An Examination of the Effects of Leuprolide Acetate Used in the Treatment of Central Precocious Puberty on Bone Mineral Density and 25-Hydroxy Vitamin D

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ABSTRACT

Aim: *Leuprolide acetate is a gonadotropin-releasing hormone (GnRH) analogue frequently used in the treatment of central precocious puberty. Research is currently taking place into its effects on endocrine systems. The aim of this study is to investigate the effect of leuprolide acetate on vitamin D and bone mineral density.*

Methods: *Twenty-three children diagnosed with central precocious puberty and receiving leuprolide acetate therapy for at least 12 months, and a control group of 17 healthy children were enrolled. In the study group, calcium, phosphorus, alkaline phosphatase, parathormone and 25-hydroxy vitamin D levels and bone mineral density were measured. The results were compared with those of the control group.*

Results: *25-Hydroxy vitamin D levels in the study and control groups were 15.17 ± 7 mg/dL and 22.2 ± 6.1mg/dL, respectively (*$p < 0.05$*). In terms of bone mineral density, osteopenia was determined in 13 (56.5%) patients in the study group and osteoporosis in one (4.3%), while osteopenia was identified in seven patients in the control group, with no osteoporosis being identified (*$p > 0.05$*).*

Conclusion: *Gonadotropin-releasing hormone agonists may have an adverse effect on bone health. They may exhibit these effects by impacting on vitamin D levels. These levels should be periodically monitored in patients receiving treatment, and vitamin D support should be given in cases where the deficiency is identified.*

Keywords: Bone mineral density, leuprolide acetate, vitamin D

Examen de los Efectos del Acetato de Leuprolide Usado en el Tratamiento de la Pubertad Precoz Central sobre la Densidad Mineral Ósea y la 25-hidroxivitamina D

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RESUMEN

Objetivo: *El acetato de leuprolide es un análogo de la hormona liberadora de la gonadotropina (GnRH) utilizado con frecuencia en el tratamiento de la pubertad precoz central. Actualmente se investigan sus efectos sobre el sistema endocrino. El objetivo de este estudio fue investigar el efecto del acetato de leuprolide sobre la vitamina D y la densidad mineral ósea.*

Métodos: *Veintitrés niños diagnosticados con pubertad precoz central, que recibieron terapia de acetato de leuprolide durante al menos 12 meses, y un grupo control de niños sanos 17, fueron los sujetos de la investigación. En el grupo de estudio, se midieron los niveles de calcio, fósforo, fosfatasa alcalina, parathormona, y 25-hidroxivitamina D, así como la densidad mineral ósea. Los resultados fueron comparados con los del grupo control.*

Resultados: *Los niveles de 25-hidroxivitamina D en los grupos de estudio y control fueron 15.17 ± 7 mg/dL y 22.2 ± 6.1 mg/dL, respectivamente (*$p < 0.05$*). En términos de la densidad mineral ósea, se de-*

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*terminó la existencia de osteopenia en 13 pacientes (56.5%) en el grupo de estudio, y osteoporosis en uno (4.3%), en tanto que se identificó osteopenia en siete pacientes en el grupo control, sin que se identificara osteoporosis (*p > *0.05).*

Conclusión: *Los agonistas de la hormona liberadora de la gonadotropina pueden tener efectos adversos sobre la salud ósea, por su impacto sobre los niveles de vitamina D. Estos niveles deben vigilarse periódicamente en los pacientes que reciben tratamiento, y debe apoyarse el suministro de vitamina D en los casos en que se detecte su deficiencia.*

Palabras claves: Densidad mineral ósea, acetato de leuprolide, vitamina D

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INTRODUCTION

Bone mass exhibits a progressive rise in childhood, particularly accelerating in adolescence. The time when peak bone mass is reached has not been fully determined, although it is thought to be largely completed toward the end of adolescence. Measures that can be taken to increase bone mass after the peak has been reached are limited (1). Much of the skeletal mineralization and epiphyseal maturation take place during puberty. Growth hormone, thyroid hormone and sex steroids (particularly oestrogen) have to be at sufficient levels for this to be completed normally (2).

In additional to gonadal steroids, vitamin D plays an important role in bone health in all ages. 25-hydroxy (25-OH) vitamin D level and bone mineral density measurement are techniques used to analyse bone health in children. The best-known of these techniques is dual-energy X-ray absorptiometry [DEXA] (2, 3). Gonadotropin-releasing hormone (GnRH) agonists are frequently used for gonadal suppression in order to prevent the development of early puberty in girls with central precocious puberty. Gonadotropin-releasing hormone agonist therapy is thought to be capable of affecting bone mineral density while lowering gonadotropin and oestrogen levels, and various data on the subject have been reported. Some studies have determined decreases in bone mineral density with suppression of puberty, while others have reported that the therapy has no effect on bone marrow density (4, 5).

There are very few studies examining the effect of long-term leuprolide acetate in central precocious puberty on 25-OH vitamin D levels and bone mineral density in children. This study investigated the probable effects of leuprolide acetate therapy on bone health in childhood using vitamin D levels and DEXA.

