

# Correlativity between osteocalcin and coronary artery calcification in middle-aged and elderly population

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## Research article

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**Article title:** Correlativity between osteocalcin and coronary artery calcification in middle-aged and elderly population

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**Abstract:** Background and Objective: With the increase of aging, osteoporosis and cardiovascular diseases are common diseases in middle-aged and elderly people. The purpose of this study is to explore the correlation between osteocalcin and coronary calcification.

Methods: A total of 150 middle-aged and elderly physical examination subjects who had undergone coronary artery CT angiography (CTA) were selected as the research objects. The calcification score of the coronary artery was calculated according to the Agatston (AS) integral method, and the total score was divided into the coronary artery calcification group and the non-calcification group. The indexes of N-terminal midfragment of osteocalcin (N-MID), routine biochemical indexes and basic conditions of each patient were recorded.

Results: In the comparison of general data between the non-calcification group and the calcification group, the differences in age ( $P < 0.001$ ), pressure difference ( $P = 0.01$ ) and low density lipoprotein cholesterol (LDL-C) ( $P < 0.001$ ) were statistically significant, the differences in smoking history ( $P < 0.001$ ) and drinking history ( $P < 0.001$ ) were statistically significant, and the differences in N-MID ( $P < 0.001$ ) were statistically significant. Coronary artery calcification score (CACS) was correlated with age, pressure difference, LDL-C, N-MID, smoking history and drinking history. The four variables, age, LDL, N-MID, and smoking history, have certain effects on coronary artery calcification (CAC).

Conclusions: In middle-aged and elderly people undergoing physical examination, N-

MID is correlated with CAC and can be used as a predictive indicator.

**Abbreviations:**

AS: Agatston

BGLAP: Bone glutamate protein

BMI: Body mass index

CABG: Coronary artery bypass grafting

CAC: Coronary calcification calcification

CACS: Coronary artery calcification score

CAD: Coronary artery disease

CHD: Coronary heart disease

CKD: Chronic renal failure

cOC: Carboxylated osteocalcin

CTA: Coronary artery CT angiography

CVD: Cardiovascular disease

FBG: Fasting blood glucose

GGCX: Glutamate  $\gamma$ -carboxylase

HDL-C: High density lipoprotein cholesterol

HR: Heart rate

LDL-C: Low density lipoprotein cholesterol

N-MID: N-terminal midfragment of osteocalcin

OC: Osteocalcin

TC: Total cholesterol

TG: Triglyceride

uOC: Uncarboxylated osteocalcin

VC: Vascular calcification

VKDPs: Vitamin k-dependent proteins

VSMCs: Vascular smooth muscle cells

$\bar{x} \pm s$  : Mean  $\pm$  standard deviation

**Keywords:** vascular calcification, Coronary artery calcification, osteocalcin, N- MID

## 1. Background

By the end of 2017, there were 2.409 million elderly people over the age of 60 in the country<sup>1</sup>. With the increase of aging, the health problems of the elderly become more and more prominent. Osteoporosis and cardiovascular disease (CVD) are the two most common diseases among the elderly, and some studies have suggested that there may be a link between the two<sup>2-5</sup>. As people get older, the calcium lost from bones is deposited in the cardiovascular system<sup>6</sup>. Vascular calcification (VC) is a recognized risk factor for cardiovascular morbidity and mortality<sup>7</sup> and is currently considered an active process<sup>8-11</sup>. VC has some similarities to bone mineralization and is related to the transformation of vascular smooth muscle cells (VSMCs) into osteoblast-like phenotypes<sup>12</sup>.

There are 17 types of vitamin k-dependent proteins (VKDPs), including osteocalcin (OC) [also known as bone glutamate protein (BGLAP)]. OC is divided into two kinds: carboxylated osteocalcin (cOC) and uncarboxylated osteocalcin (uOC). uOC is converted into cOC under the action of glutamate  $\gamma$ -carboxylase (GGCX) and vitamin K<sup>13</sup>. OC is the most abundant non-collagenous protein in the bone mineralization matrix<sup>14</sup>. The characteristic of atherosclerosis is associated with circulating OC<sup>15</sup>. OC mediates calcification of VSMCs<sup>16</sup>. These studies show that OC is correlated with VC.

