

Serum 25-hydroxyvitamin D and biochemical markers of bone metabolism in patients with juvenile idiopathic arthritis

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Abstract

Our objective was to evaluate the concentrations of serum 25-hydroxyvitamin D [25(OH)D], serum calcium, serum phosphorus, alkaline phosphatase, and parathormone (PTH) in patients with polyarticular juvenile idiopathic arthritis (JIA) and to associate them with disease duration and activity, bone mineral density and use of medications. In a cross-sectional and controlled study, 30 patients with polyarticular JIA were evaluated and compared to 30 healthy individuals matched for age and gender. Clinical status, anthropometry, laboratory markers in both patients and controls, and bone mineral density, only in the patients, were measured. Of the 30 patients included in the study, 23 (76.7%) were female and 16 (53.3%) non-Caucasian; mean age was 14 years (range = 4 to 20 years). Mean disease duration was 5 years (range = 1 to 12 years). The mean concentrations of serum albumin-corrected calcium (9.04 ± 0.41 mg/dL) and alkaline phosphatase (153.3 ± 100.1 IU) were significantly lower in patients with JIA than in controls ($P < 0.0001$ and $P = 0.001$, respectively). No differences in 25(OH)D, PTH or serum phosphorus were observed between JIA and control subjects. Regarding 25(OH)D concentration, 8 patients (26.7%) and 5 controls (16.7%) had 25(OH)D concentrations compatible with deficiency (lower than 20 ng/mL) and 14 patients (46.7%) and 18 controls (60%) had concentrations compatible with insufficiency (20-32 ng/mL). These values were not associated with disease activity, use of medications or bone mineral density. We observed a high frequency of 25(OH)D insufficiency and deficiency in the study sample. The compromised bone metabolism emphasizes the importance of follow-up of JIA patients.

Key words: Juvenile idiopathic arthritis; Serum vitamin D deficiency; Calcium; Alkaline phosphatase

Introduction

Juvenile idiopathic arthritis (JIA) is the most common chronic rheumatic disease of childhood in developed countries and an important cause of short- and long-term disability.

The effects of vitamin D have been studied in experimental models of autoimmunity. In humans, there are some difficulties in establishing a direct relationship between vitamin D deficiency and an increase in the frequency of rheumatic diseases, considering the limitations of available studies, such as different cut-offs used and small subject samples (1).

Although intake is not the best method for the assessment of the nutritional status of vitamin D, a higher intake of this vitamin was found to be inversely related to the development of rheumatoid arthritis in a cohort study of 29,368 women (2). In patients with rheumatoid arthritis, available results suggest a high frequency of vitamin D

insufficiency or deficiency (3-9) and show that vitamin D supplementation in subjects with active disease may relieve the symptoms and reduce inflammation (10).

Studies performed in healthy children and adolescents have shown lower serum concentrations of 25-hydroxyvitamin D [25(OH)D] at frequencies ranging from 9 to 62% (11-13). However, only a few studies have assessed the serum concentrations of 25(OH)D in children and adolescents with JIA (14-17).

The aim of the present study was to determine the serum concentrations of 25(OH)D, serum calcium, serum phosphorus, alkaline phosphatase, and parathormone (PTH) in patients with polyarticular JIA and to associate them or not with disease activity and duration, bone mineral density and use of medications (glucocorticoids and hydroxychloroquine) and supplements containing vitamin D and calcium.

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Patients and Methods

In a cross-sectional and controlled study, 30 patients of a total of 50 patients with polyarticular JIA were evaluated according to the criteria of the International League of Associations for Rheumatology (ILAR) (18). The patients were followed at the Pediatric Rheumatology Clinic from September to November 2008. Twenty patients were excluded due either to other chronic diseases interfering with the calcium metabolism, or because they could not perform bone density measurements due to their stature or they did not consent to participate in the study.

Thirty healthy individuals, matched for gender and age to the patients, not using supplements containing vitamin D or calcium were included as controls.

