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ORIGINAL ARTICLE

Decreased Cathepsin K Plasma Level may Reflect an Association of Osteopoenia/osteoporosis with Coronary Atherosclerosis and Coronary Artery Calcification in Male Patients with Stable Angina

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Background The aim of this study was to evaluate the plasma levels of bone turnover markers (BTMs) in male patients with stable angina depending on the bone mineral density (BMD), coronary atherosclerosis (CA) and coronary artery calcification (CAC).

Methods We recruited 112 males with verified stable angina. All the patients underwent coronary angiography, multislice spiral computed tomography, and dual-energy X-ray absorptiometry. Plasma levels of BTMs were measured by enzyme-linked immunosorbent assay.

Results Osteopoenia and osteoporosis were reported in 90 (80.4%) and 34 (30.4%) patients, respectively. Multivessel coronary artery disease, severe CA and CAC, decreased cathepsin K plasma level, and increased osteocalcin plasma level were significantly more prevalent in patients with osteopoenia/osteoporosis compared to the subjects with normal BMD. Patients with severe CA and CAC had significantly reduced cathepsin K plasma levels.

Conclusions We revealed a significant association of osteopoenia/osteoporosis with severe CA and CAC in males with stable angina. Cathepsin K and osteocalcin plasma levels may be suggested as the significant markers of osteopoenia/osteoporosis. In addition, cathepsin K plasma level can be also a valuable marker of severe CA and CAC.

Keywords Stable angina • Calcification • Osteopoenia/osteoporosis • Cathepsin K • Osteocalcin

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Introduction

Decrease of bone mineral density (BMD), clinically defined as osteopoenia and osteoporosis, is amongst the most common diseases in the Western world, particularly in the elderly [1]. Fractures are the most significant problem associated with osteopoenia and osteoporosis: they are highly prevalent, costly, and become a chronic burden on both individuals and society [1]. Moreover, osteopoenia and osteoporosis are now considered as the independent risk factors of coronary atherosclerosis (CA), coronary artery calcification (CAC), and cardiovascular events [2,3]. It was suggested that vascular calcification may be caused by the bone turnover alterations related to osteopoenia and osteoporosis [4]. The levels of osteocalcin, osteonectin, osteopontin, and other bone-specific proteins are considerably higher in atherosclerotic plaques compared to healthy tissues [5]. In addition, ossification is the common finding in atherosclerotic plaques and calcified heart valves [6]. Therefore, we carried out this study with the aim to evaluate the plasma levels of bone turnover markers (BTMs) in patients with stable angina depending on BMD, CA, and CAC.

Materials and Methods

We recruited 112 male patients with stable angina who were admitted to the Research Institute for Complex Issues of Cardiovascular Diseases (Kemerovo, Russian Federation) in 2014 (Table 1). Mean age was 59.8 (from 55 to 70) years. The study was performed in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki, was approved by the local ethical committee, and written informed consent was provided by all the participants after a full explanation of the study was given to them.

The criteria of inclusion into the study were 1) age ≤75 years; 2) diagnosis of stable angina according to the Canadian Cardiovascular Society (CCS) guidelines [7]; 3) written informed consent to participate in the study. Criteria of exclusion were 1) age >75 years; 2) refusal to write an informed consent to participate in the study; 3) past medical history of cancer, concomitant rheumatic, endocrine, digestive, blood and/or mental disorders, coronary revascularisation, prolonged immobility, chronic obstructive pulmonary disease, alcoholism; 4) use of glucocorticoids >3 months.

