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Sex-related differences in bone metabolism in osteoporosis observational study

Kyu Hwan Choi, MD[a], Jong Ho Lee, MD[b], Dong Gyu Lee, MD, PhD[a,*]

Abstract

Although the incidence is lower in men than women, osteoporosis remains a significant health issue in men as it may give rise to severe complications if not managed appropriately. As men and women show different biological and social backgrounds, we retrospectively evaluated the differences in the bone metabolism between men and women using bone biomarkers.

Bone mineral density (BMD) was determined in all patients using dual-energy X-ray absorptiometry (DXA) and analyzing various bone biomarkers such as carboxyl-terminal collagen crosslinks (CTX), osteocalcin (OCT), and alkaline phosphatase (ALP). The CTX/OCT ratio was used to estimate the association between bone absorption and formation.

OCT, CTX, and ALP levels were elevated in patients with osteoporosis. Women displayed a higher incidence of osteoporosis and greater reduction in BMD than men. The mean OCT level in men was lower than that in women. Moreover, men showed significantly lower OCT levels than women of aged 65 and under 80 years old. Among patients with osteoporosis, men had a higher ratio of bone markers than women.

Levels of biomarkers of bone formation and absorption were increased in the osteoporosis group. However, men showed lower increases in bone formation biomarkers than did women, indicating that the rate of bone formation relative to bone absorption did not increase in men compared with that in women. Therefore, we suggest that men and women have different bone metabolism in old age.

Abbreviations: ALP = alkaline phosphatase, BMD = bone mineral density, CTX = carboxyl-terminal collagen crosslinks, DXA = dual-energy X-ray absorptiometry, OCT = osteocalcin, OPG = osteoprotegerin, RANK = receptor activator of nuclear factor-kappa B, TRPV4 = transient receptor potential vanilloid 4.

Keywords: bone marker, osteoblast, osteoclast, osteoporosis, osteoporosis sex-related differences, sex hormone

1. Introduction

With the increasing life expectancy, osteoporosis has become an important public health issue.[1] Hormonal changes, kidney disease, and a sedentary lifestyle due to frailty produce an imbalance in bone metabolism, resulting in decreased bone mineral density (BMD). Decreased BMD can lead to osteoporosis, which increases the vulnerability to fracture.[2] Bone fracture in elderly individuals increases the rate of mortality and makes daily life activities more difficult.[3] Therefore, effective treatment and prevention of osteoporosis in elderly people are important.

Bone tissues maintain the bone structure by the processes of bone absorption and formation.[4] Bone remodeling renews the old bone by generating new bone tissue following the absorption of existing bone tissue. Bone biomarkers allow the effective evaluation of the state of bone turnover.[5] Carboxyl-terminal collagen crosslinks (CTX) is used as a biomarker of bone absorption.[6] Osteocalcin (OCT, also termed bone gamma-carboxylglutamic acid-containing protein) and alkaline phosphatase (ALP) are used as biomarkers of bone formation.[7–9] The diagnostic value of bone biomarkers remains controversial, in part because of the significant variation between individuals.[10,11] However, evaluation of bone biomarkers is essential for understanding the normal biologic processes and for the evaluation of therapeutic outcomes in the treatment of osteoporosis.[12,13] Several studies aimed to evaluate the diagnostic value of bone biomarkers for the osteoporosis or the risk of bone fracture.[14,15]

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The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

[a] Department of Physical Medicine and Rehabilitation, [b] Department of Laboratory Medicine, College of Medicine, Yeungnam University, Daegu, Republic of Korea.

[*] Correspondence: Dong Gyu Lee, Department of Rehabilitation Medicine and Spine Center, College of Medicine, Yeungnam University, 170, Hyeonchung-ro, Nam-gu, Daegu, 42415, Republic of Korea (e-mail: painfree@yu.ac.kr).

