ORIGINAL ARTICLE

Association of Bone-related Biochemical Markers and Risk of Prehypertension in Osteoporotic Elders

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Abstract

Context: The association of bone turnover markers (BTMs) and cardiovascular disease had been representing. **Aims:** The study aimed to evaluate the association of osteoporosis status and prehypertension in elders, who were indicated by bone related - and cardiovascular risked - biochemical markers. **Settings and Design:** A cross-sectional study was carried out in Samut Songkhram, Thailand, and 105 elders were joined. **Materials and Methods:** Bone mineral density (BMD) was measured by calcaneal quantitative ultrasound. BTMs (osteocalcin [OC], Procollagen type 1 N-terminal pro-peptide, Beta-crossLaps [CTX], and alkaline phosphatase [ALP]); bone-related biochemical markers (25-hydroxyvitamin D [25-(OH)D], calcium, phosphorus, and magnesium); lipid profile (cholesterol, triglyceride, high density lipoprotein-cholesterol, and low density lipoprotein); and high sensitive C-reactive protein (hs-CRP) were analyzed using automatic analyzers. Blood pressure measurement was done after resting. **Statistical Analysis Used:** Descriptive data were represented as a mean ± standard deviation. One-way ANOVA was compared parameters within three elders groups. Pearson's correlation was tested for the relations of BMD with other parameters. Odds ratio was calculated for risk of prehypertension. **Results:** BMD was significantly correlated to calcium, phosphorus, and triglyceride in high, low, and very low levels, respectively. Inversely correlations of BMD with CTX and hs-CRP were significant in medium level. Risk of prehypertension was increased 1.12-fold with a decrement of T-score < −1.0. Increment of CTX, hs-CRP, and LDL-C was increased 1.93, 1.94, and 1.31-fold of prehypertension risks, respectively. **Conclusions:** Prehypertension was associated with osteoporosis, which indicated by inversely correlation of hs-CRP and CTX to BMD; and correlation of serum calcium, phosphorus, and triglyceride to BMD.

Key words: Biochemical markers, bone mineral density, bone turnover markers, cardiovascular disease, osteoporosis

INTRODUCTION

The association of bone turnover markers (BTMs) and cardiovascular disease had been representing.[1] Bone calcium and phosphorus are played role of bone metabolism. Serum calcium and phosphate homeostasis are maintain many physiologic conditions, including cell interaction, cardiovascular function; and act as catalyst ions for metabolic pathways. The vascular calcification is related to serum calcium and phosphate,[2] which are link to coronary artery disease (CAD),[3] myocardial infarction (MI),[4] and heart failure,[5] and mortality is caused by CAD.[6] The level of serum calcium is positive correlate to risk factors of cardiovascular disease such as hypertension, dyslipidemia, hyperglycemia, and obesity.[7,8] The low serum 25-hydroxyvitamin D (25-OH-D), is associated with the occurrence and severity of CAD, which may due to regulation of mineral status in bone turnover mechanism.[9,10]

The serum BTMs are indication of recent bone remodeling stage. Many previous studies had been reported that osteocalcin

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(OC), a non-collagen protein is regulates various metabolic pathways including bone metabolism.[11] The OC level is associated to metabolic disorders, especially Type 2 DM and obesity.[12] The relationship of serum OC and calcification of atherosclerotic plaque had been reported in patients on the early stage of atherosclerosis. Procollagen type 1 N-terminal pro-peptide (P1NP) is secretary protein from osteoblasts during the collagen Type I synthesis, which is usually detection for osteoporosis management.[13] An extracellular matrix predominantly comprises Type I and III collagen and also surrounded with cardiac myocytes and excessive of myocardial collagen deposition cardiac hypertrophy are occurred in MI and congestive heart failure.[13] The lower levels of P1NP are associated with hyperinsulinemia and hyperglycemia in healthy individuals.[14] Beta-crossLaps (CTX) is bone resorptive marker, which is produced from osteoclasts during bone resorption and associate to diabetes. Recently, various studies were reported that serum levels of OC and CTX were lower in diabetes patients when compared to controls.[15] Moreover, CTX is demonstrating as a predictive factor of an increased carotid intima-media thickness in the elderly population.[16]

