Osteocalcin and Chinese visceral adiposity index are associated with the risk of atherosclerotic cardiovascular disease and arterial stiffness in patients with type 2 diabetes mellitus—a case control study

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Research Article

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Abstract

Background

Current risk assessments for atherosclerotic cardiovascular disease (ASCVD) in patients with type 2 diabetes mellitus (T2DM) are limit. Recent evidence strongly supports a close correlation between serum osteocalcin, the Chinese visceral adiposity index (CVAI), and T2DM, and cardiovascular events. However, their association with ASCVD risk in patients with T2DM remains unknown, and their impact on arterial stiffness also remains unclear.

Methods

An analysis of 646 T2DM patients aged 18 and older was conducted in this cross-sectional study. The ASCVD risk was assessed using the China-PAR equation, with patients categorized into low- or medium-high-risk groups. Osteocalcin was detected through electrochemical luminescence, whereas arterial stiffness was defined using ankle-brachial index and brachial-ankle pulse wave velocity. Logistic regression analysis was conducted to examine the correlation between serum osteocalcin levels, CVAI, ASCVD risk, and arterial stiffness.

Results

Osteocalcin levels were significantly lower in men patients with T2DM in the medium-high-risk ASCVD group compared to the low-risk ASCVD group, whereas CVAI levels was significantly higher in women patients with T2DM in the medium-high-risk ASCVD group than the low-risk ASCVD group. Logistic regression analysis identified osteocalcin and CVAI as independent risk factors for both medium-high-risk ASCVD (osteocalcin: men, OR, 0.958, 95%CI 0.923, 0.99, women, OR, 0.788, 95%CI 0.645, 0.96, respectively) (CVAI: men, OR, 1.010, 95%CI 1.00, 1.02, women, OR, 1.084, 95%CI 1.00, 1.17, respectively) and arterial stiffness (osteocalcin: men, OR, 0.958, 95%CI 0.92, 1.00, women, OR, 0.925, 95%CI 0.86, 0.99, respectively) (CVAI: men, OR, 1.011, 95%CI 1.003, 1.02, women, OR, 1.0217, 95%CI 1.00, 1.03, respectively) in both men and women patients with T2DM. Combining osteocalcin levels and CVAI improved the prediction accuracy of arterial stiffness in men patients with T2DM (difference of AUC (Model 4 vs. Model 1): 1.4%). All P-values were < 0.05.

Conclusion

Osteocalcin levels and CVAI are independent risk factors for ASCVD risk and arterial stiffness in T2DM. Combining osteocalcin and CVAI can enhance the early detection of atherosclerosis through men patients with T2DM.
1. Introduction

Atherosclerotic cardiovascular disease (ASCVD) is the leading cause of morbidity and mortality in patients with type 2 diabetes mellitus (T2DM), and diabetes increases the risk of ASCVD greatly [1]. Research shows that cardiovascular risk assessments using traditional risk factors are inadequate. Furthermore, most assessment models of ASCVD risk are based on the general population and focus on predicting coronary heart disease and stroke, whereas initial diabetic manifestations of cardiovascular disease (CVD) are more likely to be peripheral artery disease and heart failure [2, 3]. Meanwhile, arterial stiffness, a sign of arterial wall damage and endothelial dysfunction in early atherosclerosis, is associated with adverse cardiovascular outcomes [4]. Identifying risk factors that are consistent with the representative characteristics of T2DM may facilitate the accurate evaluation of ASCVD risk and improve the early identification of atherosclerosis in T2DM.

Osteocalcin (OC), which is synthesized and secreted by osteoblasts, is the most abundant non-collagenous protein in the extracellular bone matrix. Recently, an increasing number of studies have found that osteocalcin regulates glucose [5] and lipid [6] metabolism, vascular calcification, and atherosclerotic formation, thereby affecting the rate of CVD events and mortality. Very recently, a study involving serum osteocalcin and ASCVD risk in men aged 40 and over revealed an inverse relationship between osteocalcin and ASCVD risk [7]. Yet, the correlation among these factors in T2DM remains unclear, and the relationship between osteocalcin and arterial stiffness needs to be investigated.