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Men and women show social and biological differences. Sex hormone is one of the important factors which affect bone metabolism. Osteoclasts possess estrogen receptors but no androgen receptors. Moreover, postmenopausal women show a rapid decrease in sex hormone levels relative to that in men in the same climacteric period. Women commonly develop severe post-menopausal symptoms, as compared with men after andropause.[16,17] Therefore, the incidence of osteoporosis is relatively low in men compared with that in women.[18] Smoking and excessive consumption of alcohol tend to be more prevalent in men than in women.[19,20] These collective findings support the strong possibility of sex-related differences in bone metabolism.

We sought to address this possibility by examining sex-related differences in bone metabolism using bone biomarkers in the context of osteoporosis.

2. Subjects and methods

2.1. Subjects

This retrospective observational study used medical records obtained from Yeungnam University Hospital from January 2017 to December 2019. Ethical approval of the study was obtained from the hospital's institutional review board (YUMC-2020-04-126). Measurements of OCT and CTX were available for 139 patients (64 men, 75 women) who met the inclusion and exclusion criteria. Other inclusion criteria were the availability of BMD data and no prior use of osteoporosis medications other than vitamins or calcium. The exclusion criteria were history of kidney disease, history of cerebral stroke, spinal cord injury, history of prostate cancer, thyroid disease, and metastatic cancer involving bone.

2.2. Assessments

BMD was assessed using dual-energy X-ray absorptiometry (DXA), as the mean T-score from L2–4 in the lumbar spine, and femur neck and total hip in the femur area. Osteoporosis was classified if at least one T-score in the 3 sites was <–2.5. The T-score was the number of standard deviations from the mean BMD of a control population given in the manufacturer's reference values.

2.3. Laboratory measurements

OCT (N-MID OCT) and CTX (β–CrossLaps) were analyzed in serum with automated electrochemiluminescence assays using the Cobas e601 apparatus (Roche Diagnostics, Mannheim, Germany). ALP was assessed using the AU5800 analyzer (Beckman Coulter Inc., Brea CA). The CTX/OCT ratio was used to estimate the association between bone absorption and formation.

2.4. Statistical analyses

Data input and statistical calculations were performed using SPSS ver. 23.0 (SPSS Inc., Chicago, IL). The difference of bone biomarkers between men and women was analyzed using the *t* test. The difference between age and bone biomarkers by sex was also analyzed using the *t* test. Age differences were based on an age of 65 years. It is recommended to conduct a screening test for osteoporosis at the age of 65 to prevent osteoporotic bone fracture considering incidence of osteoporosis and the financial aspects of it.[21] Moreover, patients who were 80 years or older are at an increased risk for high hip fracture. Therefore, the age was divided into 3 groups (under 65 years old, 65 and under 80 years old, and 80 years old and over) to analyze the sex-related bone metabolism differences. A *P*-value <.05 was considered to be significant.

3. Results

Age distribution between men and women did not statistically significant differences. Women displayed significantly higher incidence of osteoporosis than men. T-scores of BMD in the femur neck, total hip, and lumbar region were lower in women than in men. The reduction of BMD was more significant in women than in men (Table 1). Men displayed a lower mean plasma concentration of OCT than did women. This resulted in a higher CTX/OCT ratio in men. The level of 25-dihydroxyvitamin D (Vitamin 25-D) was not significantly different in men and women.

Patients diagnosed with osteoporosis displayed higher plasma levels of OCT, CTX, and ALP than patients without osteoporosis (Table 2). However, the CTX/OCT ratio was not significantly different. These results suggest that although the levels of individual bone biomarkers increased, bone absorption and

Table 1

Demographic data and bone biomarkers differences between men and women.