The demographic, clinical and treatment data obtained with a standardized questionnaire were collected from the patients' charts. To assess if the disease was active, we used the remission criteria described in previous papers (19,20).

The anthropometric assessment included weight and height measurements using as reference those proposed by the World Health Organization (WHO) (21-23). Pubertal development was determined as recommended by Marshall and Tanner (24).

Blood samples were collected from patients and controls after a 12-h fast for the following laboratory tests: serum phosphorus and serum albumin-corrected calcium by a colorimetric method with reactant molibdate and arsenazo, respectively, and alkaline phosphatase measured by an enzyme activity method with reactant p-nitrophenyl phosphate (Biochemical Analyzer Olympus AU640, USA). Urinary calcium and creatinine were determined in the patients by a colorimetric method using a specified apparatus. All biochemical analyses of patients and controls were performed from September to November 2008, corresponding to the Brazilian spring months. Since there is seasonal variability in 25(OH)D, we opted, as done in other studies, to collect the samples in the spring.

Serum 25(OH)D was determined in 2009 by electrochemiluminescence immunoassay using the commercial kit Elecsys 25(OH)D3 (USA) and serum intact PTH was determined by immunofluorimetry using the same kit. Reference 25(OH)D values below 20 ng/mL indicate deficiency, values between 20 and 32 ng/mL indicate insufficiency, and values above 32 ng/mL are considered to be ideal (25). The normal range for PTH is 15 to 65 pg/mL.

The bone mineral density of the spine (L1-L4) was measured by dual-energy X-ray absorptiometry (DXA) using a Lunar™ DPX-MD plus densitometer (GE-Lunar Radiation Corporation, USA) equipped with pediatric software (version 8.5). Bone mineral density was adjusted for height by the method of Khan et al. (26).

All statistical calculations were done using the statistical software SPSS 18.0. The chi-square test or, when indicated, the Fisher test was applied to evaluate the association between dichotomous variables. The normality of continuous variables was established by the Kolmogorov-Smirnov test and is reported as means \pm SD. The Student *t*-test was used to compare groups regarding the variables under study. Pearson's correlation coefficient was used for the quantitative variables with normal distribution. An $\alpha < 5\%$ was adopted ($P < 0.05$).

An informed consent form was signed by all patients and controls and/or their legal guardians. The Ethics Committee of the Universidade Federal de São Paulo, Hospital São Paulo (CEP No. 0178/09) approved the study protocol.

Results

Table 1 shows the characteristics of the study group. In the JIA group, the mean age at diagnosis was 9 years (3 to 16 years) and the mean disease duration was 5 years (1 to 12 years); 12/30 (40%) patients had active disease at the time of evaluation. Six (20%) patients had positive rheumatoid factor.

No differences in serum 25(OH)D, PTH or phosphorus concentrations were observed between the JIA and control groups (Table 2). The mean concentrations of serum albumin-corrected calcium and alkaline phosphatase

Table 1. Demographic characteristics, nutritional status, and pubertal stage of patients with juvenile idiopathic arthritis.

Variables	JIA	Controls
Females	23 (76.7)	23 (76.7)
Age at evaluation (range)	14 (4-20)	14 (4-19)
Ethnic origin		
Caucasian	14 (46.7)	19 (63.3)
Non-Caucasian	16 (53.3)	11 (36.7)
BMI Z score		
Eutrophic	20 (66.7)	30 (100)
Non-eutrophic	10 (33.3)	0
Malnutrition	5 (16.6)	0
Overweight	3 (10.0)	0
Obesity	2 (6.7)*	0
S/A Z score		
Low height	5 (17.9)	0
Adequate height	23 (82.1)	28 (100) ^a
Pubertal stage		
Prepubertal stage	6 (20.0)	6 (20.0)
Pubertal stage	24 (80.0)	24 (80.0)

Data are reported as number with percent in parentheses for 30 patients in each group. JIA = juvenile idiopathic arthritis; BMI = body mass index; S/A = stature for age. * $P < 0.05$ compared to controls (chi-square test). ^aTwo patients were older than 19 years.

