

Relationship Between Bone Mineral Density and Angiotensin Converting Enzyme Polymorphism in Hypertensive Postmenopausal Women

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Background: The purpose of this study was to assess the relationship between bone mineral density and insertion/deletion (I/D) angiotensin converting enzyme polymorphism (ACE) in hypertensive postmenopausal women.

Methods: Blood and urine samples from the study subjects were analyzed for calcium metabolism related parameters. Densitometry studies were conducted in the lumbar spine (L2 to L4). The ACE polymorphism was analyzed by polymerase chain reaction.

Results: Women with II genotype showed a higher intact parathyroid hormone (76 ± 33 v 55 ± 27 pg/mL and

52 ± 26 pg/mL, $P = .034$) without a decrease in calciuria, and higher bone mineral density than women with ID and homozygous deletion genotype (1.138 ± 0.08 v 1.051 ± 0.16 pg/mL and 1.053 ± 0.16 pg/mL).

Conclusion: The ACE polymorphism could be one of the factors causing bone mass variations. Am J Hypertens 2003;16:233-235 © 2003 American Journal of Hypertension, Ltd.

Key Words: Bone mineral density, hypertension, women, calcium excretion, ACE I/D polymorphism.

Many alterations in extracellular calcium metabolism have been associated with hypertension. Such alterations may determine the bone mineral density in hypertensive individuals; they may also cause a decrease in ionic calcium, calciuria, and urinary cyclic adenosine monophosphate, parathyroid hormone serum concentration, and plasma calcitriol, as well as an increase of intestinal absorption of calcium.¹⁻³ Furthermore, hypertension and osteoporosis are caused by the interaction of genetic and environmental factors. The polygenic heredity pattern of these diseases determines the interaction of several genes. The number of studies on the genetic factors related to these two diseases is surprisingly low. As 50% of the hypertensive population is formed by postmenopausal women at high risk for osteoporosis, hypertension represents a considerable health problem in this population.

Although angiotensin converting enzyme (ACE) polymorphism has not been associated to a higher frequency of arterial hypertension, a relationship with the frequency of

complications in arterial hypertension has been found.⁴ In the nonhypertensive population, the presence of allele D increased less in bone mass but not in the initial mass after treatment with estrogens.⁵ The purpose of this study was to determine the relationship between ACE insertion/deletion (I/D) polymorphism and bone mass in this population group.

Methods

Subjects and Study Protocol

According to the criteria of the Sixth Report of the Joint National Committee, 48 postmenopausal women with mild-to-moderate hypertension were included in the study. Exclusion criteria were ethylism, neoplasia, secondary arterial hypertension, chronic renal insufficiency, hypercalcemia and hypocalcaemia, diabetes, hyperparathyroidism, and use of modifying bone mass drug. The average time of hypertension development was 7 ± 8 years. Of the patients, 88% were under antihypertensive treatment. However, the patients un-

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derwent a washout period of 2 weeks before analyses and densitometric measurements were conducted.

This study was approved by the hospital's ethics committee, and patients signed an informed consent form to participate.

Measurements

Blood samples were obtained after 8 and 9 h of fasting. Total calcium, phosphorous, magnesium, and alkaline phosphatase were measured by a Hitachi 917 autoanalyzer (Hitachi, Tokyo, Japan). Levels of ionic calcium and ion (sodium, creatinine, calcium, and phosphorus) in urine were assessed with an ion-selective electrode (Nova-7, Nova Instruments, Waltham, MA). Osteocalcin was measured using a commercial radioimmunoassay (RIA) test (Schering, Berlin, Germany) with a 6.7% interassay variation coefficient. The intact parathyroid hormone (PTHi) levels were determined by chemoimmunoluminescence (Immulite DPC, Los Angeles, CA) with 6% variation coefficient. The 25-hydroxyvitamin D levels were determined by high performance liquid chromatography (HPLC) with 12% variation coefficient and 1.25-hydroxyvitamin D levels were determined by RIA with 12% variation coefficient.