At present, the relationship between OC and VC has not been determined. Research on cOC is still lacking. In this paper, one of the indexes of bone metabolism —N-terminal midfragment of osteocalcin (N-MID) was included to investigate its relationship with coronary artery calcification (CAC).

## 2. Methods

### 2.1 Study population

A total of 150 middle-aged and elderly physical examination subjects who had undergone coronary artery CT angiography (CTA) were selected as the research objects. The exclusion criteria for all selected candidates are: diseases that affect bone metabolism, such as hyperthyroidism, hyperparathyroidism, chronic renal failure or malignancy; or use drugs that affect bone metabolism, such as glucocorticoids,

estrogens, or bisphosphonates; patients with incomplete clinical data; previous history of fracture; acute heart, liver, kidney, lung and other important organ complications occurred within the last 3 months. All participants in this study agreed in writing.

## 2.2 General data

Retrospective data analysis was used to record the subjects' gender, age, body mass index (BMI), systolic blood pressure, diastolic blood pressure, differential blood pressure, heart rate (HR), fasting blood glucose (FBG), triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), smoking history, drinking history and medication history. Measurement of blood pressure: after resting for at least 10 minutes, take an average of two readings of blood pressure under the sitting position. Measurement of routine biochemical indicators: After one night of fasting, each participant collected blood samples of forearm veins in the morning and sent them to the laboratory of our hospital to complete relevant index tests.

## 2.3 Measurement of N-MID

The complete osteocalcin (amino acid 1-49) is not very stable in the peripheral blood. The amino acid chain between the carboxyl terminus 43 and 44 is easily hydrolyzed by protease. The large fragment (amino acid 1-43) after cleavage is called N-MID. N-MID is relatively stable and is an internationally recognized marker of bone metabolism. The detection of N-MID can better reflect the recent bone transformation. We used the third generation N-MID assay kit to detect the whole 1-49 of cOC and 1-43 of degraded cOC fragments by enzyme-linked immunoassay.

## 2.4 Measurement of CAC score

Multi-slice spiral CT was used to detect the calcification score of coronary arteries<sup>17</sup>, The calcification integral was calculated by the Agatston (AS) integral method, AS calcification integral condition: CT value  $\geq 130$ HU, calcification area  $\geq 1$ mm<sup>2</sup>, calcification score = calcification area  $\times$  calcification peak score(130~199Hu=1, 200~299Hu=2, 300~399Hu=3,  $\geq 400$ Hu=4). The coronary artery was divided into four parts: left main trunk, left anterior descending branch, left circumflex branch, and right coronary artery, and calcification scores were calculated

for each part. The integral results of each part are added to obtain a total integral<sup>18</sup>. According to the total score, this study was divided into the coronary artery calcification group and the non-calcification group (calcification group: > 10; non-calcification group: ≤10).

## 2.5 Statistical analysis

Statistical software SPSS19.0 was used for statistical analysis. The measurement data is expressed in mean ± standard deviation ( $\bar{x} \pm s$ ), the counting data is represented by probability, and the grade data by frequency. For comparison of measurement data, the independent data t test is used if the data conforms to normal distribution and uniform variance, and the Wilcoxon rank sum test is used if data does not conform to normal distribution or uneven variance. The comparison of counting data was conducted by  $\chi^2$  test, and Spearman rank correlation analysis was used for correlation analysis. Binary Logistic regression was used to analyze the correlation between multiple variables and coronary artery calcification score. The test level was  $\alpha=0.05$ , and  $P < 0.05$  was considered statistically significant.

## 3. Results

3.1 Baseline characteristics of the study population are shown in Table 1. Comparison of the general data between the two groups showed statistically significant differences in age ( $P < 0.001$ ), pressure difference ( $P = 0.01$ ) and LDL-C ( $P < 0.001$ ), and differences in smoking history ( $P < 0.001$ ) and drinking history ( $P < 0.001$ ) were statistically significant. There was no statistically significant difference in gender, height, weight, BMI, systolic blood pressure, diastolic blood pressure, HR, FBG, TG, TC and HDL-C ( $P > 0.05$ ).

Tab.1 Comparison of baseline characteristics of 150 subjects. Data are given as n (%) or  $\bar{x} \pm s$ .

