Vitamin D receptor gene polymorphism and bone metabolism during low-dose oral contraceptive use in young women

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Abstract

With the aim to determine whether bone metabolism in young women using low-dose oral contraception is influenced by vitamin D receptor (VDR) genotype, we designed the prospective clinical study of 41 healthy women aged 20–27 years. Twenty-one women of the study group were prescribed an oral contraceptive (30 μg ethynyl estradiol and 150 μg levonorgestrel) and 20 women of the control group a nonhormonal contraceptive or none. Biochemical markers of bone metabolism (bone-specific alkaline phosphatase, osteocalcin, deoxypyridinoline) and VDR genotype, using BsmI endonuclease, were determined. After 3 months in the study group, the BB genotype subgroup showed significantly decreased osteocalcin (p < 0.010), in the Bb genotype subgroup bone-specific alkaline phosphatase (p < 0.043) and osteocalcin (p < 0.006) decreased, and in the bb genotype subgroup no changes were observed. In the control group, there were no significant changes in markers of bone metabolism regarding VDR genotype. In conclusion, our study shows that in young women VDR gene polymorphism could influence bone metabolism during low-dose oral contraceptive use. © 2003 Elsevier Science Inc. All rights reserved.

Keywords: Hormonal contraception; Genotype; Biochemical markers; Osteocalcin; Deoxypyridinoline; Vitamin D receptor gene

1. Introduction

Combined oral contraception is one of the most frequently used and also most evaluated contraceptive methods. However, the influence on bone metabolism in women of reproductive age has not yet been clarified.

Most studies report on beneficial effect of oral contraceptive use on bone metabolism in premenopausal women [1–3] and on reduced risk for postmenopausal osteoporosis development in past oral contraception users [4–6]. However, the influence is not so obvious in women of reproductive age [7–9]. Some studies revealed reduced bone metabolism with oral contraceptive use in women of reproductive age, evident in reduced levels of biochemical markers of bone metabolism [10–12] or no change in bone mineral density (BMD) [13,14]. In younger women, the process of bone gain has not yet been completed [15] and therefore, the biological impact of reduced bone metabolism in oral contraception users could be important. Polatti et al. [13] suggested that long-term treatment with a low-dose combined oral contraceptive did not modify the bone mineral density but prevented the occurrence of physiologic peak of bone mass in young women. Similarly, Burr et al. [16] found out that oral contraception exerts a suppressive effect on bone mass gain in young women. The influence of oral contraception on peak bone mass may be important because peak bone mass, together with the bone loss later on, is one of the principal factors determining bone mass later in life, osteoporosis and fracture risk [17].

The peak bone mass is mostly determined by genetic factors. Morrison et al. [18,19] were the first who revealed that vitamin D receptor (VDR) gene could be one of the genetic determinants of bone metabolism and BMD. Several studies have shown that pre- and postmenopausal women with BB genotype, determined by restriction fragment length polymorphism (RFLP) using BsmI endonuclease-
ase, have lower bone mineral density than women with bb genotype [20–23], and are prone to accelerated postmenopausal bone loss [24–27]. Studies in prepubertal girls also suggest that bone density is determined by VDR genotype [28–30]. Other studies revealed that women with allele B are more sensitive to calcium and calcitriol supplementation [31,32], hormonal replacement therapy and to antiresorption therapy with bisphosphonates as compared to women with allele b [33,34].

The question is whether vitamin D receptor gene polymorphism could significantly influence bone metabolism during low-dose oral contraceptive use in young women. Therefore, we designed a prospective clinical study with the aim to determine whether bone metabolism in young women using low-dose oral contraception is influenced by VDR genotype.

2. Materials and methods

In the prospective clinical study, 41 healthy women, aged 20–27 years, were included. The subjects were recruited among the women attending the outpatient clinic at the Department of Obstetrics and Gynecology at the University Medical Center in Ljubljana. The exclusion criteria were: absolute and relative contraindications for oral contraception, irregular menstrual cycles, pregnancy, delivery or breastfeeding within previous 12 months, abortion or miscarriage within previous 6 months, use of drugs influencing bone metabolism (including oral contraceptives) during previous 6 months, all subjects were fully informed on the study procedure and design, and written informed consent was given by each subject. The study protocol was approved by the Committee for Medical Ethics at the Ministry of Health of the Republic of Slovenia.

Twenty-one women in the study group were prescribed a combined oral contraceptive containing 30 μg ethinyl estradiol and 150 μg levonorgestrel starting from the first day of a normal menstrual cycle. Twenty women in the control group were given a nonhormonal contraceptive or none, if not needed. At the beginning of the study, reproductive history (age at menarche, number of pregnancies, parity), nutrition (diary products), life-style habits (smoking, use of alcohol, exercise) and anthropometrical characteristics (weight, height, body mass index - BMI) were collected. The biochemical markers of bone metabolism were evaluated in all the women enrolled in the study: serum bone-specific alkaline phosphatase (bone ALP), serum osteocalcin (OC), and urinary deoxypyridinoline (DPD). In all subjects, VDR genotype was determined. After 3 months, nutrition, life-style habits, anthropometrical characteristics and the biochemical markers of bone metabolism were re-evaluated.