		Total	Males	Females	*P*-value
Sex	N	139	64 (46.0%)	75 (54.0%)	
Osteoporosis		79 (56.8%)	27 (42.2%)	85 (69.3%)	.002*
Age		72.31 ± 0.71	72.53 ± 0.92	72.97 ± 1.06	.315
BMD (T-score)					
Femur neck		−2.32 ± 1.23	−1.80 ± 1.11	−2.77 ± 1.16	.000*
Total hip		−1.20 ± 1.13	−0.94 ± 1.05	−1.41 ± 1.16	.013*
Lumbar		−1.44 ± 1.51	−1.0 ± 0.64	−1.82 ± 1.28	.001*
OCT, ng/mL			15.27 ± 7.41	18.31 ± 6.72	.012*
CTX, ng/mL	Mean ± SD		0.47 ± 0.24	0.46 ± 0.23	.890
ALP, IU/L			98.31 ± 50.94	100.39 ± 40.27	.791
Vitamin D (25-D), ng/mL			19.95 ± 10.74	18.80 ± 9.93	.515
Ratio of bone marker			3.66 ± 2.26	2.78 ± 1.73	.013*

BMD = bone mineral density, CTX = carboxyl-terminal collagen crosslinks, ratio of CTX/OCT bone marker, OCT = osteocalcin.

* *P* < .05, *P*-value between men and women.

Table 2

Bone biomarker differences according to sex in osteoporosis patients.

	OCT, ng/mL	CTX, ng/mL	ALP, IU/L	Vitamin D (25-D), ng/mL	Ratio of bone marker
Male (N=27)	16.36±7.58	0.53±0.27	117.59±64.35	19.85±11.69	3.97±2.73
Female (N=52)	18.89±5.94	0.50±0.25	107.64±42.54	18.39±10.08	2.80±1.66
P-value	.106	.678	.474	.566	.048*

Values are presented as the mean±standard deviation. CTX=carboxyl-terminal collagen crosslinks, ratio of CTX/OCT bone marker, OCT=osteocalcin.
* $P<.05$, *P*-value between men and women in the osteoporosis patients.

formation increase in proportion to each other. The accompanying increase in bone absorption and formation indicated increased bone turnover.

When the biomarker changes in patients with and without osteoporosis were analyzed according to sex, the results differed from those of the total sex analysis. In non-osteoporosis patients, OCT, CTX, and CTX/OCT showed no significant differences between men and women. However, in patients with osteoporosis, the CTX/OCT ratio was higher in men than women (Table 2). Mean plasma concentration of OCT in men and women was 16.36±7.58, and 18.89±5.94, respectively. Men displayed a significantly lower mean plasma level of OCT than women, which resulted in an increased CTX/OCT ratio.

Changes in bone biomarkers according to sex were analyzed in patients of under 65 years old, 65 and under 80 years old, and 80 years old and over. There were no significant differences, except for in OCT levels and CTX/OCT ratio on 65 and under 80 years old (Table 3, Fig. 1). In patients <65 years of age and 80 years old and over, there were no differences between men and women concerning bone biomarkers. In patients of 65 and under 80 years old, the OCT level was significantly lower in men than in women. Correspondingly, the CTX/OCT ratio was higher in men than in women.

4. Discussion

In this study, OCT, CTX, and ALP levels were increased in osteoporosis patients. Women showed a higher incidence of osteoporosis and greater reduction in BMD than men. The mean OCT level in men was lower than that in women. Although OCT and CTX did not differ in men and women with osteoporosis, men with osteoporosis had a higher CTX/OCT ratio than women with osteoporosis. Moreover, men of 65 and under 80 years of age showed a significant decrease in OCT levels compared with women of the same age group. An increase in the ratio of the bone biomarkers indicated that bone formation relative to the bone absorption did not increase as much in men as in women.