SUBJECTS AND METHODS

Twenty-three children with central puberty praecox (study group) under observation by the Regional Training and Research Hospital, Erzurum, Turkey, and 17 healthy children (control group) were enrolled in the study. Ethical Committee approval was obtained before the study began. Informed consent forms were received from the parents of all participants. Children were deemed eligible for inclusion when they were aged 5 to 12 years. Patients and healthy groups had the same parameters. The groups were gender and age-matched. The children in the control group were from the same geographical area, and they were admitted to the paediatric outpatient clinic for other reasons than systemic problems. Controls were similar to patients except for leuprolide acetate.

We viewed the records of all patients and looked at the following details: age of onset; how long drug used, laboratory parameters that included calcium (Ca), phophorus (P), alkaline phosphatase (ALP), parathormone (PTH) and 25-OH vitamin D. All patients in the study group were selected from those regularly attending check-ups and whose growth parameters were within normal limits. Patients with central puberty praecox received leuprolide acetate (Lucrin Depot 3.75 mg, Takeda Pharmaceutical Company, Ltd., Japan) replacement therapy for different periods at a dosage of 0.3 mg/kg.

The exclusion criteria were: use of any medications known to interfere with liver or renal and thyroid functions, thyroid, kidney or liver disease and endocrine disorders. Blood sample was obtained from patients at least 12 months after leuprolide acetate treatment was started between 8:00 and 10:00 am after 12-hour fasting to avoid diurnal variations.

All blood samples were stored at -80 ˚C until analysis. In accordance with laboratory reference values, normal serum values were determined at 8.8–10.8 mg/dL for Ca, 2.8–6.0 mg/dL for P and 75–400 U/L for ALP.

In terms of 25-OH vitamin D levels, 15 ng/mL and below was classified as severe deficiency, 15–20 ng/mL as deficiency and values of 20 ng/mL and above as normal (6). All the tests were performed according to the manufacturer's instructions. Serum Ca, P and ALP levels were determined in serum using a Roche Cobas 8000 System (Tokyo, Japan) using Roche Diagnostics kits. 25-Hydroxy vitamin D levels (ng/mL) were determined in an E-170 ECL system (Roche, Japan) with an electrochemiluminescence method. Parathormone (pg/mL) was measured by chemiluminescent enzyme immunoassay, IMMULITE (DPC Co, USA) autoanalyzer.

For all patients and controls, Z-score was measured by DEXA using the Hologic QDR 2000. The Z-score is the standard deviation of the individual bone mineral density (BMD) compared to the mean BMD score of a similar gender-, age-, weight- and height-matched population. According to World Health Organization (WHO) classification, Z-score > –1 was

considered normal, Z-score between –1 and –2.5 was considered osteopenia and Z-score = –2.5 was considered osteoporosis.

Statistical analysis

SPSS software package version 18 (SPSS Inc., Chicago, IL) was used for statistical analysis. Data are presented as means ± standard deviation. One-way analysis of variance and post-hoc least significant difference (LSD) option test were used to compare between cases and controls and between the different groups. Pearson Chi-square test and independent samples test were used to analyse multiple variants. Significance was declared at $p \leq 0.05$.

RESULTS

Mean age was 8.18 ± 1.48 years in the study group and 9.35 ± 1.65 years in the control group. Twenty (87%) of the study group were girls and three (13%) were boys, compared to 14 (82.4%) girls and three (17.6%) boys in the control group. The differences between the gender and mean age value in the study and control groups were not statistically significant ($p > 0.05$).

Mean length of leuprolide acetate use in the study group was 2.48 ± 1.08 months (min-max, 1–5 months, 95% confidence interval (CI); 2.01, 2.95). The difference between the vitamin D levels in the study and control groups was statistically significant (Table 1).

Table 1: Comparison of vitamin D levels in the study and control groups

Groups	Vitamin D				*P*-value
	Normal	Mild	Severe	Total	
	n (%)	n (%)	n (%)	n (%)	
Central precocious puberty	5 (21.7%)	5 (21.7%)	13 (56.5%)	23 (100%)	0.005*
Control	12 (70.6%)	3 (17.6%)	2 (11.8%)	21 (100%)	

The Pearson Chi-square test was applied. *$p \leq 0.05$ was regarded as significant

25-Hydroxy vitamin D levels in the study and control groups were 15.17 ± 7 mg/dL and 22.2 ± 6.1 mg/dL, respectively ($p < 0.05$). Mean PTH level in the study group was 43.5 ± 9.9 and 37 ± 10.6 in the control group. The difference was not statistically significant ($p > 0.05$). Study and control group mean Ca, P and ALP values were 9.1 ± 0.7 and 9.6 ± 0.6, 3.8 ± 0.8 and 4.8 ± 0.1, and 407 ± 104 and 229 ±112, respectively. In terms of Ca, there was no statistically significant difference between the groups ($p > 0.05$). The differences between the groups in terms of serum P and ALP were statistically significant ($p < 0.001$). The difference between the study and control group in terms of Z-scores was not statistically significant ($p > 0.05$) [Table 2]. $P \leq 0.05$ was regarded as significant.