Elevating of Thai aging population is a trend to increase with the number of osteoporosis in elderly[17] and will be fast increase along with common chronic diseases, such as diabetes mellitus and cardiovascular diseases.[18,19] From Thai nation-wide survey (during 2000–2001), the age-adjusted prevalence of osteoporosis in Thai women ranging in age from 40 to 80 years was 13.6% and 19.8% for femoral neck and lumbar spine, respectively. For men, the age-adjusted prevalence of osteoporosis was 12.6, 4.6, and 3.9% at the femoral neck, lumbar spine, and both sites, respectively. The Asian osteoporosis study was multi-national research surveillance, which had documented the incidence of hip fracture in Thailand. The report of age-adjusted rates (per 100,000) was 114 and 289, in men and women, respectively.[17,20] Vitamin D insufficiencies of Thai elderly women in the urban area were higher than in rural area because of the difference in lifestyle.[21,22] Study on the multi-centers from 5 provinces of Thailand which cover all region of Thailand except southern area which represented calcidiol level of Thai premenopausal women was 29.09 (±0.42) ng/ml, and with the cut point of <or = 35 ng/ml; the prevalence of vitamin D insufficiency was 77.81%, and parathyroid hormone (PTH), and bone resorption markers were trended to increase.[9] Thai premenopausal women were commonly hypovitaminosis D and low level of 25-(OH)D and risk will be increased in elderly women who are living in the rural area.[22] The prevalence of vitamin D insufficiency among elderly males was 13.6%, while the BTM (P-CTX and PINP) levels were in the normal Thai reference range.[23] The numbers of elders in Samut Songkhram province are trend to increase, and approximately 16.7% of elders were home, and bed bounded elders, which were difficult to medical service due to the limitations of medical staff and equipment. Early diagnoses of osteoporosis before fractures occurring and early assessment of bone mineral density (BMD) with osteoporosis treatment can be providing a better clinical outcome. The hypertension and dyslipidemia were also usually occurred in this coastal area including Samut Songkhram province due to high salt and lipid food consumption, especially seafood intake.[24,25] This recent study aimed to evaluate the association of osteoporosis status and prehypertension in elders, who were indicated by bone related - and cardiovascular risked - biochemical markers.

MATERIALS AND METHODS

Participated elders and demographic data

The cross-sectional study was carried out from December 2017 to February 2018 (this period including public relation and health service), to determine BMD; BTMs including OC, P1NP, CTX, and alkaline phosphatase (ALP); bone-related biochemical markers, including 25-(OH)-D, calcium, phosphorus, and magnesium; lipid profile (including cholesterol, triglyceride, and high-density lipoprotein-cholesterol [HDL-C] and low-density lipoprotein-cholesterol [LDL-C]) and high sensitive C-reactive protein (hs-CRP); and blood pressure from 105 elders who joined as volunteers in academic health service program at Amphawa district, Samut Songkhram, Thailand, which was responsible by Samut Songkhram campus, Suan Sunandha Rajabhat University. Informed consent of all participants was done, and study protocol was approved by the Ethical Review Committee. The inclusion criteria: (1) Male and female elders were aged ≥60 years old; (2) consciousness; and interactive; (3) had not severe medical conditions. The exclusion criteria: (1) Elder was combined with metabolic bone disease (osteomalacia, Paget disease, or primary hyperparathyroidism); (2) had an accident especially with fracture; (3) taking daily calcium and Vitamin D supplements; and (4) pathological fracture (secondary osteoporosis). Anthropometric data, medical history, and health behaviors (including related risks) were recorded by physical examined and interviewed. The elders with poor literacy and/or unable to read as well were helped for filling the form of questionnaire.

