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Effects of isotretinoin on bone turnover markers and bone mineral density in women with acne vulgaris and vitamin D deficiency: a preliminary study.

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Abstract

The prolonged use of retinoids is associated with changes in bone turnover markers and toxic skeletal effects. Although the effect of short-term oral isotretinoin therapy on bone loss is not well established, caution is recommended when it is used in patients with metabolic bone disease. We examined prospectively the effect of short-term oral isotretinoin therapy on bone turnover markers and bone mineral density (BMD) in women affected by severe acne and vitamin D deficiency. Serum bone Tartrate-resistant Acid Phosphatase (TRACP), bone specific alkaline phosphatase (Bone ALP), calcium, phosphorus, parathyroid hormone (PTH) and 25-hydroxyvitamin D [25(OH)D] were measured in 10 women at baseline, 6 weeks, and end of isotretinoin treatment. BMD was measured in 5 subjects at baseline and end of treatment. Mean serum 25(OH)D at baseline was 16.3 ± 7.5 nmol/L. Mean TRACP and Bone ALP increased at end of treatment but this was only statistically significant for TRACP (1.18, 1.13, 1.64 U/L; P<0.001). Mean calcium decreased slightly at end of treatment but no significant changes occurred in PTH, 25(OH)D and phosphorus. BMD decreased in all studied patients at the femur (range -2.5 to -7.6%), and in all but one patient at the lumbar spine (range +3.2 to -6.8%). Mean BMD decreased at all measured sites but this was statistically significant only for the femur (-5.3 ± 1.9%; P=0.002). Our preliminary study suggests that short-term oral isotretinoin therapy in women with vitamin D deficiency is likely associated with increased bone resorption and decreased BMD. Correction of vitamin D deficiency may be necessary before starting isotretinoin therapy.

Key words*: isotretinoin; acne vulgaris; vitamin D deficiency; bone turnover markers; bone mineral density*

Introduction

The prolonged use of retinoids has been reported to be associated with changes of bone biochemical markers and toxic skeletal effects.[1-5] Animal studies show that excess vitamin A increases bone resorption and number and size of osteoclasts, and results in a decrease in osteoid surface and deterioration of cartilage.[6,7] Hypervitaminosis A may also promote the development of osteoporosis and hip fractures in humans.[8-11] Isotretinoin, a synthetic 13-cis-retinoic acid, is highly effective in the treatment of severe acne vulgaris. Spontaneous reports of osteoporosis, bone fractures and delayed healing of fractures have been reported in subjects treated with isotretinoin.[12-17] Although a negative effect on bone of low-dose short-term oral isotretinoin for the treatment of acne vulgaris is not well-established, [18] the manufacturer recommends that it should be used cautiously in patients with history of metabolic bone disease such as osteomalacia.[17] Vitamin D deficiency is highly prevalent in Middle Eastern women and this is attributed to insufficient sunlight exposure and low dietary vitamin D intake.[19-23] In this preliminary observational report, we evaluated the effect of low-dose short-term oral isotretinoin treatment on bone remodeling markers and bone mineral density in Emirati women affected by severe acne and vitamin D deficiency**.**

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Subjects and Methods

Subjects

Study subjects were consecutive Emirati women diagnosed by one of the authors (LH) with severe acne requiring isotretinoin treatment at the time they were attending the Dermatology Clinic at Tawam hospital in Al Ain, United Arab Emirates during the period of October 2005 to February 2006. Exclusion criteria included pregnancy, lactation and treatment with vitamin D (other than multivitamins) within the past 1 year. Women known to have vitamin D deficiency, osteomalacia, osteoporosis, major systemic chronic diseases (such as renal, liver, thyroid, and parathyroid disorders, and cancer) or were taking other medications known to affect skeletal metabolism (such as glucocorticoids, bisphosphonates,

anticonvulsives, fluoride, heparin, thyroxin, or vitamin D metabolite) were also excluded. All sexually active women were required to have 2 methods of contraception. All patients received both oral and written information and gave informed consent. Patients were treated with isotretinoin (Accutane®, Roche) initially at the dosage of 0.5 mg/kg/day in 2 divided doses. The dose was increased to 1 mg/kg/day after 6 weeks if no significant improvement in acne was noted. The maximum total accumulative dose was set at 120 mg/kg/course of therapy.