The global incidence of obesity is increasing sharply every year, and the incidence of abdominal obesity alone in patients with T2DM can reach 45.4% [8]. Despite often having a lower body mass index (BMI) than their Western counterparts, Asians tend to accumulate visceral fat [8]. This accumulation has been linked to the advancement of cardiovascular complications in T2DM, as demonstrated by recent studies [9]. The Chinese visceral adiposity index (CVAI), a reliable parameter for visceral fat area (VFA) in Chinese individuals, is the most reliable indicator of increased ASCVD risk in adults among various cardiovascular and metabolic indicators [10]. Yet, a clear understanding of the association between the CVAI and ASCVD risk in T2DM patients remains to be determined. What's more, the capacity of the CVAI combined with osteocalcin levels to pinpoint arterial stiffness in T2DM patients is yet to be established. Studies have verified that there are age and gender differences in osteocalcin, VFA and the cardiometabolic risk profile. While the underlying mechanisms for these differences are currently unclear [11], the distinctions in osteocalcin based on age and gender are believed to arise from variations in bone turnover rates. The menopausal status could be pivotal in explaining the disparity in bone turnover rate [12]. The correlation between osteocalcin levels, VFA, ASCVD risk, and arterial stiffness should be interpreted according to sex and age.

This study aimed to examine the association between serum osteocalcin levels, CVAI, ASCVD risk, and arterial stiffness in men and women with T2DM. The predictive accuracy of serum osteocalcin and the Chinese visceral adipose index for arterial stiffness are also discussed.
2. Methods

Subjects

In this study, we conducted a retrospective analysis of data from adult patients with T2DM who were admitted to the Endocrinology Department of the First Hospital of Lanzhou University between August 2021 and September 2022. The diagnosis of T2DM was based on the 1999 criteria set by the World Health Organization (WHO). The exclusion criteria were as follows: 1) age < 18 years; 2) patients with incomplete data on high-density lipoprotein cholesterol (HDL-C), osteocalcin, ASCVD family history, age, systolic blood pressure (SBP), fasting blood glucose (FBG), BMI, WC, triglycerides (TG), brachial-ankle pulse wave velocity (ba PWV), and ankle-brachial index (ABI); 3) combined with any known disease that affects skeletal metabolism exogenously, including benign and malignant tumors of the pituitary, parathyroid, and adrenal glands, as well as traumatic fractures, bone tuberculosis, hyperthyroidism, and hypothyroidism; 4) take any drugs that are known to affect bone metabolism, including thiazolidinedione and hormonal drugs, within 3 months[12]; and 5) pregnant and lactating women [13]. Finally, 646 participants with complete data were included. Upon admission, trained professionals completed questionnaires, physical examination, examination of the abdominal fat region, and arteriosclerosis for all participants, and blood samples were collected the next morning. The questionnaire included: (1) basic information: sex, age, and course of diabetes; (2) previous history of ASCVD, hypertension, and other diseases, and family history of ASCVD; (3) lifestyle: current smoking and current alcohol consumption; and (4) medication history. This study was conducted in accordance with the World Medical Association Declaration of Helsinki and was approved by the Ethics Committee of the First Hospital of Lanzhou University. All participants provided written informed consent.

Biochemical parameters

The patient fasted for a minimum of 8 h before venous blood was obtained. All blood samples were centrifuged within 2 h of collection and frozen at -20°C after serum extraction. All biochemical indices were tested at our hospital’s endocrine laboratory or transported to the biochemical laboratory on dry ice within 2–4 hours of collection.

Leukocyte counts were analyzed using a fully automatic blood cell analyzer (XFA6000; Plant, China). An automatic biochemical analyzer (AU5831, Beckman Coulter, USA) was used to detect total cholesterol (TC), serum creatinine, blood calcium (Ca), TG, blood phosphorus (P), HDL-C, ferritin (FT), low-density lipoprotein cholesterol (LDL-C), FBG, and uric acid (UA) levels. High-performance liquid-phase analysis (Bole-D10, Bio-Rad, USA) was used to detect hemoglobin (HbA1c). Vitamin D (Vit D) levels were analyzed using an enzyme-linked immunoassay (AU5831, Beckman Coulter, USA). Fasting insulin (FINS) and fasting C-peptide (FCP) levels were measured using chemiluminescence (Centaur-XP, Siemens, Germany). The detection of osteocalcin was accomplished using the Elecsys N-MID osteocalcin kit, utilizing electrochemical luminescence technology from Roche Diagnostics in China; the coefficient of variation within and between the measurements was 1.2-4.0% and 1.7–6.5%, respectively.
Anthropometric measurements and examination parameters