All the patients underwent coronary angiography, multislice spiral computed tomography, and dual-energy X-ray absorptiometry. Coronary angiography was performed using GE Healthcare Innova 3100 Cardiac Angiography System (General Electric Healthcare, USA). Multislice spiral computed tomography was conducted by SOMATOM Sensation 64 (Siemens Healthcare, Germany) using Leonardo multimodality workstation (Siemens Healthcare, Germany). Coronary artery calcification was assessed by Agatston score. Patients with Agatston score >75th percentile for their age were considered as having a high risk of cardiovascular events. Dual-energy X-ray absorptiometry was carried out using Norland XR-46 bone densitometry system (Orthometrix, USA), and the results were interpreted according to the International Society for Clinical Densitometry Official Positions [8]. Bone mineral density of not more than 1 standard deviation (SD) below the mean peak bone mass (average of healthy young adult of the corresponding gender) at the lumbar spine (L_I-L_{IV}) and femoral neck was considered as

Table 1 Clinicopathological features of the patients

Feature	Value
Age, median with interquartile range, yrs	61 (55.5;66)
Arterial hypertension, n (%)	109 (97.3)
Past medical history of myocardial infarction, n (%)	95 (85)
Diabetes mellitus, n (%)	12 (11)
Family history of myocardial infarction or stroke in males < 55 yrs and females < 65 yrs, n (%)	15 (12)
Left ventricular ejection fraction, median with interquartile range, %	56 (48;63)
Polyvascular disease, n (%)	17 (15)
Stable CCS class I angina, n (%)	5 (4.5)
Stable CCS class II angina, n (%)	59 (52.5)
Stable CCS class III angina, n (%)	48 (43)
NYHA class of chronic heart failure, median with interquartile range	2 (1;3)
NYHA class I chronic heart failure, n (%)	39 (34.6)
NYHA class II chronic heart failure, n (%)	49 (44)
NYHA class III chronic heart failure, n (%)	22 (19.6)
NYHA class IV chronic heart failure, n (%)	2 (1.8)
Body mass index, median with interquartile range, kg/m^2	28 (25;30)
Glomerular filtration rate (MDRD formula), median with interquartile range, mL/min/1.73 m^2	102 (85;123)

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normal. Osteopoenia and osteoporosis were defined as a BMD of 1-2.49 SDs and ≥2.5 SDs below the mean peak bone mass, respectively.

Venous blood was withdrawn at the time of hospital admission. Plasma was obtained with a centrifugation for 15 min at 1,780 × g and −4 °C; 300 µl aliquots were stored at −80 °C until determination. Plasma levels of BTMs (parathyroid hormone, Diagnostic System Laboratories, USA; osteocalcin, IDS, USA; bone alkaline phosphatase, Quidel Corporation, USA; osteopontin, Enzo Life Sciences, USA; cathepsin K, Biomedica, Austria; insulin, Monobind Inc., USA; estradiol and testosterone, Diagnostics Biochem Canada Inc., Canada; calcitonin and osteoprotegerin, Biomerica, USA) were measured in all the patients by enzyme-linked immunosorbent assay according to manufacturer's instructions. All samples were plated in duplicates, and average concentrations were used for further analysis. Plasma levels of total and ionised calcium level, phosphorus, and alkaline phosphatase were measured by commercially available kits (Bio-Sys, Germany) using Konelab 20i clinical chemistry analyser (Thermo Scientific, USA).

Statistical analysis was performed using STATISTICA 6.0 (StatSoft, USA). A sampling distribution was assessed by D'Agostino-Pearson test. Data were represented by the median, the interquartile range (25th and 75th percentiles), the mean and the confidence intervals (CIs) for both the median and mean. Two independent groups were compared by Mann-Whitney U-test, three and more independent groups were compared using Kruskal-Wallis test with the further pairwise multiple comparisons by Mann-Whitney U-test if statistically significant differences were revealed by Kruskal-Wallis test. An adjustment for multiple comparisons was performed using false discovery rate (FDR). P-values, or q-values if FDR was applied (q-values are the name given to the adjusted p-values found using an optimised FDR approach), ≤ 0.05 were regarded as statistically significant.

