9-13). Nevertheless, some studies have reported that the decrease of serum concentrations of phosphorus, calcium, vitamin D, alkaline phosphatase and PTH may not be associated with low BMD (3,12).

The purpose of this study was to explore the association between BMD and biochemical and hormonal indicators of bone metabolism in children with quadriplegic CP treated at a University Hospital in the metropolitan area of Guadalajara, Mexico.

METHODS

Analytical cross-sectional study including 62 participants from six to 18 years of age with quadriplegic CP with or without spasticity who attended the Pediatric Neurology Outpatient Clinic of the Nuevo Hospital Civil de Guadalajara. The results of the present study are part of a study conducted in the same population of children and adolescents with quadriplegic cerebral palsy (2). The sample size was calculated in 58 participants with an alpha level of 0.05, power of 0.80, an expected frequency of low bone mineral density in children with CP of 42% (14), and a type II error of 0.20. We did not include patients with diagnoses unrelated to CP (autism, Down syndrome, degenerative disorders, hypothyroidism), moderate or severe congenital malformations, CP of postnatal origin and/or other diseases that affect bone mineral metabolism.

ETHICAL CONSIDERATIONS

The protocol did not expose participants to risk and adhered to the guidelines of the Declaration of Helsinki in its last correction made during the 64th Annual Assembly organized by the World Medical Association (2013). The persons legally responsible for

the participants signed the informed consent and the research protocol was approved by the Bioethics and Research Committee of the Nuevo Hospital Civil de Guadalajara with registration number 66/HCJIM-JAL/2016.

DETERMINATION OF BONE MINERAL DENSITY

The measurement of BMD expressed in g/cm² and age-adjusted Z-score was performed by dual-energy X-ray absorptiometry (DXA) using the GE Medical Systems Lunar software by a certified technician. The lumbar spine (L₁-L₄) was used as the region of interest, with the following criteria: low BMD for age, a Z-score defined as bone mass reduction (< -2 Z-score), and osteoporosis defined as bone mass reduction plus the alteration of bone architecture (< -2 Z-score plus a significant fracture history: two or more long bone fractures before ten years of age or three or more long bone fractures before 19 years of age) (15-17).

LABORATORY ANALYSIS

We obtained 5 ml of peripheral blood for the determination of serum concentrations of biochemical indicators and hormones with standardized methods. The 25OHD metabolite and PTH were determined by the chemiluminescence immunoassay method LIAISON[®] 25OH Vitamin D Total and LIAISON[®] 1-84 PTH Assay, DiaSorin (USA). The cut-off points for 25OHD metabolite were as follows: deficiency (< 30 nmol/l), insufficiency (30-50 nmol/l), sufficiency (51-75 nmol/l) and optimal (> 75 nmol/l) (14,18); the PTH reference values were 9 to 52 pg/ml.

The serum concentrations of phosphorus, calcium and alkaline phosphatase were determined by spectrophotometry (SYNCHRON[®], Beckman Coulter, USA), with the following reference values: calcium 8.7-10.7 mg/dl, phosphorus 2.4-5.6 mg/dl and alkaline phosphatase 30-400 IU/l. TSH, triiodothyronine (T₃) and thyroxine (T₄) were determined by the chemiluminescence immunoassay method using the Access Immunoassay System (Beckman Coulter, USA) with the following reference values: TSH 0.27-3.1 uIU/ml, T₃ 0.26-0.62 ng/ml, and T₄ 4.5-10.8 µg/ml.

NUTRITIONAL STATUS

The weight/age (W/A) and BMI anthropometric indexes were calculated using the Brooks reference (19). Those participants whose BMI and W/A scores were between the 10th and 90th percentiles were considered as normal and those who were located < 10th percentile were considered as malnourished.

STATISTICAL ANALYSIS

The normality of data distribution was determined with the Kolmogorov-Smirnov test. The unpaired Student's t-test was used

for quantitative variables with normal distribution. The Chi-square test was used for qualitative variables. Odds ratios were used to identify the probability of association and its epidemiological significance. The Pearson's correlation test and the coefficient of determination (R^2) were used to explain the variability of BMD in relation to the biochemical indicators studied. The multiple linear regressions were used to explain the variability between BMD and several independent variables. A p value ≤ 0.05 was considered as significant. Data capture and analysis was done with the SPSS program version 20 (SPSS, Inc., Chicago, IL, USA).