In both the sexes, hormones restrain an overactivity of bone remodeling to maintain bone density. Therefore, hormones are considered as the principal factor for bone health in men and women. Most cells involved in the bone metabolism possess an estrogen receptor that acts to modulate the activity of cells. However, osteoclasts do not have an androgen receptor.[22] Osteoclasts are modulated by the receptor activator of nuclear factor-kappa B, (RANK) ligand (RANKL), and osteoprotegerin (OPG) produced by osteoblasts and osteocytes. OPG is a decoy of the RANKL, which activates osteoclasts. Increased RANKL/OPG ratio activates osteoclasts. Androgen maintains osteoclast activity by suppressing the production of OPG in a dose-dependent manner.[23] Thus, androgen acts directly on osteoblasts by stimulating their proliferation, but also acts indirectly on osteoclasts.[24,25] Androgen does not directly affect osteoclast. However, estrogen stimulates production of OPG in osteoblasts.[26] As a result, sex hormones exhibit sexual dimorphism in bone metabolism.

Transient receptor potential vanilloid 4 (TRPV4) is a Ca^{2+}-permeable non-selective cation channel expressed in osteoblast and osteoclast cells.[27] TRPV4 protein has been implicated in unloading induced bone loss. In an animal study, male TRPV knockout mice attenuate the osteoblast activity resulting in osteoblast-osteoclast uncoupling.[28]*TRPV* gene variant affects the fracture in elderly men but not in women. Therefore, this genetic factor can influence an attenuation in bone formation activity corresponding to the bone resorption in men with osteoporosis.

Table 3

Differences in bone biomarkers between sexes according to age group.

	OCT, ng/mL	CTX, ng/mL	ALP, IU/L	Vitamin D (25-D), ng/mL	Ratio of bone marker
N<65					
Male (N=9)	20.34±6.66	0.54±0.25	93.33±31.81	20.80±8.87	2.84±1.25
Female (N=11)	16.24±6.65	0.40±0.22	86.18±22.72	80.81±9.59	2.68±1.47
P-value	.18	.22	.56	.99	.796
65≤N<80					
Male (N=46)	15.26±7.41	0.48±0.24	98.31±50.94	19.94±10.74	3.65±2.25
Female (N=48)	18.31±6.71	0.46±0.23	100.38±40.27	18.80±9.92	2.78±1.73
P-value	.01*	.89	.79	.51	.01*
N≥80					
Male (N=9)	13.00±3.97	0.74±0.20	92.55±42.05	22.25±14.98	4.32±3.24
Female (N=16)	17.69±6.75	0.62±0.30	116.56±61.08	15.79±11.09	3.69±1.93
P-value	.07	.195	.30	.23	.55

Values are presented as the mean±standard deviation. CTX=carboxyl-terminal collagen terminal collagen crosslinks, ratio of CTX/OCT bone marker, OCT=osteocalcin.
* $P<.05$, *P*-value of *t* test between men and women in >65 years old groups.