Participants' height and weight were measured while they stood shoeless and wore light clothing. Waist circumference was measured at the mid-axillary line and midpoint between the lowest rib and the highest level of the ilium with a precision of 0.1. Automatic electronic equipment (HEM-752 FUZZY, Omron, China) was used to measure blood pressure in the non-dominant arm (units: mmHg). Participants were instructed to rest for 5 minutes prior to the blood pressure measurement. This procedure was repeated three times, with one-minute intervals between each reading, and the average of the three readings was calculated. The DUCALSCAN Bioimpedance technique (HDS-2000, Omron, China) measured the areas of visceral and subcutaneous abdominal fat. A color ultrasound diagnostic instrument (IU22; Philips, Holland) was used to detect fatty liver disease. Screening for diabetic retinopathy was performed using an aphakia binocular indirect ophthalmoscope (TRC-NW 400, Topcon, Japan).

Definition

Current smoking was defined as having smoked at least 100 cigarettes in the past and continuing to smoke currently. Current alcohol consumption was defined as the intake of more than 20 g per week for more than 3 months. Hypertension was defined as an SBP ≥ 140 mmHg, DBP ≥ 90 mmHg, or a self-reported diagnosis. TC ≥ 5.7 mmol/L, TG ≥ 1.8 mmol/L, LDL ≥ 3.7 mmol/L or HDL < 0.8 mmol/L was considered as dyslipidemia. The deficiency of diabetic retinopathy was indicated by fundus photographs without abnormalities. In contrast, the presence of diabetic retinopathy was determined by findings under a fundus microscope that displayed characteristics such as inner retinal microaneurysms, hemorrhages, venous beading, noticeable abnormal microvessels, neovascular formation, and changes like retinal hemorrhage or detachment. Chronic kidney disease (CKD) was diagnosed as a urinary albumin/creatinine ratio > 30 mg/g or an glomerular filtration rate (eGFR) < 60 mL/min / 1.73 m². A VFA ≥ 100 cm² was judged to be visceral fat type obesity. ASCVD includes acute coronary syndrome, myocardial infarction, stable or unstable angina pectoris, arterial revascularization, stroke, transient ischemic attack, and atherosclerotic peripheral vascular disease. Insulin resistance (HOMA-IR) and insulin sensitivity (HOMA-β) according to homeostasis models were determined using the following formula: HOMA-IR = FPG×FINS/22.5, HOMA-β = 20×FINS/(FBG-3.5). The glomerular filtration rate was calculated according to the CG-GFR formula: eGFR = (140- age) × Body weight (kg) ×1.23/ serum creatinine (umol/L) (male), eGFR = (140- age) × Body weight (kg) ×1.08/ serum creatinine (umol/L) (female). BMI was calculated by formula: weight/height²(kg/m²). The CVAI, triglyceride-glucose (TyG) index, and waist-height ratio (WHR) were calculated as follows:

CVAI:

Male: -267.93 + 0.68×age + 0.03×BMI + 4.00×WC + 22.00 × LgTG – 16.32 ×HDL-C
Female: -187.32 + 1.71×age + 4.32×BMI + 1.12×WC + 39.76 × LgTG – 11.66 ×HDL-C

TyG index = Ln [TG × FBG ÷ 2]
WHR = WC (cm) ÷ Height (cm)

**ASCVD risk**

The 10-year atherosclerotic cardiovascular Risk Assessment Equation (China-PAR) was used to assess ASCVD risk [13]. The equation was established using four large cohort studies that are more appropriate for the Chinese population and widely used in epidemiological studies. In detail, the 10-year ASCVD risk of the subjects was evaluated based on 11 indicators, including sex, age, WC, geographic location of residence (north/South, urban/rural), TC, HDL-C, BP, whether they were taking hypotensive drugs, smoking status, family history of ASCVD and diabetes (13). 10-year ASCVD risk < 5% was a low risk and ≥ 5% was a medium-high risk.