Table 2: Comparison of the study and control groups in terms of Z-scores

Groups	Z-scores				*P*-value
	Normal	Osteopenia	Osteoporosis	Total	
	n (%)	n (%)	n (%)	n (%)	
Central precocious puberty	9 (39.1%)	13 (56.5%)	1 (4.3%)	27 (100%)	0.368
Control	10 (58.8%)	7 (41.2%)	0 0%	21 100%)	

The Pearson Chi-square test was applied. Z-scores > -1 were normal, while scores between -1 and -2.5 were regarded as osteopenia and scores < -2.5 as osteoporosis; $p \leq 0.05$ was regarded as significant

DISCUSSION

Several factors, such as diet, physical activity, race, sex hormones, growth hormone and insulin-like growth factor (IGF) affect bone mineralization and bone mineral density. Bone mineralization defects have been observed in patients with oestrogen receptor deficiency, untreated structural puberty and delayed growth. Gonadotropin-releasing hormone analogues reduce the release of sex hormones by affecting the production of the hypophyseal gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH). From that perspective, the question arises if whether interventions performed in puberty, when peak bone mass is achieved, have an adverse effect on bone health. Significant bone losses have been shown to take place in adult women after three to six months of GnRH agonist therapy. Additionally, this loss did not improve after cessation of treatment. The effect of these analogues, which are frequently used in the treatment of genuine precocious puberty, on bone health in children is the subject of debate (4, 6, 7).

While the incidence of osteopenia and osteoporosis in patients using leuprolide acetate in this study was greater than in the control group, the difference was not statistically significant. However, vitamin D levels were significantly lower in the patients using leuprolide acetate compared to the control group. Looked at from this perspective, bone mineralization that is likely to be impaired in association with vitamin D deficiency may be a risk factor for osteoporosis in later years, even if GnRH agonist therapy is stopped.

Heger *et al* (8) stated that the lumbar spine BMD of central precocious puberty patients treated with GnRH analogue was not significantly different from control subjects, but they noticed that 15% of their patients had osteopenia. Bertelloni *et al* (5) reported that it causes no change in BMD in female children with central precocious puberty receiving treatment. Studies have evaluated the effect of calcium support on GnRH therapy. These have reported that the adverse effects of GnRH agonist therapy on bone health can be prevented with Ca supplementation (9–11). According to these studies, the adverse effects on bone health of GnRH agonist therapy in children are lower in adults. The reason for this may be the low case numbers or the short treatment period involved. Vitamin D levels

in our study were lower than in the control group. Vitamin D may be a better marker of the adverse effects of leuprolide acetate on bone health than BMD. This suggests that vitamin D, in addition to leuprolide acetate therapy, may be beneficial.

A study performed with triptorelin or leuporelin showed that five of the 11 patients (45%) had their BMD below -1 SD (standard deviation), which was defined as osteopenia. It was explained that this reduced BMD is a possible major side effect that should be seriously considered during long-term GnRH analogue therapy (12). Yanovski *et al* (13) reported that 82% of patients treated with GnRH analogue had BMD more than 1 SD below the population. Unal *et al* (4) compared vertebral bone mass values of patients with central precocious puberty with healthy age and puberty matched controls to determine the effect of leuprolide acetate on bone mass in patients who had been treated for at least one year. In this study, BMD values of patients compared to the control group were normal. No significant change in BMD values was observed after treatment. Neither osteopenia nor osteoporosis was observed in patients taking leuprolide acetate. However, Boot *et al* (14) reported that mean ALP was decreased after six months. Mean 1,25 dihydroxyvitamin D level was decreased after 6–12–24 months. Serum calcium and phosphate were normal at baseline and did not change significantly during time. And mean lumbar spine BMD SD scores were significantly higher than zero at baseline and did not differ from normal, after two years of treatment. Mean spinal BMD SD scores and total body BMD SD scores were not significantly different from zero at baseline and had not changed significantly after two years of treatment. van der Sluis *et al* (15) also studied 47 children with central precocious puberty and 11 children with early puberty and reported that peak bone mass would not be impaired in patients with precocious puberty or early puberty after GnRH agonist therapy.

In adults with GnRH analogue therapy, an absolute decrease in BMD has been reported (16, 17); whereas, in children, the effects of GnRH analogue on bone system are a controversial issue. Absolute BMD increased (8, 18), equalled (4, 5) or decreased (10, 19) during GnRH analogue therapy in children.

In conclusion, the use of GnRH analogues should be revised considering the side effects on bone health that can develop in the long term. Apart from those cases in which therapy is definitely required, the advantages and disadvantages should be set out in uncertain cases, and therapy should be commenced once the family has been informed. Vitamin D levels should be reviewed in patients receiving therapy and vitamin D added to treatment in those cases where it is required.

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