Results

All the patients had no clinical manifestations of osteoporosis. However, only 22 (19.6%) patients had normal BMD, whilst 56 (50%) and 34 (30.4%) individuals had osteopoenia and osteoporosis, respectively (Table 2). According to the International Society for Clinical Densitometry Official Positions [8], we formed these groups using the SDs from the mean peak bone mass at the femoral neck since it was more

Table 2 Bone mineral density in patients with osteopoenia/osteoporosis and normal bone mineral density

Features	Osteopoenia/osteoporosis, n=90	Normal bone mineral density, n=22	P-value	Q-value
Femoral neck				
T-score	-2.07 (-3.0;-1.86)	-0.66 (-1.16;-0.33)	<0.0001	<0.0001
Bone mineral density, g/cm^3	922.1 (797.7;976.3)	1106 (1042;1183)	<0.001	<0.001
Lumbar spine (L_I-L_{IV})				
T-score	-1.08 (-1.53;-0.7)	0.09 (-0.45;0.42)	<0.0001	<0.0001
Bone mineral density, g/cm^3	1099 (996.3;1171)	1329 (1153;1372)	<0.001	<0.001

Table 3 Plasma levels of bone turnover markers depending on bone mineral density (median and interquartile range)

Feature	Osteopoenia/osteoporosis, n=90	Normal bone mineral density, n=22	P-value	Q-value
Cathepsin K, pmol/L	15.89 (0.01;19.69)	29.1 (6.77;42.46)	0.02	0.027
Bone alkaline phosphatase, U/L	20.96 (14.30;28.80)	13.40 (10.00;23.10)	>0.05	>0.05
Insulin, ng/mL	4.41 (2.45;10.32)	2.48 (0.25;4.24)	>0.05	>0.05
Estradiol, pg/mL	62.83 (45.75;91.34)	58.53 (44.47;67.62)	>0.05	>0.05
Testosterone, ng/mL	4.09 (3.45;4.80)	5.20 (3.68;5.56)	>0.05	>0.05
Osteocalcin, ng/mL	26.89 (15.83;30.13)	16.5 (8.03;18.14)	0.022	0.031
Osteopontin, ng/mL	6.71 (6.01;7.34)	7.05 (5.89;11.36)	>0.05	>0.05
Osteoprotegerin, pg/mL	139.24 (59.60;268.20)	125.77 (39.80;202.25)	>0.05	>0.05
Calcitonin, pg/mL	7.54 (6.97;8.64)	8.55 (7.41;9.68)	>0.05	>0.05
Parathyroid hormone, pg/mL	37.68 (16.86; 49.50)	36.7 (14.21;46.55)	>0.05	>0.05
Ca, mmol/L	2.43 (2.20;2.57)	2.50 (2.22;2.58)	>0.05	>0.05
P, mmol/L	0.91 (0.78;1.01)	0.81 (0.71;1.04)	>0.05	>0.05
Alkaline phosphatase, U/L	121.4 (39.5;202.5)	111.8 (47.0;177.6)	>0.05	>0.05
Ionised Ca, mmol/L	0.38 (0.36;0.39)	0.36 (0.32;0.40)	>0.05	>0.05

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Table 4 Severity of coronary atherosclerosis depending on osteopoenia/osteoporosis

Feature	Osteopoenia/osteoporosis, n=90	Normal bone mineral density, n=22	P-value	Q-value
Number of affected coronary arteries				
One	13 (14.4)	11 (50.0)	0.023	0.032
Two	23 (25.5)	6 (27.3)	>0.05	>0.05
Three	54 (60.1)	5 (22.7)	0.041	0.048
SYNTAX score				
Low (0-22)	35 (38.9)	15 (68.1)	>0.05	>0.05
Intermediate (23-32)	32 (35.5)	2 (9.0)	>0.05	>0.05
High (≥33)	23 (25.6)	5 (22.9)	>0.05	>0.05

significant compared to the lumbar spine. Antiplatelet drugs, beta-blockers, angiotensin-converting enzyme inhibitors, statins, and long-acting nitrates were administered to 109 (97.3%), 110 (98.2%), 108 (96.4%), 106 (94.6%), and 107 (95.5%) patients, respectively; there were no significant differences between the groups regarding the pharmacological treatment. There also were no significant differences in family history of cardiovascular events or glomerular filtration rate (GFR) between the groups, with a median GFR of 103 (87;122) and 101 (84;123) in patients with normal BMD and osteopoenia/osteoporosis, respectively.