	Non-calcification group (n=72)	Calcification group (n=78)	P Value
Gender (male/female)	31/41(47.00/48.80)	41/28(53.00/51.20)	0.82
Age (years)	54.17±6.97	60.19±9.21	<0.001
Height (cm)	164.40±7.85	164.62±7.09	0.86

Weight (kg)	72.33±12.71	69.49±12.14	0.16
BMI(kg/m <sup>2</sup> )	26.67±3.74	25.54±3.59	0.06
Systolic blood pressure(mmHg)	136.74±20.32	140.79±18.62	0.20
Diastolic blood pressure(mmHg)	81.64±12.15	79.08±12.22	0.20
Pressure difference(mmHg)	55.10±13.38	61.72±17.09	0.01
HR(beats/min)	73.88±10.56	74.31±12.20	0.82
FBG (mmol/L)	5.86±1.34	6.14±1.93	0.30
TG (mmol/L)	1.61±0.73	1.61±0.85	0.99
TC (mmol/L)	4.64±1.01	4.74±1.07	0.54
HDL-C(mmol/L)	1.28±0.28	1.23±0.24	0.22
LDL-C(mmol/L)	2.52±0.71	3.14±0.74	<0.001
Smoking history	12 (1.40)	38 (27.50)	<0.001
Drinking history	6 (19.40)	25 (80.60)	<0.001

BMI= Weight/height<sup>2</sup>(kg/m<sup>2</sup>); Pressure difference= Systolic blood pressure- Diastolic blood pressure(mmHg).

3.2 The relationship between CAC and N-MID is shown in table 2. The relationship between CAC and N-MID was statistically significant when comparing without gender distinction ( $P < 0.001$ ). When comparing the relationship between CAC and N-MID in male ( $P = 0.005$ ) and female ( $P < 0.001$ ), the difference was still statistically significant.

Tab.2 The relationship between N-MID and CAC was discussed by gender stratification.

	Non-calcification group ( <i>n</i> =72)	Calcification group ( <i>n</i> =78)	<i>P</i> Value
Without gender distinction	10.49±5.47	16.24±5.87	<0.001
Male	10.72±6.57	14.66±4.19	0.005
Female	10.31±4.55	17.52±6.73	<0.001

3.3 Correlation analysis of CAC with N-MID and general data in table 3. Correlation analysis was performed by Spearman rank correlation analysis. The results showed that the difference between CAC and age( $r = 0.35$ ,  $P < 0.001$ ), pressure difference( $r = 0.21$ ,  $P = 0.01$ ), LDL( $r = 0.39$ ,  $P < 0.001$ ), N-MID( $r = 0.45$ ,  $P < 0.001$ ), smoking history( $r = 0.29$ ,

$P < 0.001$ ) and drinking history ( $r = 0.34$ ,  $P < 0.001$ ) at the  $P = 0.05$  level was statistically significant. Binary Logistic regression was used to evaluate the influence of multiple variables on CAC, and the difference of Logistic model was statistically significant ( $\chi^2 = 82.098$ ,  $P < 0.001$ ). Among the independent variables (or predictors) included in this model, the four variables of age ( $P = 0.02$ ), LDL ( $P = 0.002$ ), N- MID ( $P < 0.001$ ) and smoking history ( $P = 0.02$ ) had statistically significant differences at the level of  $P = 0.05$ .

Tab.3 Results of Logistic model analysis

	<i>OR</i>	<i>95%CI</i>	<i>P Value</i>
Age	1.08	(1.02~1.15)	0.02
Pressure difference	1.02	(0.99~1.05)	0.27
LDL	2.71	(1.45~5.10)	0.002
N- MID	1.23	(1.11~1.37)	<0.001
Smoking history	4.38	(1.33~14.45)	0.02
Drinking history	2.20	(0.81~5.97)	0.12

## Discussion

VC is very common in patients with ischemic cardiovascular disease, cerebrovascular disease and renal failure. Vascular calcification is a major factor in the progression of cardiovascular disease<sup>19</sup>. Calcified vascular cells are derived from local VSMCs and circulating hematopoietic stem cells (especially intimal calcification)<sup>20</sup>. Vascular calcification is a marker of atherosclerosis<sup>21</sup>. In calcified atherosclerotic lesions, VSMCs express a variety of osteogenic differentiation markers<sup>22</sup>, similar to the osteogenic mineralization process<sup>23</sup>. VC is caused by the imbalance of vascular wall mineralization promoters<sup>24</sup> and inhibitors and is a serious health problem with serious clinical consequences<sup>25</sup>. CAC is an independent predictor of coronary heart disease (CHD) / CVD<sup>26</sup>. Compared with other risk markers, it has better identification and risk reclassification ability<sup>27-29</sup>, which is related to the degree of coronary artery disease (CAD)<sup>30</sup>. Studies have shown that in the early stage of dialysis patients with chronic renal failure (CKD), CAC is closely related to the incidence of CVD events and



all-cause mortality<sup>31</sup>.