RESULTS

We included 62 participants from six to 18 years of age (11 ± 4 years) with quadriplegic CP; three were excluded because they presented BMD < 0.208 g/cm² (< -7 SD). Of the 59 participants, 25 (42.4%) were female and 34 (57.6%) were male. By age groups, 61% were school children (six to eleven years old) and 39% were adolescents (12 to 18 years old). According to the Gross Motor Function Classification System (GMFCS), in the total population, 6.8% belong to level III, 20.3% to level IV and 72.9% to level V. Most of the participants with low and normal BMD received anticonvulsants (98% and 90%, respectively).

Biochemical and hormonal indicators according to BMD. The nutritional status of vitamin D was deficient in 27.1% of children with low BMD and 10% of children with normal BMD, while it was insufficient in 35.4% of children with low BMD and 30% in children with normal BMD. Serum calcium and phosphorus concentration were normal in most children with normal and low BMD; only 14.9% of children with low BMD presented hypocalcemia and 10.6% had low serum concentrations of phosphorus. In children with low BMD, 11.4% had elevated PTH concentrations while 4.5% had low PTH concentrations; 13% of children with low BMD had elevated concentrations of T_4 . In contrast, 11.1% of children with normal BMD had low concentrations of T_4 . Serum T_3 concentrations were elevated in all the children with low BMD and normal in all the children with normal BMD. Elevated TSH serum concentrations were found in children with low and normal BMD (Table I).

The serum calcium concentration was lower in children with low BMD vs children with normal BMD ($p = 0.02$). Similarly, there was a trend towards lower concentrations of phosphorus and alkaline phosphatase in children with low BMD ($p < 0.1$) (Table II).

Biochemical and hormonal indicators and malnutrition. According to the BMI, 43% of the total sample had malnutrition and 57% had normal nutritional status. Similarly, using the W/A index, 48% of the children had malnutrition and 52% presented a normal nutritional status. Children with malnutrition, according to BMI, had significantly lower serum concentrations of calcium, phosphorus, and alkaline phosphatase vs non-malnourished children. With the W/A index, the serum concentrations of phosphorus and alkaline phosphatase were significantly lower in children with malnutrition (Table III).

The odds of alterations of biochemical and hormonal indicators according to sex, age group, motor impairment, use of anticonvulsants, feeding method and nutritional status are described in table IV.

No significant differences in the biochemical and hormonal indicators by sex, GMFCS, use of anticonvulsants, type of therapy and feeding methods were found. Nevertheless, adolescents had lower serum concentration of phosphorus than school children ($p = 0.004$). According to the GMFCS, 62% children from level V and 44% of children from levels III and IV had lower concentrations than the average of 25OHD metabolite. Males were more likely to have high-

Table I. Serum concentrations of calcium, vitamin D, alkaline phosphatase, phosphorus, PTH and thyroid hormones in participants with quadriplegic CP according to BMD

Variable	Low BMD		Normal BMD	
	n	%	n	%
<i>Vitamin D status (nmol/l) (n = 48)</i>				
Deficiency	13	27.1	1	10.0
Insufficiency	17	35.4	3	30.0
Sufficiency	13	27.1	4	40.0
Optimal	5	10.4	2	20.0
<i>Calcium (mg/dl) (n = 47)</i>				
Low	7	14.9	0	0.0
Normal	40	85.1	10	100.0
High	0	0	0	0.0
<i>Alkaline phosphatase (U/l) (n = 47)</i>				
Low	3	6.4	0	0.0
Normal	41	87.2	10	100
High	3	6.4	0	0.0
<i>Phosphorus (mg/dl) (n = 47)</i>				
Low	5	10.6	0	0.0
Normal	40	85.1	10	100
High	2	4.3	0	0.0
<i>PTH (pg/ml) (n = 44)</i>				
Low	2	4.5	0	0.0
Normal	37	84.1	10	100
High	5	11.4	0	0.0
<i>T₃ (ng/ml) (n = 46)</i>				
Low	0	0	0	0.0
Normal	0	0	9	100
High	46	100	0	0.0
<i>T₄ (µg/ml) (n = 46)</i>				
Low	1	2.2	1	11.1
Normal	39	84.8	8	88.9
High	6	13.0	0	0.0
<i>TSH (uIU/ml) (n = 42)</i>				
Low	0	0	0	0.0
Normal	25	59.5	5	62.5
High	17	40.5	3	37.5

¹BMD: bone mineral density; PTH: parathyroid hormone; T₃: triiodothyronine; T₄: thyroxine; TSH: thyroid-stimulating hormone.