Methods

Blood samples were collected at baseline, 6 weeks, and end of isotretinoin treatment (just before discontinuation), and the serum was separated and frozen at -80°C till biochemical testing at a later stage. Serum calcium and phosphorus were measured with Beckman Synchron autoanalyzer. Serum 25-hydroxyvitamin D [25(OH)D] concentration was measured by radioimmunoassay (DiaSorin; Stillwater, Minnesota). The intra- and inter-assay coefficients of variation (CVs) were 8.3% and 3.2%, respectively. Serum 25(OH)D concentration <50 nmol/L was considered as vitamin D deficiency based on studies in the literature.[24] Serum intact parathyroid hormone (PTH) was measured by chemiluminiscent assay (Access 2, Beckman Coulter). The normal adult range is 1.2-6.5 pmol/L. The intra- and inter-assay CVs were 6% and 5.1%, respectively. Serum bone Tartrate-resistant acid phosphatase (TRACP), a marker of bone resorption, was measured by enzyme-linked immunosorbent assay and bone specific alkaline phosphatase (Bone ALP), a marker of bone formation, was measured by enzyme immunoassay (Immunodiagnostic Systems; Tyne and Wear, UK). The intra- and inter-assay CVs were <10% for TRACP and Bone ALP. Bone mineral density (BMD) measurements were determined at the lumbar spine (L2-L4) anterior-posteriorly and the right and left hip by dual-energy X-ray absorptiometry (DXA) using the Lunar Expert-XL DXA system (Lunar Corp., Madison, WI, USA) according to the manufacturer's operator manuals. The manufacturer provided data for calculation of BMD Z-score (BMD of subject-BMD of age-matched control/SD). The precision of DXA was 1% for the lumbar spine and the femur. The least significant change (2.77 x precision) [25] was 2.77% for the spine and femur.

Statistical analysis

Data were analyzed using SPSS software (versions 15; SPSS Inc, Chicago). The methods used included, student's *t* test and analysis of variance (ANOVA). Adjustments for multiple-comparisons were carried out using Tukey's test for post-hoc pairwise comparisons. A two-tailed P value < 0.05 was considered statistically significant.

Results

Of the 25 eligible patients, 11 agreed to participate. There were no differences in the baseline characteristics between participating and non-participating subjects (data not shown). One patient was subsequently excluded from the analysis as she was treated with vitamin D during the study period. Table 1 shows the baseline characteristics of the 10 patients studied. Mean age was 23.9 ± 4.5 (range 16-33) years. Patients were treated with isotretinoin for a mean period of 19.3 ± 5.0 (range 12-24) weeks at a mean dose of 0.59 ± 0.11 mg/kg/d and a mean cumulative dose of 80.8 ± 28.8 mg/kg/course (range 40.3-112.4). All patients showed a good clinical response to the treatment and no major side effects were noted except for elevation in liver function tests in one patient that returned to normal after discontinuation of treatment. Mean 25(OH)D at baseline was 16.3 ± 7.5 nmol/L (range 7.0-29.0). Only three patients had biochemical evidence of osteomalacia at baseline [25(OH)D ≤ 25 nmol/L, PTH > 6.5 pmol/L, and low to normal calcium and phosphorus]. Two patients did not have baseline BMD measurements and only 1 patient had a baseline BMD at any of the measured sites below the expected range for age (Z-score ≤ -2.0). Mean serum calcium decreased slightly at end of treatment, no significant changes were detected in serum PTH, 25(OH)D and phosphorus in response to treatment with isotretinoin (Table 2). Mean serum TRACP and Bone ALP concentrations increased at end of treatment (Table 2) but this was only statistically significant for TRACP (1.18, 1.13, 1.64 U/L; P <0.001 by ANOVA). Serum TRACP concentrations did not change significantly at 6 weeks but increased significantly in each of the seven patients who had TRACP concentrations measured at end of treatment (Fig. 1). Only five patients had baseline and follow-up BMD measurements (case numbers 1-5 in Table 1). BMD decreased in all these patients at the femur (range -2.5 to -7.6%), and in all but one patient at the lumbar spine (range +3.2 to -6.8%). This decrease was in excess of the least significant change in 4 of the 5 patients. Mean BMD decreased at all three measured sites (Table 3) but this was statistically significant for the right femur (-3.7 ± 2.0%; P=0.015), left femur (-6.9 ± 2.4%; P = 0.002), mean (right and left) femur (-5.3 ± 1.9%; P = 0.002), but not for the lumbar spine (-3.0 ± 3.8%; P = 0.17).