There are many risk factors for CVD, including age, diabetes, hypertension, hyperlipidemia and so on. The results of this study showed that age, LDL and other general data were significantly higher in the calcified group than in the non-calcified group. In this study, differential pressure was added, and the results showed that the differential pressure was significantly different between the two groups and correlated with the CACS.

Some studies have found that the progressive calcification of atherosclerotic plaque is accompanied by the accumulation of insignificant calcitonin and osteocalcin, and the concentration of OC is significantly increased during the calcification process<sup>32</sup>. A meta-analysis<sup>33</sup> evaluated the relationship between circulating OC(total OC, uOC, and cOC) and cardiovascular outcomes. Patients with CVD had significantly lower circulating total OC levels than patients with non- CVD. Data on uOC and cOC were limited and there was no evidence of association. Studies have evaluated the OC levels of subjects undergoing coronary artery bypass grafting (CABG) compared with the normal control group. Regardless of diabetes status, serum uOC levels have decreased, and there is no difference in cOC levels between the two groups<sup>34</sup>. A prospective cohort study examining the association of various bone markers with CVD risk in type 2 diabetes found no association between OC and CVD risk<sup>35</sup>. Studies have found that there is no difference in serum OC levels between stroke patients and healthy controls<sup>36</sup>. A meta-analysis found no clear association between OC and VC or the degree of atherosclerosis<sup>14</sup>.

OC is a non-specific collagen produced by osteoblasts in the bone and consists of 49 amino acids. Previous studies have not come to a uniform conclusion. This study included the N-MID indicator, we used the third generation n-mid assay kit to detect the whole 1-49 of cOC and 1-43 of degraded cOC fragments by enzyme-linked immunoassay. The metabolism of bone tissue can be understood by detecting the level of n-mid in blood. In this paper, the relationship between N-MID and CAC was compared. We found that compared with the non-calcification group, the value of N-MID in the calcification group was higher, and the difference was statistically

significant. This article also proves that N-MID is related to CAC and has a certain impact on CAC. This paper also discusses the relationship between n-mid and CAC through gender stratification, and finds that there is still a correlation between the two, whether male or female. Future research may focus on the relationship between different types of OC and VC to draw relevant conclusions.

Why come to a different conclusion? The forms of osteocalcin used in each study were different, including total OC, cOC and Uoc; the reagents used to detect OC varies; different gender selection; study population diversity (kidney disease, diabetes or glucose intolerance, postmenopausal women, and hypertension); ethnic and regional variability; blood sampling time varies; The calcification or atherosclerosis measurement methods used vary, including calcification scoring, intima-media thickness measurement, pulse wave velocity measurement, plaque presence, and coronary angiography or echocardiography; Comorbidities increase with age, which may influence the findings of some studies. In addition, OC concentrations were also affected by drug treatments, including glucocorticoid therapy, antiabsorbents, and vitamin D therapy. Not all researchers believe that these are potential confounders.

### **Conclusion**

In middle-aged and elderly people undergoing physical examination, N-MID is correlated with CAC and can be used as a predictive indicator.

### **Declarations:**

**Ethics approval and consent to participate:** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the Declaration of Helsinki and have been approved by Ethics committee of Shandong university Qilu hospital. Written informed consent was obtained from individual or guardian participants.

**Consent for publication:** Not applicable.

**Availability of data and materials:** All data generated or analyzed during this study are included in this published article.

**Competing interests:** The authors declare that they have no competing interests.

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**Authors' contributions:** Na Li collected a variety of clinical information and analyzed it to reach conclusions. Na Li was a major contributor in writing the manuscript. Zhi - Mian Zhang provided this topic and conducted a clinical study. All authors read and approved the final manuscript.

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