Table II. Serum concentrations between participants with low BMD vs normal BMD

Biochemical variables	Low BMD			Normal BMD			p
	n	Mean	SD	n	Mean	SD	
Calcium (mg/dl)	47	9.15	0.45	10	9.51	0.36	0.020
Phosphorus (mg/dl)	47	4.30	0.84	10	4.58	0.25	0.063
Alkaline phosphatase (U/l)	47	151	62	10	188	60	0.088
PTH (pg/ml)	44	28.4	18.7	10	23.0	8.1	0.162
Vitamin D (nmol/l)	48	47.0	29.8	10	55.1	20.8	0.419
T ₃ (ng/ml)	46	1.29	0.40	9	1.21	0.26	0.573
TSH (uIU/ml)	42	3.36	2.29	8	2.97	1.87	0.657
T ₄ (µg/ml)	46	8.21	1.93	9	8.01	2.05	0.789

Statistics: unpaired Student's t-test. BMD: bone mineral density; PTH: parathyroid hormone; T₃: triiodothyronine; TSH: thyroid-stimulating hormone; T₄: thyroxin.

Table III. Serum concentrations of biochemical indicators in participants with quadriplegic CP according to nutritional status

Biochemical variables	Malnourished			Non-malnourished			p
	n	Mean	SD	n	Mean	SD	
BMI (kg/m²)							
Calcium (mg/dl)	24	9.00	0.48	33	9.36	0.37	0.003
Phosphorus (mg/dl)	24	4.09	0.87	33	4.54	0.65	0.030
Alkaline phosphatase (U/l)	24	138	51	33	171	67	0.050
PTH (pg/ml)	22	28.1	18.2	32	26.9	16.9	0.804
Vitamin D (nmol/l)	25	44.1	30.9	33	51.6	26.4	0.326
T ₃ (ng/ml)	23	1.25	0.36	32	1.29	0.40	0.722
TSH (uIU/ml)	22	3.44	2.14	28	3.18	2.31	0.689
T ₄ (µg/ml)	23	8.42	1.47	32	8.00	2.22	0.427
Weight/age (percentiles)							
Calcium (mg/dl)	27	9.18	0.54	30	9.24	0.37	0.656
Phosphorus (mg/dl)	27	4.08	0.84	30	4.60	0.63	0.010
Alkaline phosphatase (U/l)	27	132	47	30	180	67	0.003
PTH (pg/ml)	25	26.1	18.0	29	28.6	16.9	0.597
Vitamin D (nmol/l)	28	45.5	29.5	30	51.1	27.6	0.458
T ₃ (ng/ml)	26	1.30	0.46	29	1.25	0.30	0.584
TSH (uIU/ml)	24	3.44	1.98	26	3.17	2.45	0.672
T ₄ (µg/ml)	26	8.33	2.17	29	8.04	1.72	0.581

Statistic: unpaired Student's t-test. Malnourished: percentile < 10th. Non-malnourished percentile 10-90th. PTH: parathyroid hormone; T₃: triiodothyronine; TSH: thyroid-stimulating hormone; T₄: thyroxin.

er than the average serum concentrations of alkaline phosphatase. By age group, the likelihood of lower than the average of serum phosphorus concentrations was higher in adolescents than in school children. With the W/A index, malnourished children were found to have a higher likelihood of lower than the average serum phosphorus concentrations. In contrast, children without malnutrition were more likely to have higher than the average serum alkaline phosphatase concentrations. With BMI, children with malnutrition were more likely to have lower than the average of serum calcium concentrations.