BMD, blood pressure, and biochemical measurements

BMD was measured by calcaneal quantitative ultrasound, ultrasound bone densitometer (SONOST-2000, OsteoSys, Korea) and the instrument protocol and data interpretations were followed manufacturer instruction. According to BMD status, elders were divided to osteoporotic (T score at or below −2.5), osteopenia (T score between −1.0 and −2.5), and normal (T score at -1.0 and above) elders. Each blood pressure measurement was done at resting (after 5 minute resting and average value calculated from 2 times), and interpretation of blood pressure value for hypertension

was >139 mmHg of systolic blood pressure (SBP) and between >90 mmHg of diastolic blood pressure (DBP) and prehypertension was defined as SBP >120 and <140 mmHg or DBP >80 and <84 mmHg.[26] Each 6 ml of fasting blood sample was obtained by venipuncture from median cubital vein during morning (7–9 a.m.). 6 ml of blood sample was drawn into clotting blood tube for centrifuged and processed within 2 h after phlebotomy and stored at −20°C.[27] Each serum concentrations of BTMs (P1NP, ALP, CTX, and OC), 25-(OH)D and bone-related minerals, calcium, phosphorus, and magnesium were determined by Cobas E411, Cobas E601, and Cobas E501 automatic analyzers (Roche Diagnostics, Switzerland), respectively. The determination of lipid profile was including triglyceride, cholesterol, HDL-C, and LDL-C, and hs-CRP, which was analyzed by automatic analyzer, COBAS Integra ® a 400 plus (Roche-diagnostics, Switzerland). The within-run and between-run CVs were <10%. Control materials and pooled serum were also done prior sample test for accuracy checking.

Statistical analysis

Descriptive data of BMD, biochemical parameters and blood pressure from all elders were represented as mean ± standard deviation. The Kolmogorov–Smirnov test was used to test for normal distribution. One-way ANOVA was used to compare continuous variables within normal, osteopenia, and osteoporosis elders, significance level at $P < 0.05$. Pearson's correlation was tested for the relations of BMD with continuous variables. Odds ratio (OR) was calculated for risk of prehypertension, which indicated by BMD, CTX, hs-CRP, and LDL-C. All statistical analysis was operated under SPSS 20.0 software (SPSS, Chicago, Illinois, USA).

RESULTS

According to T-score of BMD, the number of normal, osteopenia, and osteoporosis were 48, 21, and 36, respectively [Table 1], and more than 95% were female. Mean of serum phosphorus, magnesium, ALP, 25-(OH)D, OC, total P1NP, hs-CRP, and lipid profile from all elders was within normal range. However, average of T-score (BMD) and serum calcium in elders was lower than the normal range, while CTX was higher level than normal [Table 2]. Moreover, mean of SBP and DBP values were ranged as prehypertension. Serum calcium, phosphorus, 25-(OH)D, CTX, hs-CRP, triglyceride, SBP, and DBP among normal, osteopenia and osteoporosis group were significantly different [Table 2]. BMD was significant correlated to serum calcium, phosphorus, and triglyceride in high ($r = 0.7–0.9$), low ($r = 0.3–0.5$), and very low ($r = 0.00–0.3$) levels, respectively. Inversely correlations of BMD with CTX and hs-CRP were significant in medium level ($r = 0.5–0.7$) [Table 3]. However, other markers were not significantly correlated to BMD. The risk of prehypertension was increased 1.12-fold with decrement of T-score < −1.0 (OR = 1.12, 95% confidence interval [CI] = 0.65–1.92). Increment of CTX, hs-CRP, and LDL-C was increased 1.93, 1.94, and 1.31-fold of prehypertension risks (OR = 1.934, 95% CI = 1.10–3.42; OR = 1.94, 95% CI = 0.77–4.89; OR = 1.31, 95% CI = 0.63–2.73), respectively [Table 4].

DISCUSSION

More than half of elders in this study were low BMD rather than normal. Normal serum phosphorus, magnesium, ALP, 25-(OH)D, OC, and total P1NP were summarized that elders in the study area had a normal bone formation with sufficient Vitamin D intake. Moreover, lipid profile and hs-CRP of elders were also trended to normal. However, low serum calcium and high CTX levels were represented increment rate of bone resorption, especially in osteopenia and osteoporosis. In addition, mean of SBP and DBP were within prehypertension. Hence, there were implied that osteoporosis or low BMD may relate to prehypertension, which was defined as blood pressure elevated above normal, but not to the level considered hypertension. When compared the bone-related - and cardiovascular risked - biochemical markers among three elder groups, we were found serum calcium, phosphorus, 25-(OH)D, CTX, hs-CRP, and triglyceride, SBP and DBP were significantly different, which were confirmed bone turnover status associate to blood pressure; and cardiovascular risks. The strong and inversely medium correlation of serum calcium, CTX and hs-CRP to BMD were represent bone resorption and risk of cardiovascular diseases may implied association. In addition, low and very low correlation of serum phosphorus and triglyceride were supported the association of osteoporosis and risk of cardiovascular diseases by bone and lipid metabolisms. 25-(OH)D level was slightly different among three elder groups, however, no correlation of 25-(OH)D to BMD, which may due to average value within the normal range. Further, we were calculated OR for evaluate effects of BMD, CTX, hs-CRP, and LDL-C level on prehypertension risks and all of them had OR >1.0 in out of normal range group. Thus, BMD, CTX, hs-CRP, and LDL-C levels were the markers, indicated risk of prehypertension and also may be markers of cardiovascular diseases in osteopenia and osteoporotic elders.