Discussion

We observed that the use of short-term low-dose oral isotretinoin for the treatment of severe acne in patients with vitamin D deficiency was associated with increased bone resorption and decreased BMD. Although a cause and effect relationship cannot be concluded from this study, the absence of significant changes in 25(OH)D concentrations suggest that these effects are related to the isotretinoin treatment. In studies of vitamin D-deficient rats retinoid-induced bone resorption appears to be independent of the vitamin D endocrine system.[26] An antagonistic relationship, albeit weak, between retinol and vitamin D is also well established.[27, 28] Although previous studies indicate that retinol may affect the metabolism of vitamin D,[29, 30] recent studies suggest that retinol interferes with vitamin D action at the molecular level rather than by altering vitamin D metabolism.[31]

We are not aware of any studies of isotretinoin use in vitamin D-deficient patients. Previous studies of short-term oral isotretinoin treatment in patients not known to have vitamin D deficiency have shown that bone turnover

Table 1: Baseline characteristics of women with vitamin D deficiency treated with isotretinoin for severe acne.

Case No.	Age (yr)	Weight (kg)	Duration (wk)	Dose (mg/kg/ course)	25(OH)D (nmol/L)	PTH (pmol/L)	Bone ALP (U/L)	TRACP (U/L)	Spine (Z-score)	Rt femur (Z-score)	Lt femur (Z-score)
1.	33	81.0	24	68.8	7.0	14.7	3.0	1.09	-0.6	+0.1	0.0
2.	23	50.0	22	112.4	7.0	5.1	14.9	1.36	-1.1	-0.7	-0.5
3.	25	59.0	12	49.6	24.0	4.8	25.7	1.53	-0.9	+0.4	+0.2
4.	22	51.5	21	110.3	25.0	6.9	11.1	1.18	+0.7	+0.6	+1.4
5.	23	71.0	15	58.5	29.0	4.7	14.1	1.01	-1.4	-0.7	-0.7
6.	25	67.5	23	109.5	12.0	6.2	2.2	1.05	-2.2	-1.3	-1.4
7.	27	72.0	12	109.2	13.0	4.3	18.4	1.29	+1.5	+0.1	0.0
8.	25	70.5	15	92.4	16.0	8.0	6.5	1.14	+0.3	-0.1	0.0
9.	16	58.0	24	56.7	16.0	3.1	58.3	1.10	NA	NA	NA
10.	20	63.0	24	40.3	17.0	NA	3.8	1.09	NA	NA	NA

Abbreviations: TRACP, bone Tartrate-resistant Acid Phosphatase; Bone ALP, bone specific alkaline phosphatase; PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D; NA, not available.

Table 2: Bone turnover markers in women with severe acne and vitamin D deficiency at baseline, 6 weeks and end of isotretinoin treatment.*

Time (month)	Baseline (n)	6wks (n)	End of treatment (n)	P value†
Serum 25(OH)D (nmol/L)	16.3 ± 7.5 (10)	12.0 ± 6.0 (9)	16.6 ± 8.3 (7)	0.4
Serum PTH (pmol/L)	6.4 ± 3.4 (9)	6.9 ± 27 (9)	6.5 ± 3.5 (6)	0.9
Serum calcium (mmol/L)	2.35 ± 0.1 [a,b] (9)	2.42 ± 0.1 [b] (10)	2.32 ± 0.1 [a,c] (9)	0.04
Serum phosphorus (mmol/L)	1.0 ± 0.2 (7)	1.1 ± 0.2 (9)	1.1 ± 0.1 (9)	0.6
Serum Bone ALP (U/L)	15.7 ± 16.8 (10)	20.1 ± 15.7 (10)	31.7 ± 16.5 (7)	0.2
Serum TRACP (U/L)	1.18 ± 0.16 [a] (10)	1.13 ± 0.19 [a] (10)	1.64 ± 0.25 [b] (7)	<0.001

* Mean ± SD. † P value by ANOVA; means within a row not sharing the same superscript letter are significantly different at P < 0.05 by Tukey's pairwise post-hoc tests.