CORRELATIONS BETWEEN BMD AND BIOCHEMICAL INDICATORS

The association between biochemical and hormonal indicators was analyzed and there were no significant associations. There were significant correlations between BMD (g/cm²) and serum phosphorus concentrations (r = 0.310, R² = 0.096, p = 0.019) and alkaline phosphatase (r = 0.327, R² = 0.107, p = 0.013). The linear regression between BMD (g/cm²) and TSH was inverse

Table IV. Serum concentrations of biochemical indicators by sex, age group, use of anticonvulsants and nutritional status: probability of higher or lower values than average

Indicator	Sex						
	Males		Females		OR	CI (95%)	p
	n/N	%	n/N	%			
ALP (U/l) (> 157)	18/33	54.5	6/24	25	3.6	1.14, 11.4	0.027
Indicator	Age group						
	Adolescents		School-aged children		OR	CI (95%)	p
	n/N	%	n/N	%			
Phosphorus (mg/dl) (< 4.35)	14/22	63.6	11/35	31.4	3.8	1.2, 11.8	0.018
Indicator	Use of anticonvulsants						
	Poly-therapy		Monotherapy		OR	CI (95%)	p
	n/N	%	n/N	%			
T ₄ (µg/ml) (< 8.17)	18/28	64.3	11/27	40.7	2.6	0.88, 7.8	0.083
Indicator	Nutritional status (weight/age)						
	Malnourished		Non-malnourished		OR	CI (95%)	p
	n/N	%	n/N	%			
Phosphorus (mg/dl) (< 4.35)	16/27	59.3	9/30	30	3.39	1.13, 10.1	0.028
ALP (U/l) (> 157)	4/27	14.8	20/30	66.7	11.5	3.1, 42.4	< 0.001
Indicator	Nutritional status (BMI)						
	Malnourished		Non-malnourished		OR	CI (95%)	p
	n/N	%	n/N	%			
Phosphorus (mg/dl) (< 4.35)	11/33	33	14/24	58.3	0.35	0.12, 1.1	0.06
Vitamin D (nmol/l) (< 48.4)	17/25	68	16/33	48.5	2.3	0.8, 6.7	0.14
Calcium (mg/dl) (< 9.21)	16/24	66.7	11/33	33	4.0	1.3, 12.2	0.013
ALP (U/l) (> 157)	7/24	29.2	17/33	51.5	2.6	0.8, 7.9	0.09

ALP: alkaline phosphatase; T₄: thyroxin. Almost all biochemical indicators had serum concentrations lower than average among different variables. ALP serum concentration was higher than average among variables as sex, weight/age and BMI.

($r = -0.316$, $R^2 = 0.099$, $p = 0.030$). There was a direct and potentially significant correlation between the serum concentrations of vitamin D and BMD ($p = 0.056$). No correlations were observed between BMD (g/cm²) and calcium, T₃, T₄ and PTH. The linear regressions between BMD expressed in Z-score and the biochemical indicators showed some different findings; 22% of the variance in BMD was explained by phosphorus ($p < 0.001$), 20% by alkaline phosphatase ($p < 0.001$) and 8% by calcium ($p = 0.036$) (Fig. 1A-C), respectively. No correlations were observed between BMD (Z-score) with vitamin D, T₃, T₄, TSH, and PTH.

MULTIPLE LINEAR REGRESSIONS WITH BMD AS DEPENDENT VARIABLE

Four multiple regression models were performed with BMD expressed in g/cm² and in Z-score as dependent variable. The first multiple regression model with BMD (g/cm²), with the stepwise method, accepted the variables phosphorus concentration, alka-

line phosphatase and TSH, which explained 17% of the variability in BMD. The second model accepted the variables weight and serum phosphorus concentration, which explained 54% of the variability in BMD. Contrary, when Z-score was used, the variables phosphorus and alkaline phosphatase explained 28% of the variability in BMD and the last model accepted the variables BMI, age and serum phosphorus concentration, which explained 41% of the variability (Table V).

DISCUSSION

Children with CP have been found to have decreased BMD due to various factors, such as the degree of motor impairment, malnutrition, the use of anticonvulsants, and feeding problems (2,3). In the present study, it was observed that 8.5% of the participants had osteoporosis (BMD < -2 Z-score + history of fractures) and 74.6% had low BMD for age (< -2 SD). This finding is consistent with other studies that have shown a similar frequency of low