### Detection and evaluation criteria for arterial stiffness indicators

For the ba PWV measurement, subjects were required to rest for 5 min in the supine position. Cuff straps were placed around the subject's arms and ankles, and both left and right brachial and ankle blood pressures were recorded. A volumetric splenogram (BP-203RPE II; Colin, Komaki, Japan) was used to record the pressure waves in the brachial artery. During ABI detection, the patient rested in a recumbent position for approximately 10 min. The left and right brachial and ankle joint artery SBP (Omron Colin BP-203RPE III, Omron Health Care, China) and ABI were calculated as the ratios of the SBP of the ankle artery to that of the brachial artery. Arterial stiffness was considered as baPWV ≥ 1600 cm/s or ABI < 0.9. All the surgeries were performed by trained professionals. The measurements were repeated 3 times, and the average double-sided ba PWV and ABI values were recorded. The ba PWV value was the upper value from the double side, and the ABI value was the lower value.

### Statistical analysis

All steps are analyzed separately for women and men. Continuous variables with a normal distribution are presented as mean ± standard deviation, while those with skewed distributions are represented by the median and interquartile ranges. The t-test or Mann-Whitney U test was employed for group comparisons. Categorical variables are described using frequency and percentage, with chi-square tests used for between-group comparisons. Before linear regression analysis, the existence of a linear relationship was confirmed using a scatterplot of the two variables to be tested. The tolerance or variance inflation factor was used to diagnose collinearity. Tolerance ≥ 0.1 or variance inflation factor < 10 indicates no collinearity. For the binary logistic regression analysis, the included variables were decided carefully according to clinical importance and multifactorial stepwise logistic regression. By building various models, the recognition ability of the variables was assessed using the receiver operating characteristic (ROC) curve and area under the curve (AUC). The differential recognition performances of the models were compared with those of the Delong test. When examining the correlation between different variables and 10-year ASCVD risk, the Crude Model only controls for the female menopausal status; Model 1 included traditional atherosclerosis risk factors: current smoking (male), menopausal status (female), hypertension, antihypertensive therapy, history of ASCVD, HDL, and osteocalcin or CVAI; Model 2 added
osteocalcin and CVAI to the traditional atherosclerosis risk factors in Model 1; Model 3 added statin therapy, Ca, and HOMA-β to Model 1; Model 4 adds statin therapy, Ca, and HOMA-β to Model 2. SPSS software (version 26.0) was used for the statistical analysis. Differences were considered statistically significant when all P-values were double-tailed (P < 0.05).

3. Results

3.1 General characteristics of the participants

In total, 646 patients with T2DM were included in this study. The group consisted of 432 men and 214 women, with a mean age of 58.72 ± 10.13 years (ranging from 19 to 85 years old). Compared to the low-risk of ASCVD group, men with T2DM in the medium-high-risk of ASCVD group had higher age, SBP, DBP, weight, height, ba PWV level, ASCVD history, hypertension, antihypertensive treatment, and DR incidence, lower TyG index, ferritin, HbA1c, TG, Ca, LDL, eGFR and osteocalcin levels, as shown in Table 1. Meanwhile, women with T2DM in the medium-high-risk of ASCVD group had higher age, WC, WHR, SBP, DBP, BMI, diabetes duration, CP-2,2-Fin, CVAI, abdominal and subcutaneous fat area, VFA, ba PWV, menopausal status, hypertension, antihypertensive therapy, and DR incidence, and lower height, as shown in Table 2.

Furthermore, for women, postmenopausal individuals exhibited higher levels of CVAI, ba PWV, and a medium-high risk of ASCVD compared to premenopausal women, as shown in Supplementary Table 1. Among women with T2DM at a medium-high-risk of ASCVD, the postmenopausal group had higher levels of ba PWV, lower TyG index, and eGFR levels compared to the premenopausal group, as shown in Supplementary Table 2. All P values < 0.05.