The relationship between osteoporosis and atherosclerosis had been report in cardiovascular disease patients.[28,29] The aortic calcification is commonly occur in women with osteoporotic fractures, and degree of calcification is inversely related to BMD.[28,30] Low BMD in menopause is a risk factor of cardiovascular disease, and an increase of mortality[31] and both of osteoporosis and aortic calcification are increasing with age.[32] Reduction of estrogen is affect to decrease BMD in postmenopause and then be associated with oxidized LDL-C production.[33] Bone osteoblast differentiation is minimally inhibited by oxidized LDL, however, there can induce osteoblast-like cell differentiation, which locates

Table 1: The summary of BMD screening within elders for interpretation as normal, osteopenia, and osteoporosis groups

Group	BMD	Mean±SD of BMD	Frequency
Normal	T score > −1.0	−0.72±0.17	48
Osteopenia	T score ≤ −1.0 to ≥ −2.5	−1.30±0.56	21
Osteoporosis	T score < −2.5	−2.89±0.26	36

BMD: Bone mineral density, SD: Standard deviation

Table 2: Descriptive data of BMD, biochemical parameters, and blood pressure from all elders (*n*=105); and significantly different of each parameter within normal, osteopenia, and osteoporosis group

Parameter	Normal range	Mean±SD	*P* value
Age	-	65.7±12.0	-
BMD	T score > −1.0	−1.57±1.08	-
Calcium	8.6–10.2 mg/dL	7.81±1.50	<0.0001*
Phosphorus	2.7–4.5 mg/dL	3.18±0.72	<0.0001*
Magnesium	1.40–2.10 mEq/L	1.60±0.31	0.121
ALP	39–105 U/L	53.54±8.70	0.308
Vitamin D (25-OH-D)	≥30 ng/mL	42.24±7.68	0.045*
Osteocalcin (OC)	11.5–29 ng/mL	16.58±3.54	0.975
Total P1NP	15.0–74.0ng/ml	46.21±14.91	0.366
Beta-CrossLaps (CTX)	0.0–0.32 ng/ml	0.38±0.16	<0.0001*
hs-CRP	<1.0 mg/L	0.63±0.57	<0.0001*
Cholesterol	<200 mg/dL	167.68±27.89	0.33
Triglyceride	50–200 mg/dL	114.90±42.77	0.008*
HDL-C	>35 mg/dL	57.37±11.11	0.141
LDL-C	<130 mg/dL	88.50±25.38	0.790
SBP	≤120 mmHg	126.12±6.84	<0.0001*
DBP	≤80 mmHg	82.87±2.11	0.002*

*Significant at *P*<0.05 01 level (2-tailed). BMD: Bone mineral density, ALP: Alkaline phosphatase, P1NP: N-terminal extension pro-peptide of type-I collagen, hs-CRP: High sensitive C-reactive protein, HDL-C: High-density lipoprotein-cholesterol, LDL-C: Low-density lipoprotein-cholesterol, SBP: Systolic blood pressure, DBP: Diastolic blood pressure

in the artery wall and increase vascular mineralization and calcification.[34] Low level of estrogen is also related to increase parathyroid hormone and affect to BMD reduction;[35] and associate to cardiovascular disease by an increase of homocysteine in elders.[36] As our results, increase of hs-CRP in osteoporosis elders with prehypertension was corresponded to the previous study by increase of oxidative markers as cardiovascular risk or hypertension. Moreover, inversely correlation of hs-CRP and CTX to BMD was revealed the risk of cardiovascular disease associated to the rate of bone resorption. In addition, the relation of serum calcium, phosphorus, and triglyceride to BMD were also supported the role of calcium in lipid metabolism. In this study, no relation of 25-(OH)D of to BMD and also the risk of cardiovascular disease in elders may due to sufficient consumption.