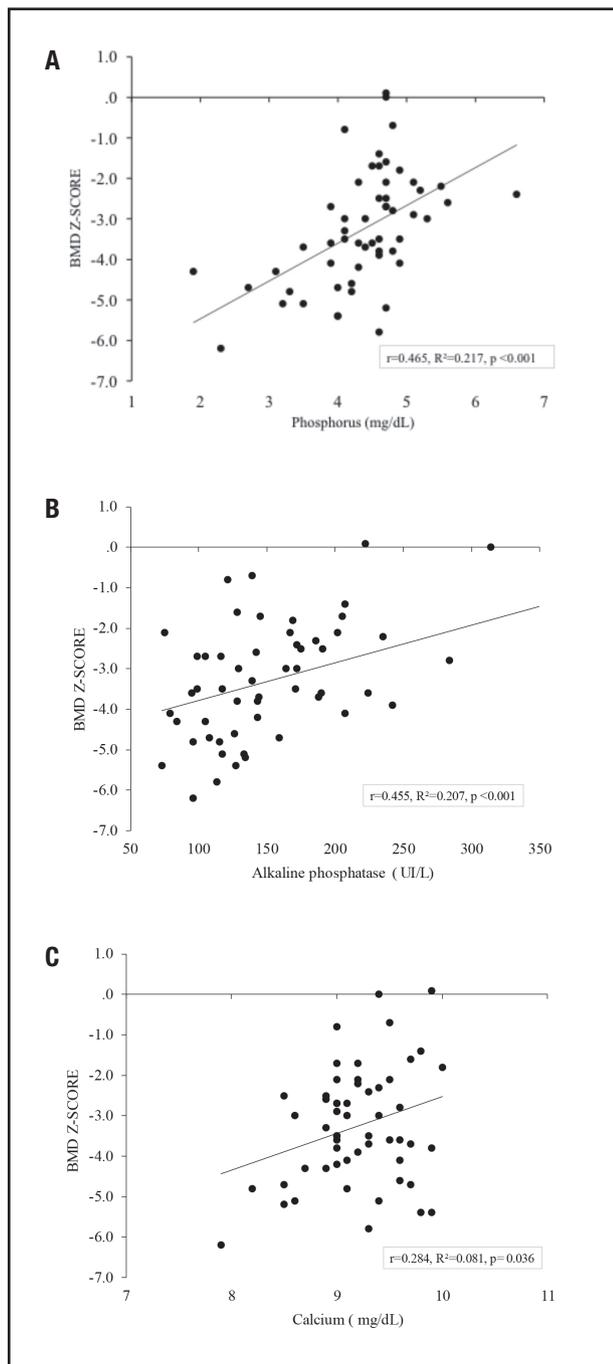


Figure 1.

Relationship between BMD (Z-score) and phosphorus, alkaline phosphatase, and calcium concentrations.

BMD in these patients. Tosun (13) reported that 63% of children with motor impairment had low BMD; likewise, Jacob (20) and Coppola (21) found that 80.7% and 70.2% of their participants had low BMD, respectively. Children with CP and acute malnutrition (BMI < 10th percentile) showed lower serum concentrations of calcium, phosphorus, and alkaline phosphatase. With the W/A index (composite indicator whose deficit reflects a chronic and

acute malnutrition), lower phosphorus and alkaline phosphatase serum concentrations were also observed. The probability of serum concentrations lower than the average of phosphorus, calcium and alkaline phosphatase was higher in malnourished *versus* non-malnourished children. It is known that malnourished children, especially those with severe grade malnutrition, have hypocalcemia, probably because the total calcium concentration must be adjusted to the concentration of albumin (22). In addition, it is common for the serum concentration of alkaline phosphatase to be decreased in these patients (23).

Adolescents had lower serum phosphorus concentrations than school children. This finding may be explained because at puberty the BMD increases significantly and reaches its maximum peak of calcium accumulation in the bone (40 to 60%) (24). It has also been observed that the serum concentration of phosphorus is higher in school children and progressively decreases at the concentrations observed in adults during the late phase of puberty (25). There were no significant differences in the serum concentrations of calcium, phosphorus, alkaline phosphatase, thyroid hormones, and PTH between the different GMFCS levels. Chen (9) found differences in serum calcium concentrations between children belonging to level I-II vs III vs IV-V of GMFCS ($p = 0.042$). Children belonging to levels IV and V had lower serum calcium concentration vs children belonging to level III. Finbraten (14) did not find differences in serum concentrations of calcium, phosphorus, alkaline phosphatase and PTH between children belonging to levels I-III vs IV-V of GMFCS.