Table 2. Serum concentrations of 25(OH)D, PTH, calcium, phosphorus, and alkaline phosphatase of patients with juvenile idiopathic arthritis.

	JIA	Controls
25(OH)D (ng/mL)	25.70 ± 8.65	26.92 ± 7.62
PTH (pg/mL)	31.21 ± 12.35	30.04 ± 15.63
Albumin-corrected calcium (mg/dL)	9.04 ± 0.41*	9.48 ± 0.48
Serum phosphorus (mg/dL)	4.45 ± 0.61	4.17 ± 0.86
Alkaline phosphatase (IU)	153.3 ± 100.1*	285.2 ± 169.9

Data are reported as means ± SD for 30 patients in each group. JIA = juvenile idiopathic arthritis; 25(OH)D = 25-hydroxyvitamin D; PTH = parathormone. *P < 0.05 compared to control group (Student *t*-test).

tase were significantly lower in the JIA patients than in the controls (Table 2). All but 2 patients had calciuria/creatinuria ratios within the normal range.

Regarding serum 25(OH)D concentrations, 8 patients (26.7%) and 5 controls (16.7%) had values compatible with deficiency (<20 ng/mL) and 14 patients (46.7%) and 18 controls (60%) had values compatible with insufficiency (20-32 ng/mL). Three patients with deficiency and 6 with insufficiency of 25(OH)D had active disease at the time of evaluation.

Only 1 patient (3.3%) and 1 control (3.3%) had elevated PTH levels (>65 pg/mL), with 25(OH)D concentrations of 24.4 and 39.9 ng/mL, respectively.

25(OH)D concentrations were not different in relation to ethnicity ($P = 0.896$), Z body mass index ($P = 0.111$), Z stature/age ($P = 0.136$), or pubertal stage ($P = 0.398$).

In the JIA group, 3/30 (10%) patients were taking chloroquine, 11/30 (36.7%) were taking a calcium supplement and 6/30 (20%) were taking vitamin D. No patients were on glucocorticoids and/or biphosphonate at the time of evaluation.

There was no association between 25(OH)D concentrations and disease activity ($P = 0.737$), use of vitamin D supplements ($P = 0.124$), use of chloroquine ($P = 0.661$) or bone mineral density ($P = 0.350$) in the JIA patients (Student *t*-test).

Three patients had low bone mass density for age; of those, 1 had insufficient 25(OH)D concentration and 1 had deficiency. Nine patients had osteopenia; of those, 5 had 25(OH)D insufficiency and 2 had deficiency. One patient did not perform the DXA. We did not find any correlations between low bone mass and disease duration or corticotherapy.

Neither the laboratory tests nor the disease duration correlated with serum 25(OH)D concentrations: PTH ($r = -0.020$, $P = 0.877$); serum albumin-corrected calcium ($r = 0.057$, $P = 0.765$); serum phosphorus ($r = -0.030$, $P = 0.818$); alkaline phosphatase ($r = 0.109$, $P = 0.408$); calciuria/creatinuria ratio ($r = -0.155$, $P = 0.414$);

disease duration ($r = 0.079$, $P = 0.680$).

Discussion

In this study involving patients with JIA, we found a high frequency of 25(OH)D insufficiency and deficiency; however, these findings did not differ significantly between patients and controls. Hillman et al. (14), also assessing serum concentrations of 25(OH)D, did not report any difference between JIA patients and controls. Potential explanations for the high frequency of deficiency/insufficiency in both groups may be the frequent use of sunblocks (27), dietary and life style changes with consequent adiposity and overweight (28) and season when the analyses were performed (29,30).

It is important to emphasize that vitamin D deficiency is a current public health issue that has been increasing, including in healthy individuals of all ages in developed and developing countries (11-13). These findings recently motivated a revision of the recommendations for vitamin D (31).