However, disagreement results from recent studies had been reported the lack of significant association of PTH, 25-(OH)D, OC, CTX and P1NP with coronary atherosclerosis (CAD); and increment of serum calcium levels were independently associated with CAD in postmenopausal women.[1] Moreover, only CTX and OC were proper bone markers in CAD patients with Type 2 DM,[19] however, no significant relation of OC to cardiovascular risk in our study. Study in Thai population was conducted to compare bone turnover between healthy people and CAD patients using biochemical markers of bone formation and resorption; and found that CAD patients have no higher risk for osteoporosis than healthy people.[37] Due to the limitation of our study, such as small sample size and cross-sectional study (not longitudinal study), we did not monitoring of biochemical markers and the variation of clinical appearance including blood pressure and BMD. Lack of more evidence are support and control confounding of data for a study on the association of osteoporosis and cardiovascular disease still needs, such as data from cardiovascular patients, behavior risk, and medical history records.

Table 3: The correlation of BMD with bone-related - and cardiovascular risked - parameters in elders (*n*=105)

Dependent parameter	BMD relation (Pearson correlation coefficient, *r*)	*P* value
Calcium	0.873	<0.0001**
Phosphorus	0.382	<0.0001**
Magnesium	0.179	0.680
ALP	−0.011	0.909
Vitamin D (25-OH-D)	0.041	0.677
Osteocalcin (OC)	−0.051	0.608
TotalP1NP	0.068	0.493
Beta-CrossLaps (CTX)	−0.611**	<0.0001**
hs-CRP	−0.685**	<0.0001**
Cholesterol	0.108	0.273
Triglyceride	0.263**	0.007**
HDL-C	−0.052	0.601
LDL-C	0.064	0.517
SBP	−0.043	0.665
DBP	−0.154	0.118

**Significant at *P*<0.01 level (2-tailed). BMD: Bone mineral density, ALP: Alkaline phosphatase, P1NPL: N-terminal extension pro-peptide of type-I collagen, hs-CRP: High sensitive C-reactive protein, HDL-C: High-density lipoprotein-cholesterol, LDL-C: Low-density lipoprotein-cholesterol, SBP: Systolic blood pressure, DBP: Diastolic blood pressure

Table 4: The range of BMD, hs-CRP, and LDL-C affected on risk of prehypertension[a]

Risk parameter	Range	OR	95% CI	
			Lower	Upper
BMD	T score ≥ −1.0	0.944	0.718	1.241
	T score < −1.0	1.122	0.653	1.925
CTX	≤0.32 ng/ml	0.718	0.536	0.960
	>0.32 ng/ml	1.934	1.095	3.415
hs-CRP	<1.0 mg/L	0.779	0.598	1.015
	≥1.0 mg/L	1.938	0.768	4.886
LDL-C	<130 mg/dL	0.904	0.706	1.159
	≥130 mg/dL	1.314	0.632	2.734

[a]Systolic blood pressure>120 and<140 mmHg or diastolic blood pressure>80 and<84 mmHg. CTX: Beta-CrossLaps, hs-CRP: High sensitive C-reactive protein, LDL-C: Low-density lipoprotein-cholesterol, CI: Confident interval, BMD: Bone mineral density, OR: Odd ratio

CONCLUSION

Serum calcium, phosphorus, 25-(OH)D, CTX, hs-CRP, triglyceride, SBP and DBP among normal, osteopenia and osteoporosis group were significant differences. Prehypertension was associated to osteoporosis, which indicated by inverse correlation of hs-CRP and CTX to BMD; and correlation of serum calcium, phosphorus, and triglyceride to BMD. Decrement of BMD and increment of CTX, hs-CRP, and LDL-C were increased risk of prehypertension. BMD, CTX, hs-CRP, and LDL-C were indicated prehypertension, risk of cardiovascular diseases in osteopenia and osteoporotic elders.

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