We further compared plasma levels of various BTMs depending on BMD and revealed significantly decreased cathepsin K plasma levels and significantly increased osteocalcin plasma levels in patients with osteopoenia/osteoporosis in comparison to the subjects with normal BMD (Table 3). Moreover, multivessel coronary artery disease and severe CA and CAC were also significantly more prevalent in patients with osteopoenia/osteoporosis than in individuals with normal BMD (Table 4). Then, we detected significantly decreased cathepsin K plasma levels in patients with severe CA (Table 5). However, there were no statistically significant differences regarding plasma levels of other BTMs and CA severity. We also did not find any differences in plasma levels of BTMs between the groups with and without past medical history of myocardial infarction. We suggest that osteopoenia/osteoporosis and plasma levels of BTMs may be more closely related to CAC then to CA. Indeed, the prevalence of severe CAC was significantly higher in patients with osteopoenia/osteoporosis compared to subjects with normal BMD (Table 6). In addition, a low risk of fatal coronary events was significantly more frequently detected in subjects with normal BMD (Table 6). Finally, severe CAC was significantly associated with reduced plasma levels of cathepsin K and elevated plasma levels of osteoprotegerin (Table 7).

Table 5 Plasma levels of bone turnover markers depending on the severity of coronary atherosclerosis according to the SYNTAX score (median and interquartile range)

Feature	SYNTAX score 0-22 n=61	SYNTAX score 23-32 n=30	SYNTAX score ≥33 n=21	P1-2	Q1-2
Cathepsin K, pmol/L	18.45 (l4.30;28.75)	9.86 (0.01;29.28)	9.92 (0.01;23.66)	0.023	0.032
Bone alkaline phosphatase, U/L	21.01 (13.58;27.71)	20.59 (11.58;27.10)	18.59 (13.40;29.73)	>0.05	>0.05
Insulin, ng/mL	3.94 (1.58;11.33)	4.98 (2.27;8.68)	3.38 (0.88;9.15)	>0.05	>0.05
Estradiol, pg/mL	43.8 (16.5;46.9)	42.6 (18.3;47.1)	38.6 (16.2;41.1)	>0.05	>0.05
Testosterone, ng/mL	5.6 (3.4;6.2)	4.8 (3.1;5.8)	5.9 (2.9;7.1)	>0.05	>0.05
Osteocalcin, ng/mL	17.86 (13.74;22.54)	13.57 (8.24;18.70)	15.21 (8.25;20.97)	>0.05	>0.05
Osteopontin, ng/mL	6.82 (6.26;7.73)	6.78 (6.04;7.70)	6.04 (5.54;6.91)	>0.05	>0.05
Osteoprotegerin, pg/mL	195.20 (59.65;298.40)	128.20 (67.35;246.05)	187.65 (51.50;253.05)	>0.05	>0.05
Calcitonin, pg/mL	7.73 (6.89;8.56)	7.23 (7.01;10.97)	8.16 (7.19;9.43)	>0.05	>0.05
Parathyroid hormone, pg/mL	35.5 (21.38;44.65)	36.20 (12.94;46.05)	42.75 (14.21;48.90)	>0.05	>0.05
Ca, mmol/L	2.49 (2.20;2.59)	2.39 (2.21;2.60)	2.55 (2.24;2.67)	>0.05	>0.05
P, mmol/L	0.91 (0.80;1.03)	0.86 (0.71;1.00)	0.89 (0.78;1.01)	>0.05	>0.05
Alkaline phosphatase, U/L	60.00 (42.50;71.50)	54.50 (38.50;66.00)	59.50 (44.00;71.00)	>0.05	>0.05
Ionised Ca, mmol/L	0.38 (0.32;0.39)	0.37 (0.34;0.40)	0.38 (0.37;0.40)	>0.05	>0.05