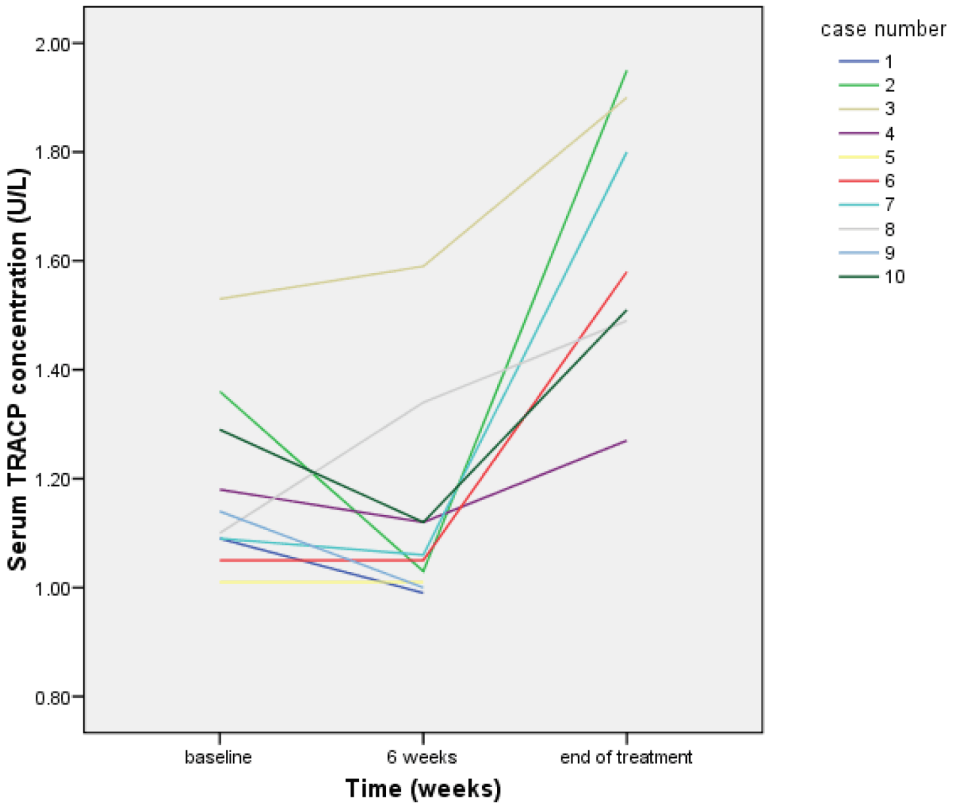

Figure 1: Serum TRACP (U/L) profiles for all studied women.
TRACP = bone Tartrate-resistant Acid Phosphatase. * Cases 1, 5, and 9 had missing TRACP values at end of treatment.

Table 3. Bone mineral density changes in lumbar spine and hip at baseline and end of isotretinoin treatment.*

Anatomic site	Baseline (n=5)	End of treatment (n=5)	Percent change	P value †
Lumbar spine (g/cm^2)	1.10 ± 0.10	1.07 ± 0.12	-3.0 ± 3.8	0.17
Right total femur (g/cm^2)	0.99 ± 0.79	0.95 ± 0.09	-3.7 ± 2.0	0.015
Left total femur (g/cm^2)	0.99 ± 0.09	0.93 ± 0.10	-6.9 ± 2.4	0.002
Mean (right and left) total femur (g/cm^2)	0.99 ± 0.08	0.94 ± 0.09	-5.3 ± 1.9	0.002

* Mean ± SD.
† P value by paired sample 2-tailed t test.