Urinary deoxyypyridinoline levels were determined by chemoimmunoluminescence after 24 h (Immulite DPC, Dipeasa, Los Angeles, CA). The results were expressed in relation with the excretion of creatinine with a variation coefficient of 14%.

Densitometry studies were conducted in the lumbar spine (L2-L4) using an x-ray densitometer (DEXA, Lunar Corp., Madison, WI). Bone mineral density was expressed in g/cm^2 and as peak bone mass percentage in normal subjects (T-score) depending on the software used in the device. Women with a T-score of less than -2.5 were considered to be osteoporotic.

ACE Polymorphism

Coagulated blood was obtained by EDTA. A nuclear pellet was then taken following the procedure described by John et al⁶ and frozen at -20°C . DNA was deduced from the pellet with the commercial QIAmp Blood Kit (Qiagen, Hilden, Germany). In a final volume of $20\ \mu\text{L}$, we used $25\ \text{mmol}/\text{L}$ of each dNTP (Amersham Pharmacia Biotech); $50\ \text{mmol}/\text{L}$ KCl; $1.5\ \text{mmol}/\text{L}$ MgCl_2 ; $10\ \text{mmol}/\text{L}$ Tris HCl ($9.0\ \text{pH}$); and 0.75 units of Taq DNA Polymerase (Amersham Pharmacia Biotech, Little Chalfont, England). Using genomic DNA, the specific sequence of insertion/deletion polymorphism of intron 16 of the ACE gene was amplified with $20\ \text{pmol}$ of each of the automatic primers described by Shanmugan et al.⁷ This is a modification of the original procedure by Rigat et al⁸ that was designed to flank the inserted segment using a third primer specific insertion to prevent the 5% typing error of both ID and DD genotypes.

A second amplification with primer specific sequence was conducted with DD genotype patients samples fol-

lowing the conditions described by Shanmugan et al.⁷ This was done to confirm that no false allocation of ID and DD genotypes derived from 5% alleles I and D amplification, described by Shanmugan et al⁷ in human ACE gene and by Perna et al⁹ in human TPA gene.

Statistical Analyses

Descriptive statistical analysis, including central and dispersion trends, were conducted for quantitative categories. Absolute and relative variables were analyzed for different categories. The Mann-Whitney *U* test was used to analyze data that matched the purpose of the study. The Tukey method was used to compare multiple variables. Results were expressed as mean \pm SD.

Results

The ACE polymorphisms were evaluated in 48 patients, of whom 10 were II (21%), 26 were ID (54%), and 12 were DD (25%). The percentage of I allele was 48%, whereas the percentage of D allele was 52%. When hypertensive patients were divided according to polymorphism (Table 1), women with II polymorphism showed higher bone mass (1.138 ± 0.08 v. 1.051 ± 0.16 and $1.053 \pm 0.16\ \text{g}/\text{cm}^2$ in ID and DD, respectively), calcium concentration in serum (9.9 ± 0.7 v. 9.6 ± 0.3 and $9.5 \pm 0.3\ \text{mg}/\text{dL}$), both nonsignificant, and a considerably higher PTHi (76 ± 33 v. 55 ± 27 and 52 ± 26 , $P = .034$) with higher urinary calcium excretion ($286 \pm 178\ \text{mg}/24\ \text{h}$). Moreover, 36% of the women in the hypertensive patients group with DD polymorphism were osteoporotic. No differences between groups were found (Table 1) when analyzing systolic blood pressure (BP) ($156 \pm 26\ \text{mm Hg}$, $153 \pm 22\ \text{mm Hg}$, $162 \pm 19\ \text{mm Hg}$) and diastolic BP ($95 \pm 7\ \text{mm Hg}$, $92 \pm 12\ \text{mm Hg}$, $95 \pm 8\ \text{mm Hg}$). In all, 80% of II polymorphism patients were under antihypertensive treatment, whereas 85% of ID patients and 100% of DD patients were receiving such treatments. However, there was no statistically significant difference between them, either in the types of therapies or in the duration of hypertension.