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Table 6 Severity of coronary artery calcification depending on bone mineral density

Feature	Osteopoenia/osteoporosis, n=90	Normal bone mineral density, n=22	P-value	Q-value
Agatston score				
Low/minimal coronary artery calcification (Agatston score 0-10)	6 (6.7)	6 (27.2)	0.016	0.024
Mild/moderate coronary artery calcification (Agatston score 11-400)	28 (31.1)	8 (36.4)	>0.05	>0.05
Severe coronary artery calcification (Agatston score >401)	56 (62.2)	8 (36.4)	>0.05	>0.05
Risk of fatal coronary events				
0-25th percentile	11 (20.75)	10 (47.62)	0.047	0.050
25th-50th percentile	8 (15.09)	3 (14.29)	>0.05	>0.05
50th-75th percentile	19 (35.85)	4 (19.05)	>0.05	>0.05
75th-100th percentile	15 (28.3)	4 (19.05)	>0.05	>0.05

Table 7 Plasma levels of bone turnover markers depending on the severity of coronary artery calcification according to Agatston score

Feature	Low/minimal coronary artery calcification (Agatston score 0-10) n=12	Mild/moderate coronary artery calcification (Agatston score 11-400) n=36	Severe coronary artery calcification (Agatston score >401) n=64	P1-3	Q1-3
Cathepsin K, pmol/L	27.08 (7.83;29.03)	27.2 (6.98;19.54)	14.83 (1.57;27.89)	0.02	0.028
Bone alkaline phosphatase, U/L	16.89 (14.30;33.37)	23.18 (17.24;27.10)	23.79 (14.90;29.52)	>0.05	>0.05
Insulin, ng/mL	5.65 (2.4;6.1)	5.28 (2.3;5.9)	6.7 (2.6;6.2)	>0.05	>0.05
Estradiol, pg/mL	56.69 (44.49;77.74)	43.21 (0.09;41.76)	42.14 (15.10;41.03)	>0.05	>0.05
Testosterone, ng/mL	5.34 (3.79;5.72)	4.69 (3.45;6.98)	5.6 (3.76;6.36)	>0.05	>0.05
Osteocalcin, ng/mL	16.07 (15.70;17.33)	21.6 (16.14;38.15)	23.18 (12.52;24.84)	>0.05	>0.05
Osteopontin, ng/mL	6.35 (6.20;7.55)	7.33 (6.83;12.33)	7.02 (6.07;7.87)	>0.05	>0.05
Osteoprotegerin, pg/mL	109.6 (41.86;230.60)	156.56 (50.32;396.0)	159.1 (106.14;408.50)	0.03	0.037
Calcitonin, pg/mL	10.54 (7.16;26.08)	10.55 (6.90;13.01)	11.6 (7.77;16.43)	>0.05	>0.05
Parathyroid hormone, pg/mL	29.34 (19.79;43.40)	37.73 (21.99;54.30)	40.16 (44.36;56.78)	0.049	>0.05
Ca, mmol/L	2.43 (2.26;2.59)	2.29 (2.19;2.40)	2.28 (2.08;2.38)	>0.05	>0.05
P, mmol/L	0.94 (0.77;1.06)	0.9 (1.00;1.34)	0.97 (0.81;1.04)	>0.05	>0.05
Alkaline phosphatase, U/L	82.1 (71.00;265.00)	139.26 (61.00;208.00)	146.21 (60.00;213.50)	0.047	>0.05
Ionised Ca, mmol/L	0.39 (0.37;0.43)	0.37 (0.36;0.43)	0.37 (0.36;0.97)	>0.05	>0.05

Discussion

The relationship between osteopoenia/osteoporosis and coronary artery disease is well-known [9]. Clinical investigations demonstrated that coronary stenosis is more prevalent in women with low BMD [9]. A number of studies showed the experimental and clinical association of BMD reduction with arterial calcification [3,4,6]. Moreover, decrease of BMD may be considered as an independent risk factor of cardiovascular complications [9,10]. In our investigation, BMD reduction was associated with CA and CAC severity whilst normal BMD was associated with a low risk of fatal coronary events in males with stable angina. Similar results were obtained in the previous studies [11,12]. Regarding the investigated calcification markers, increased osteocalcin plasma level was significantly associated with decreased BMD. Therefore, our results correspond to the data published up to now.