markers may actually decrease initially but they return to normal by the end of treatment.[32-34] Kindmark et al[32] investigated the early effects of isotretinoin in 11 patients with nodulocystic acne and found that bone turnover markers (osteocalcin, bone ALP, carboxyterminal telopeptide of type 1 collagen, and urinary hydroxyproline) decreased significantly within 5 days of isotretinoin treatment but with continued use the abnormal levels returned to baseline values within 14 days. The observed inhibitory effects of isotretinoin on bone turnover were associated with significant decrease in calcium and increase in PTH concentrations suggesting a direct effect on bone tissue. Trifiro and Norbiato [33] found no changes in serum osteocalcin, PTH, 25(OH)D or $1,25(OH)_2D$ in 10 adolescents (mean age 17.8 yrs) affected by severe acne after 3 months of isotretinoin treatment at 0.5mg/kg/day. However, urinary N-terminal telopeptide of type I collagen (NTX), a marker of bone resorption, decreased significantly. The authors speculated that the decrease in urinary NTX could be due to the effect of isotretinoin on the cutaneous component of type 1 collagen rather than an inhibitory effect on bone resorption. Margolis et al[34] noted no alterations in serum measurements of calcium, osteocalcin, PTH, 25(OH)D, or urinary hydroxyproline in 20 adult patients who were treated with isotretinoin for cystic acne for 20 weeks. While our data at 6 weeks are consistent with the above findings, we observed a significant increase in bone resorption by the end of treatment.

A negative effect on BMD of low-dose short-term oral isotretinoin treatment for acne vulgaris is not well established. In two of the above cited studies,[32, 34] no significant changes in BMD were reported. Leachman et al[35] however, observed a decrease in mean BMD at all studied sites in 18 young men with cystic acne who were treated with isotretinoin for 6 months but this was statistically significant only at the Ward's triangle (mean decrease in BMD of 4.4%; P = 0.03). Similarly, DiGiovanna et al[18] observed that a 16-20 week course of isotretinoin treatment at the recommended dose for severe acne in a large adolescent-age population resulted in a statistically significant decline in BMD of the femoral neck (-0.5 ± 3.4%, P=0.03) and Ward's triangle (-1.4 ± 4.2%, P<0.00001). There was no statistically significant decline in total hip BMD (-0.3 ± 3.0%, P = 0.2), however, and mean lumbar spine BMD actually increased by 1.4 ± 2.5% (P<0.00001). While the magnitude of change in BMD in the above-cited studies is probably not clinically significant, the observed decline in most of our patients was in excess of the least significant change,[25] and perhaps should not be ignored.

Our study is preliminary and the data must be viewed with appropriate caution. The number of patients studied is small and few had some missing data. In addition, the study is limited by its observational nature, where patients received variable cumulative doses of isotretinoin, and it lacked a control group of subjects who had normal vitamin D concentrations. Nevertheless, our results, when added to available studies raise the possibility that the use of short-term low-dose oral isotretinoin treatment for severe acne in patients with vitamin D deficiency may be associated with measurable, and potentially clinically significant, effects on skeletal homeostasis. Thus, it may be prudent to screen for vitamin D deficiency in populations with a high prevalence of this disorder before starting isotretinoin treatment. If detected then its correction before or during isotretinoin treatment may be indicated and should be studied. The appropriate dosing regimen for oral vitamin D supplementation in high-risk populations such as Emirati women is not known. Studied regimens of oral plain vitamin D therapy in vitamin D deficient subjects include 2,000 IU/d or 60,000 IU/month for 3 months,[20] 50,000 IU/d for 10 days, [36] or weekly for 8 weeks.[37] At the existing state of our understanding, it seems prudent to consider ≥ 75 nmol/L as the desirable range of 25(OH)D concentration and to provide adult patients with sufficient oral intake of vitamin D to achieve and sustain such a level.[38] Cholecalciferol is preferred over ergocalciferol since it is probably more potent,[39] and appears to be less antagonized by retinol.[31]

In conclusion, our preliminary study suggests that short-term oral isotretinoin therapy in women with vitamin D deficiency is likely associated with increased bone resorption and decreased BMD. Correction of vitamin D deficiency may be necessary before starting isotretinoin treatment for acne.

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