The 25OHD concentration below the average was 62% in level V and 44% in levels III and IV. It is likely that children belonging to level V, with greater motor impairment, would have been less exposed to the sun's rays. In addition, they would have greater problems in their feeding; consequently, an insufficient intake of vitamin D (26). Most participants with low BMD and normal BMD had deficiency (27.1% and 10%) and insufficiency (35.4% and 30%), respectively, of vitamin D. It has been shown that vitamin D deficiency causes less absorption of dietary calcium and increases the secretion of PTH, which activates the renal hydroxylation of calcidiol and increases the renal reabsorption of calcium. In addition, this deficiency induces osteoclastic activity that could increase the risk of BMD loss (27). Henderson et al. (10) showed that 33% of children studied with CP have vitamin D deficiency. Fong et al. (28) and Shellhaas et al. (11) found a similar frequency of vitamin D deficiency (22% and 25%, respectively). Tosun et al. (13) observed that 33.3% and 26.7% of their patients with CP had vitamin D deficiency and insufficiency, respectively. These findings confirm that children with CP, regardless of BMD, are at potential risk of vitamin D deficiency and require periodic monitoring and eventual substitution treatment. The serum calcium concentrations were lower in children with CP with low BMD, and there was a tendency toward lower serum concentrations of phosphorus and alkaline phosphatase. It is known that calcium normal limits are narrow; therefore, when the serum calcium concentration is 1-2 mg/dl lower than normal, hypocalcemia symptoms occur. This situation is infrequent due to the great capacity of redistribution and exchange of calcium from bone to serum and

Table V. Multiple linear regressions with BMD expressed in g/cm² and Z-score as dependent variable and phosphorus, alkaline phosphatase, TSH, weight, age and BMI as independent variables

Variables		Mean	SD	r	R ²	p
BMD (g/cm²)						
Model 1*	Phosphorus (mg/dl)	4.4	0.8	0.310	0.096	0.019
	Alkaline phosphatase (U/l)	157	63	0.381	0.145	0.014
	TSH (uIU/ml)	3.3	2.2	0.416	0.173	0.031
Model 2 [†]	Weight (kg)	18	6	0.678	0.459	< 0.001
	Phosphorus (mg/dl)	4.4	0.8	0.732	0.536	0.002
BMD (Z-score)						
Model 1 [‡]	Phosphorus (mg/dl)	4.4	0.8	0.465	0.217	< 0.001
	Alkaline phosphatase (U/l)	157	63	0.529	0.280	0.033
Model 2 [§]	BMI (kg/m ²)	13.1	2.8	0.433	0.188	0.001
	Age (years)	10	3	0.584	0.342	0.001
	Phosphorus (mg/dl)	4.4	0.8	0.643	0.413	0.004

VIF: variance inflation factor. *VIF: 1.015; Durbin-Watson: 1.608. †VIF: 1.000; Durbin-Watson: 2.061. ‡VIF: 1.189; Durbin-Watson: 1.952. §VIF: 1.164; Durbin-Watson: 1.998.

vice versa (29). It is possible that this physiological phenomenon is less effective in children with CP and low BMD. Conversely, Esen et al. (12) reported that decreased serum concentrations of phosphorus, alkaline phosphatase and PTH were not associated with a decrease in BMD. Similarly, Akhter et al. (3) reported that there were no differences in BMD among children with lower serum calcium, phosphorus, vitamin D, and alkaline phosphatase concentrations *versus* those with normal serum concentrations.

The majority of children with CP with low BMD (98%) and normal BMD (90%) received anticonvulsants. It has been shown that anticonvulsants have a negative impact on BMD (30), probably due to the adverse effects that they produce on bone mineral metabolism. These anticonvulsant drugs can produce hypocalcemia, hypophosphatemia, elevated alkaline phosphatase concentration, decreased vitamin D concentration, alterations in thyroid profile, and increased PTH concentration (7,13,31). It should be noted that in the present study, no significant association was observed between these medications and BMD, probably because the majority of participants were receiving anticonvulsant treatment. In addition, these biochemical alterations were not observed in children who received antiepileptic drugs and in children who did not receive them. Similar findings have been reported in previous studies. Cheng et al. (4) reported that there were no significant differences in biochemical parameters of bone metabolism between children who received anticonvulsants *vs* children who did not. Similarly, Chen et al. (9) conclude that there were no significant differences in bone turnover or other related to biochemical indicators of bone metabolism between children with CP and healthy children.