Serum bone-specific alkaline phosphatase was measured with a RIA using two monoclonal human-specific antibodies (Tandem-R Ostase, Hybritech, San Diego, CA, USA). The intra-assay coefficient of variation (CV) was 6.7%, the interassay CV was 8.1%, and the sensitivity was 2 μg/L. Serum osteocalcin was measured by a two-site radio-immunoassay (RIA) using two monoclonal antibodies (ELISAOSTEO, CIS Bio International, Gif-sur-Yvette, France). The intra-assay CV was 3.9%, the interassay CV was 5.2%, and the sensitivity was 0.4 μg/L. Urinary deoxypyridinoline was determined by ELISA (Pyrilinks-D, Metra Biosystem, Inc., Mountain View, CA, USA). The intra-assay CV was

<table>
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<tr>
<th>Biochem. markers</th>
<th>BB (n = 6)</th>
<th>Bb (n = 10)</th>
<th>pp</th>
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<th>pp</th>
<th>pp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone ALP (μg/L)</td>
<td>Baseline</td>
<td>End</td>
<td>p</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>8.0 (3.5)</td>
<td>8.5 (6.1)</td>
<td>NS</td>
<td></td>
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<tr>
<td>OC (ng/mL)</td>
<td>Baseline</td>
<td>End</td>
<td>p</td>
<td></td>
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<tr>
<td></td>
<td>30.6 (5.1)</td>
<td>24.5 (4.5)</td>
<td>0.010</td>
<td></td>
<td></td>
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<tr>
<td>DPD/Cr (nmol/mmol)</td>
<td>Baseline</td>
<td>End</td>
<td>p</td>
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<tr>
<td></td>
<td>10.9 (7.0)</td>
<td>8.4 (3.8)</td>
<td>NS</td>
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</tr>
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Cr, creatinine.
The VDR genotype was determined according to a previously described method [34]. Genomic DNA was isolated from peripheral blood leukocytes and the region of VDR gene carrying the polymorphic BsmI site was amplified by the polymerase chain reaction (PCR). Specific primers were used to yield an 800 bp PCR product. One μg of genomic DNA was amplified in a total volume of 30 μL. The amplification was performed as follows: incubation for 5 min at 94°C, followed by 35 cycles of 20 sec denaturation at 94°C, 40 sec annealing at 62°C, and 1 min extension at 72°C. The digestion of the amplified product with restriction endonuclease BsmI for 1 h at 65°C was performed and genotype was detected by etidium bromide-UV illumination of the fragments separated on 10% polyacrylamide gel (37:1). VDR genotype with respect to the BsmI restriction fragment length polymorphism (RFLP) was determined. Fragments of 650 bp and 150 bp resulted from the presence of BsmI restriction site and this was specified as allele b, whereas absence of BsmI restriction site was specified as allele B.

Data are expressed as mean ± standard deviation (SD) unless stated otherwise. In the statistical analysis of the women’s characteristics, t-test and χ² test were used. In the analysis of differences of the biochemical markers of bone metabolism, Student’s t-test was used. P-values of less than 0.05 were considered statistically significant. Statistical analysis was done using SPSS for Windows Version 9.0.

3. Results

There were no differences between the study group and the control group in age at menarche (12.8 ± 0.9 vs. 12.6 ± 1.2 years), body mass index (21.0 ± 2.8 vs. 22.0 ± 2.5 kg/m²), in nutritional characteristics and life-style habits. The only statistical, but not clinically significant, difference appeared in chronological age (21.2 ± 1.3 vs. 22.4 ± 1.7 years; p = 0.015). VDR genotype frequency did not differ between the two groups of patients (Table 1). In the course of the study, there was no significant change in nutrition, life-style habits and anthropometrical characteristics comparing the study group to the control group.

The baseline values of the biochemical markers (bone-specific alkaline phosphatase, osteocalcin and deoxypyridinoline) did not differ between the study and the control group, nor did the bone markers significantly change in the course of the study, either in the study or the control group.

The differences in the individual biochemical markers between the mean baseline values and the mean end values were analyzed separately in the study group and in the control group after dividing both groups to subgroups by the VDR genotype. The analysis showed that: in the study subgroup of women with the BB genotype, the osteocalcin levels decreased (p = 0.010); in the study subgroup of women with the Bb genotype, the levels of bone-specific alkaline phosphatase (p = 0.043) and osteocalcin (p = 0.006) decreased; but in the study subgroup of women with the bb genotype, no changes were observed (Table 2). In the control subgroups with BB, Bb and bb genotype, there were no changes between the mean baseline values and the mean end values of the biochemical markers of bone metabolism (Table 3).

4. Discussion

The results of our study have shown that subgroups of young women determined by VDR genotype show different susceptibility to oral contraception. In BB and Bb subgroups of oral contraceptive users, significant changes in the biochemical markers of bone metabolism occurred after 3 months of taking oral contraception, while in the bb subgroup there were no significant changes. These changes were not evident if the group was analyzed as a whole, not taking into account VDR genotyping. In addition, there were no significant changes in the VDR subgroups of the control group.

To our knowledge, there has so far been no published study in which bone metabolism was determined in young women using low-dose oral contraception considering VDR genotype. Our findings that biochemical markers of bone metabolism decreased after 3 months with low-dose oral contraceptive use in young women with BB and Bb, but not with bb genotype, are in some way consistent with studies which found that B allele is more sensitive and b allele more resistant to different pharmacological interventions, e.g.,
calcium and calcitriol supplementation, hormonal replacement therapy, antiresorption therapy with bisphosphonates [31–34].

The prevailing changes in bone formation markers, where statistical significance was reached, in contrast to bone resorption markers, where only trends were detected, could be due to the observation that in young women bone formation exceeds bone resorption, thus rendering bone formation more susceptible to inhibition [35]. This assumption could be supported by animal experiments, where in young adult female monkeys given oral contraception, bone formation was reduced to a greater extent than bone resorption [36].

In summary, our study shows that in young women, VDR gene polymorphism could influence bone metabolism during low-dose oral contraceptive use. To establish more definite conclusions, a study on a larger sample size should be performed.

References

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