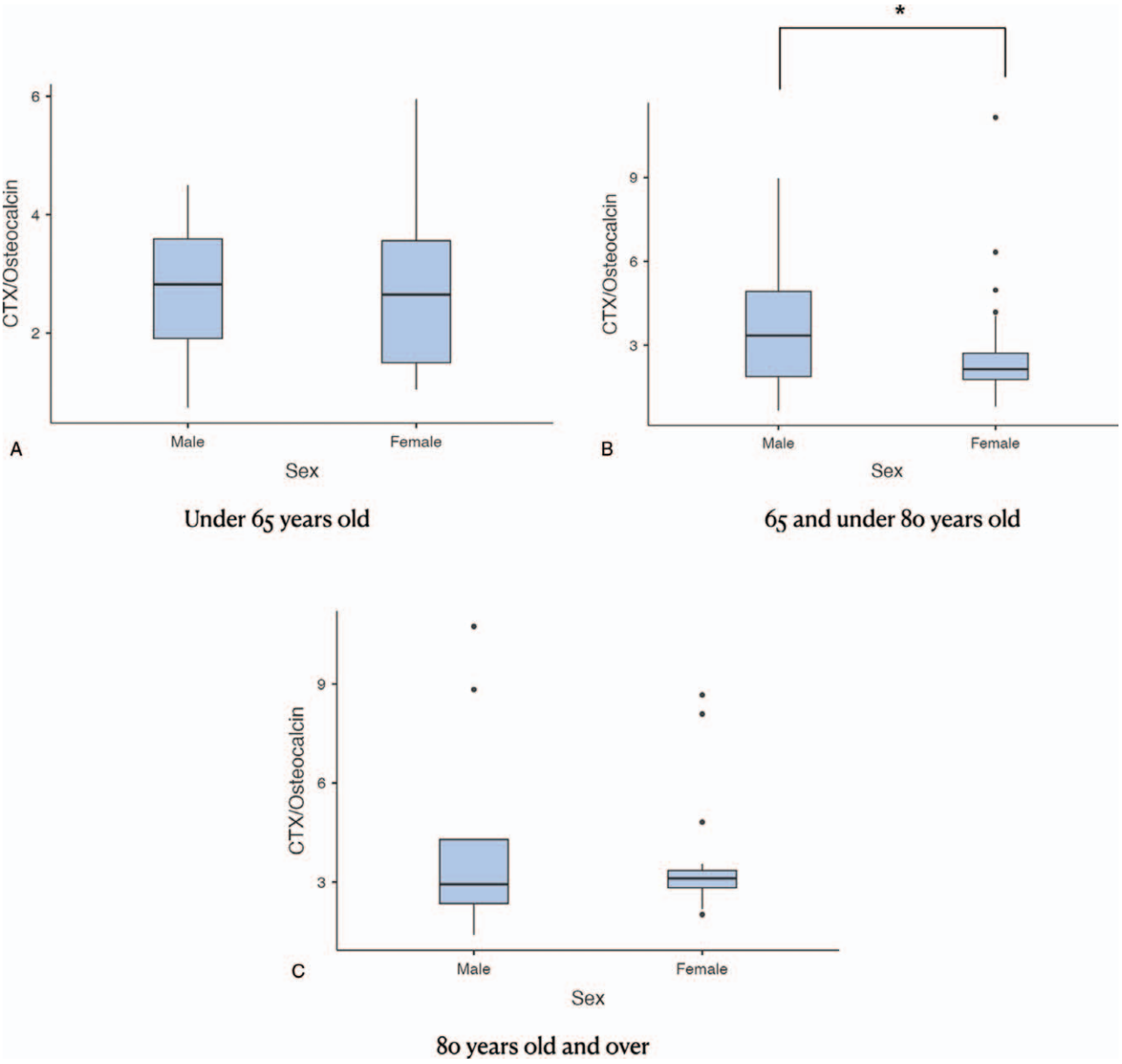

Figure 1. Men of 65 and under 80 years old showed an increased ratio of bone biomarkers compared with women. However, other age groups did not show a statistical difference in bone biomarkers' ratio between sexes. OCT = osteocalcin, $^{*}P < .05$.

Presently, as the bone remodeling increased, bone absorption and formation increased concurrently. The bone matrix factors produced by bone absorption and osteoclast-derived stimulators enhance bone formation by enhancing recruitment, proliferation, and differentiation of osteoblasts.[29] Increased bone absorption stimulates bone formation activity. Therefore, the CTX/OCT ratio remained constant with or without osteoporosis. However, men with osteoporosis showed a higher ratio of bone biomarkers than women without significant difference in CTX, indicating that bone formation in response to bone resorption does not respond effectively. Moreover, the mean plasma level of OCT was lower in men ≧65 years of age compared with those <65 years of age. The findings indicate that more elderly men are relatively less active than women in bone formation or osteoblast activity, resulting in decreased OCT level. The interplay between bone absorption and formation may not be as efficient in men.