Discussion

Although ACE polymorphism is not related to the incidence of hypertension, there are complications caused by ACE polymorphism (ie, left ventricular hypertrophy, ischemic cardiopathy, progressive renal failure).⁴ In contrast to the study carried out by Woods,⁵ we found no statistically significant variations of the initial bone mineral density related to polymorphism, probably because the number of patients was small. Patients with polymorphism II, which is associated with lower ACE activity,^{5,8} showed higher bone mass than did hypertensive women with ID and DD polymorphism. Such an increase in bone mass was not related to calciuria, a deciding factor of bone mineral density in hypertension, which indicates that other factors may have an effect on bone mineral density. Angiotensin II may be one of them.

Table 1. ACE polymorphisms, bone turnover markers, and bone mineral density

Variable	II (10)	ID (26)	DD (12)
SBP (mm Hg)	156 ± 26	153 ± 22	162 ± 19
DBP (mm Hg)	95 ± 7	92 ± 12	95 ± 8
Ca (mg/dL)	9.9 ± 0.7	9.6 ± 0.3	9.5 ± 0.3
P (mg/dL)	3.6 ± 0.7	3.7 ± 0.5	3.5 ± 0.3
Osteocalcin (ng/mL)	29 ± 6	30 ± 10	26 ± 8
PTHi pg/mL	76 ± 33	55 ± 27*	52 ± 26*
Urinary Ca (mg/24 h)	286 ± 178	242 ± 150	186 ± 70*
Ca/creatinine	0.28 ± 0.13	0.26 ± 0.17	0.20 ± 0.16
Deoxypyridinoline (nmol/mmol creatinine)	8.1 ± 2.3	8.2 ± 4.7	7 ± 4
BMD L2–L4 (g/cm ²)	1.138 ± 0.08	1.051 ± 0.16	1.053 ± 0.16
Osteoporosis (%)	0%	15%	36%

ACE = angiotensin converting enzyme; II = homozygous insertion; ID = heterozygous insertion/deletion; DD = homozygous deletion; SBP = systolic blood pressure; DBP = diastolic blood pressure; Ca = calcium; P = phosphorus; PTHi = intact parathyroid hormone; BMD = bone mineral density.

* $P = .034$.

Angiotensin II can interfere with calcium metabolism. Administration of this peptide in a group of healthy volunteer subjects caused a decrease in ionic calcium levels and an increase in PTHi levels.¹⁰ The decrease in calcemia was not related to the increase in calciuria, but it could be caused by an increase in the calcium uptake by vascular smooth muscle cells.¹¹ These data agree with the results of our study. Patients with the II + ID genotype showed higher, non-calciuria related calcemia.

Moreover, in theory, angiotensin can act indirectly on bone cells by regulating the flow in bone marrow capillaries¹² or directly by binding to angiotensin type I receptors located on osteoblasts, thus promoting the release of mediators that would activate osteoclasts.¹³ We can consider the effect on osteoblasts as contradictory: they stimulate DNA and collagen synthesis, and the number of cells by osteoblastic precursors, but they decrease mature osteoblasts mineralization.^{14,15} Increased angiotensin II levels have a harmful effect by increasing bone resorption and inhibiting mineralization.

All data suggest the possible existence of an renin-angiotensin-aldosterone system in the bone tissue, where angiotensin II can have a deleterious effect at both a cellular and humoral level. Findings in patients presenting with ID and DD polymorphism, with lower bone mass and higher ACE activity, would support this hypothesis. Furthermore, this insight allows us the opportunity to use ACE inhibitors or angiotensin type I receptor antagonists in osteoporosis treatment, at least in hypertensive patients.

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