Cathepsin K is the main proteolytic enzyme of osteoclasts and the most specific marker of bone resorption [13]. It was found that cathepsin K does not influence formation of calcium deposits but affects remodelling of mineralised tissues

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[14]. Cathepsin K inhibition during initial stages of mineral deposition retards mineralisation whilst general inhibition of cathepsin K may, on the contrary, enhance mineralisation [14]. Decrease of cathepsin K plasma levels and increase of osteocalcin plasma levels in patients with osteopoenia/osteoporosis revealed in our study may reflect the deregulation of the balance between bone formation and bone resorption. We also detected reduced cathepsin K plasma levels in patients with severe CA and CAC.

Deficiency of cathepsin K reduced atherosclerotic plaque progression and induced thickening of plaque fibrous cap but aggravated macrophage foam cell formation [15]. Normal arteries contained little or no cathepsin K whilst macrophages in atheroma contained abundant cathepsin K [16]. High and low activity of cathepsin K was detected in stable and unstable atherosclerotic plaques, respectively [17]. Cathepsin K expression and activity were increased in the left ventricle of rats with heart failure [18]. However, olmesartan, an angiotensin II receptor antagonist, attenuated cathepsin K expression and activity, restored the balance between elastin and collagen in the left ventricle, and suppressed degradation of the elastic lamina of coronary arteries [18].

In a couple of studies, patients with coronary artery disease had significantly higher serum cathepsin K levels compared to the healthy controls whilst patients with acute coronary syndrome had significantly higher serum cathepsin K levels compared to those with stable angina [19,20]. However, SYNTAX score and the proportion of patients who used beta-blockers, angiotensin-converting enzyme inhibitors, and long-acting nitrates were significantly lower in these patients compared to our study which could affect the result. Moreover, the differences revealed between the groups were mainly due to patients with acute coronary syndrome but not with stable angina [19]. Since cathepsin K levels positively correlated with percent plaque volumes and inversely with percent fibrous volumes, it could also be associated with plaque rupture and thrombosis [20].

In our study, severe CAC was associated not only with reduced cathepsin K plasma levels but also with increased osteoprotegerin plasma levels. This may reflect the deregulated balance between bone resorption and bone formation. Osteoprotegerin, also known as osteoclastogenesis inhibitory factor, is the key molecule for the inhibition of the differentiation and activation of osteoclasts [21,22]. Plasma levels of other BTMs did not differ significantly between the groups, possibly due to the preclinical osteopoenia/osteoporosis stage in all patients. All in all, our results may confirm the relationship between the decreased BMD and enhanced coronary artery calcification that was suggested before [23].

Our study has certain limitations. First, we considered only male patients in this study. Second, we did not recruit the group of age-matched healthy controls due to technical difficulties. Third, we did not determine serum levels of any other markers of calcium/phosphorus metabolism or inflammatory markers such as C-reactive protein, IL-6, or TNF-α.

Future studies may focus on the assessment of calcium/phosphorus metabolism, inflammatory markers, and CAC in patients with coronary artery disease in a short- or long-term period of follow-up. In addition, the association of osteopoenia/osteoporosis with cardiovascular events in a follow-up period also deserves to be investigated.

Conclusion

We revealed a significant association of osteopoenia/osteoporosis with severe CA and CAC in males with stable angina. Plasma levels of cathepsin K were significantly decreased in patients with osteopoenia/osteoporosis, severe CA and CAC whilst plasma levels of osteocalcin were significantly increased in patients with osteopoenia/osteoporosis. Plasma levels of various BTMs were more likely to be associated with severe CAC than with CA. Therefore, it can be suggested that BMD reduction is associated with increased CAC.

Conflict of Interest Statement

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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