Valproic acid was the most commonly used drug in all participants with low BMD (77.3%) and normal BMD (83.3%). This drug is widely used in the pediatric population and causes metabolic

acidosis, which damages the bone matrix and causes tubular renal dysfunction with greater urinary loss of calcium and phosphorus. Consequently, it could affect BMD (30,32).

The serum concentration of phosphorus and alkaline phosphatase explained 9.6% and 10% of the variability in bone mineral density (g/cm²), respectively, while the inverse relationship of TSH with BMD explained 9.9% of its variability. This finding could be explained by the fact that TSH inhibits the differentiation and function of osteoclasts by mechanisms independent of T₃ and suppresses the activity of osteoblasts (6). Chen et al. (9) and Sharawat et al. (33) did not find a correlation between BMD and serum calcium, phosphorus and alkaline phosphatase concentrations. However, in the present study, 21% of the variability in BMD (Z-score) was explained by the serum concentration of phosphorus ($p < 0.001$), 20% by alkaline phosphatase ($p < 0.001$) and 8% by calcium ($p = 0.036$).

There was a clear trend in the correlation between the serum concentration of vitamin D and BMD ($p = 0.056$). Vitamin D, through its active metabolite 1,25(OH)₂D₃, exerts a double action at the bone level, mobilizes calcium and phosphorus toward the extracellular fluid to maintain an adequate serum concentration of these inorganic nutrients, normalizes the blood calcium and favors the mineral deposit in the bone (17). However, several authors have not found this correlation between BMD and serum vitamin D concentrations (10,12-14,33).

We observed that the BMD in Z-score would be more oriented to the variables related to changes in BMD. This conclusion is based on the multivariate models designed in which it was observed that, with BMD in Z-score instead of g/cm², the variables BMI, age and serum phosphorus explained 41% of the variability. On the other hand, with the BMD in g/cm², only weight and serum phosphorus were included in the model that explains 54% of the variability.

A limitation of the study was due to the cross-sectional study design because we could not establish causality between exposure and effect. Another limitation was related to the sample size; in the analysis of some variables, a type II error was probably made. For example, in the association of greater serum concentration of phosphorus and alkaline phosphatase in non-malnourished children, in the association between malnutrition and lower serum concentration of vitamin D, in the association of gross motor function with lower serum vitamin D concentration, and in the association of polytherapy with below average serum concentrations of T_4 . In all cases, by duplicating the values of each cell of the contingency table, the probability improved significantly. Another limitation was that BMD was only measured in the lumbar spine. However, the measurement of BMD in the lumbar spine is valid (34) and has been used in different studies (10,12,20,21). The last limitation was that we did not measure serum concentrations of biomarkers of bone resorption because it was not feasible for the laboratory of the hospital to do it.

The main strength of the study was the demonstration of the association between biochemical indicators and hormonal biomarkers of bone metabolism and BMD in pediatric patients with quadriplegic CP in the Mexican population.

In conclusion, the present study demonstrated the direct correlation of BMD with serum concentrations of calcium, phosphorus, alkaline phosphatase, vitamin D and the inverse correlation with TSH in children with quadriplegic CP. BMD in Z-score would be more oriented to the variables related to changes in BMD, the variables BMI, age and serum phosphorus explained 41% of the variability.

The association of malnutrition, use of anticonvulsants and gross motor function impairment with biochemical indicators and hormonal biomarkers of bone metabolism was shown. Therefore, these variables should be monitored and controlled. For example, malnutrition may be prevented assuring a correct diet, an adequate use of anticonvulsants to prevent alteration of bone metabolism and optimized physical rehabilitation strategies to improve gross motor function. In this way, it would be possible to optimize the bone mineralization that could prevent the risk of osteoporosis and fractures in children with quadriplegic CP.

Further longitudinal studies are required to provide information about the association of BMD (measured at different skeletal regions) with biochemical and hormonal indicators of bone metabolism, as well as clinical assays to evaluate the effect of an integral treatment on those variables affecting the BMD in children with CP in medium and long terms.

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