The rate of hormone reduction differs between men and women. Climacteric women experience a prominent decline in estrogen concentration, while men have a gradual decline of androgen concentration.[30] In men of 75 years of age, the concentration of testosterone is approximately two-thirds the level at 25 years of age.[31] Bioavailable testosterone level decreases more prominently in older men relative to the slower rate of decrease in total testosterone. However, only 34% of 60-year-old men showed subnormal level of the available index. In this study, men had a higher BMD T-score than women. We speculate that the difference in the rate of hormone reduction is one of the factors for the difference in BMD between sexes.

In addition to androgen, estrogen derived from the aromatization of androgen is also involved in bone metabolism. Estrogen is the main modulator of bone absorption in men.[32] The bioavailability of estrogen produced by aromatization of androgen decreases by 47% in old age.[33] Therefore, both men and women showed increased CTX levels corresponding to decreased activation of estrogen, resulting in the upregulation of bone absorption.

Men and women have different social and biological characteristics. We anticipated that these differences would affect bone metabolism, as reflected by the differences in bone biomarker between sexes. Regardless of sex, bone biomarker levels increased in patients with osteoporosis without a change in the biomarker ratio, indicating that bone turnover was increased in both sexes, resulting in osteoporosis. The levels of bone formation and absorption biomarkers are higher in postmenopausal women.[34,35] Bone formation takes longer than bone absorption.[36] Cessation of estrogen produces high bone turnover. Therefore, extensive bone remodeling would decrease the maturation of the three-dimensional collagenous structure, which decreases bone density and quality.[37] Increased bone turnover is the cause of osteoporosis in men as well as women.

Osteoblasts contain a nicotinic receptor.[38] The cells respond differently depending on the level of nicotine. A low level of nicotine increases osteoblast activity, while a high level of nicotine inhibits osteoblast activity or enhances apoptosis.[39] Habitual smokers maintain high level of nicotine concentration,[40] which decreases the activity of osteoblasts, resulting in decreased bone formation.[41] Smoking decreases the mean serum level of OCT without affecting bone absorption biomarker.[42] The prevalence of smoking tends to be higher in men, as does their exposure to the secondhand smoke. In 1989 in South Korea, for example, the smoking prevalence of men and women was 77.8% and 2.4%, respectively.[43] Osteoblasts become less active in persons who are more exposed to a smoking environment. As bone turnover increases, an increase in bone formation may not sufficiently compensate for increased bone absorption. Therefore, smoking can be a potential factor for the low level of OCT in South Korean male patients with osteoporosis. However, we did not have data concerning smoking in this study. The correlation between smoking and OCT levels in patients with osteoporosis will have to be addressed in another study.

Our study has certain limitations. The first is the control of the bone biomarker sampling conditions. Bone biomarkers display circadian variation.[44] Thus, sampling time can affect the serum levels of bone biomarkers. In this study, sampling was done in the morning without setting an exact time. Considering that an increase in serum level of bone biomarkers was observed before sleep, the same morning sampling minimized variations in serum level of bone biomarker. The second is the adjustment for other confounders associated with bone metabolism. As our study is retrospective study based on medical record, there was not enough information concerning calcium supplement use, dietary calcium intake, obesity, alcohol abuse, etc.

In conclusion, men and women with osteoporosis showed increased bone turnover. Mean BMD was higher in men than in women. Men with osteoporosis showed a decreased mean OCT serum level compared with women with osteoporosis. Therefore, based on sex dimorphism in bone metabolism, further study is needed concerning treatment strategy in men's osteoporosis.

Author contributions

Conceptualization: Dong Gyu Lee.
Data curation: Kyu Hwan Choi, Jong Ho Lee.
Funding acquisition: Dong Gyu Lee.
Methodology: Jong Ho Lee.
Project administration: Dong Gyu Lee.
Supervision: Dong Gyu Lee.
Visualization: Dong Gyu Lee.
Writing – original draft: Kyu Hwan Choi.
Writing – review & editing: Dong Gyu Lee.

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