There is no consensus regarding the ideal serum concentrations of 25(OH)D, especially in the pediatric group, which makes it difficult to compare the results from different studies. In the present study, we decided to use the cut-offs suggested by Grant and Holick (25) as recommended in the revision by the Institute of Medicine (31).

Another important aspect is the kind of assay used for 25(OH)D analysis, which may lead to a false diagnosis of vitamin D deficiency. The electrochemiluminescence immunoassay with the Elecsys Roche's kit for polyclonal immunoassay, used in the present study, showed in previous studies a good correlation with the method considered as gold standard, i.e., liquid chromatography mass spectrometry (32,33).

An inverse association between PTH and 25(OH)D concentrations has been demonstrated in adults and the persistent increase in PTH levels may result in hyperparathyroidism secondary to the vitamin deficiency (11,12,29). In children, however, this association has not been frequently observed, a fact that limits the use of PTH as a functional marker for vitamin D deficiency (34). We found only one patient (3.3%) with elevated PTH in spite of the high frequency of vitamin D insufficiency/deficiency.

Our study showed lower levels of serum albumin-corrected calcium and alkaline phosphatase in patients with JIA compared to controls. Those alterations do not seem to be of clinical significance. A previous study showed significantly lower osteocalcin and alkaline phosphatase concentrations in individuals with JIA compared to controls, suggesting reduced bone formation (14). Lower calcium concentrations in the JIA group, with no difference in 25(OH)D concentrations may be explained by the reduced capacity for mineral absorption described in children with arthritis (17). A study in animal

models with induced arthritis revealed a reduction in 1,25(OH)₂D receptors, with a consequent reduction in calcium absorption (35).

We did not find an association between 25(OH)D concentrations with either disease activity or duration. In adults with rheumatoid arthritis there are multiple risk factors for vitamin D deficiency and there is evidence that the deficiency is associated with the activity (4,7) and severity of the disease (5,7-9).

Pelajo et al. (36) found a similar frequency of reduced vitamin D in their study with 154 JIA patients, which was also not related to disease activity. They observed an association between 25(OH)D concentrations and age, ethnicity, body mass index, and season of the year, but not with vitamin D intake.

A recent publication evaluating the vitamin D binding protein isoforms showed that some subgroups are related to the extension of arthritis (37).

The use of supplements containing vitamin D was not associated with serum 25(OH)D concentrations, with low values even in patients receiving supplementation. There is still no consensus on the ideal supplement dose of vitamin D for patients with JIA. Hillman et al. (17) observed an increase in serum calcium and 25(OH)D concentrations, with no impact on bone mineral gain, with the administration of 2000 IU vitamin D₃/day in children with arthritis. In our clinic, vitamin D supplementation at a dose of 200 to 800 IU is recommended in association with 500 to 1000 mg calcium for patients with a risk of bone mass loss (active inflammatory disease and chronic use of glucocorticoids).

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Regarding the medications used for JIA treatment, only 3 patients were on hydroxychloroquine at the time of inclusion, with no interference with vitamin D concentrations. In the literature, the effect of hydroxychloroquine on vitamin D metabolism is still controversial (38).

DXA showed that three (10%) of the 30 patients had low bone mass density for age. A cross-sectional study in the literature evaluating bone mass in children and young adults with JIA showed lower bone mass in the patients compared to healthy individuals (39). A recently published prospective study involving patients with JIA showed reduced bone mass density at the initial evaluation compared to controls (40).

There are some limitations to the present study, namely the small sample size, the cross-sectional model used that does not allow causality evaluations, and no DXA in the control group. Furthermore, the method applied for the determination of 25(OH)D concentrations may be questioned, as well as our cut-off values.

We observed serum insufficient/deficient 25(OH)D concentrations in a large number of patients, although with no relation to disease activity or duration, use of medications and vitamin D supplementation or bone mineral density. These findings, associated with the impairment of bone metabolism observed in our patients even in the absence of alterations in bone mineral density, emphasize the importance of the follow-up of JIA patients.

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