3.2 Association between general characteristics and osteocalcin and CVAI

Simple linear regression analysis was conducted using osteocalcin and CVAI as dependent variables to examine the correlation between general features, osteocalcin, and CVAI. Statistically significant variables were included in the multiple linear regression analysis.

For men with T2DM, as shown in Tables 3 and 4, HOMA-IR, creatinine and history of ASCVD were positively associated with osteocalcin (β, 0.615; β, 0.06, β, 6.465, respectively); the duration of diabetes, FBG and antihypertension were negatively associated with osteocalcin (β, -0.332; β, -0.316, β, -1.73, respectively); WHR, SBP, VFA, and TyG index were positively related to CVAI (β, 715.361; β, 0.075; β, 0.017; β, 6.724, respectively); DBP, BMI, FCP, HDL, uric acid, and eGFR were negatively related to CVAI (β, -0.092; β, -0.56; β, -1.852; β, -16.256; β, -0.01; β, -0.101, respectively). For women with T2DM, creatinine had a positive correlation with osteocalcin (β, 0.112); leukocyte counts and OC were negatively associated (β, -0.693); age and WHR were positively linked to CVAI (β, 1.719; β, 106.753, respectively), and HDL was negatively related to CVAI (β, -18.274). All P values < 0.05.
3.3 Association between osteocalcin and CVAI with ASCVD risk

Table 5 shows the crude model, Model 1, and Model 2, after controlling for traditional cardiovascular risk factors, including current smoking (male), menopausal status (female), combined hypertension, antihypertensive therapy, ASCVD history, and HDL in logistic regression analysis. Compared to the low-risk ASCVD group, osteocalcin was significantly negatively related to medium-high risk of ASCVD in males with T2DM (OR, 0.964; 95%CI 0.932,0.998), and CVAI was significantly positively linked with medium-high risk of ASCVD in females with T2DM (OR, 1.056; 95%CI 1.018,1.095).

Beyond traditional cardiovascular risk factors, Model 3 additionally adjusted for non-traditional cardiovascular risk factors such as statin therapy, Ca, and HOMA-β. Compared to the low-risk ASCVD group, osteocalcin and CVAI were significantly negatively and positively related to the medium-high risk of ASCVD in male and female patients with T2DM, respectively.

Furthermore, in Model 4, which incorporated traditional and non-traditional cardiovascular risk factors, along with osteocalcin and CVAI, the results demonstrated that osteocalcin (men: OR,0.958, 95%CI 0.923, 0.995, P < 0.05; women: OR, 0.788, 95%CI 0.645,0.962, P < 0.05) and CVAI (men: OR,1.010,95%CI 1.00,1.02, P < 0.05; women: OR, 1.084, 95%CI 1.00,1.175, P < 0.05) remained significantly negatively and positively related to the medium-high risk of ASCVD in both male and female T2DM patients, when compared to the low-risk ASCVD group. Osteocalcin and CVAI are independent negative and positive risk factors for medium-high risk of ASCVD in both men and women with T2DM, respectively.

3.4 Correlation between osteocalcin and CVAI with arterial stiffness

After controlling for confounding factors, we found a significant negative correlation between osteocalcin and arterial stiffness in male patients with T2DM (OR, 0.958,95%CI 0.92,1.00, P < 0.05), and a significant positive link between CVAI and arterial stiffness (OR,1.011, 95%CI 1.00,1.03, P < 0.05). Osteocalcin and CVAI are independent risk factors for arterial stiffness in males with T2DM.

After controlling for confounding factors, such as menopausal status, HOMA-β, hypertension, creatinine, and eGFR, osteocalcin showed a significant negative correlation with arterial stiffness in female patients with T2DM(OR, 0.925, 95%CI 0.86,0.99, P 0.05). Additionally, CVAI showed a significant positive correlation with arterial stiffness in female patients with T2DM (OR, 1.017,95%CI 1.00,1.03, P < 0.05), as shown in Table 6. Osteocalcin levels and CVAI are independent risk factors for arterial stiffness in females with T2DM.

Four models were constructed, and the predictive values of osteocalcin and CVAI for arterial stiffness were assessed using ROC curves by comparing the AUCs. Model 4’s AUC was slightly higher than that of the other models (AUC (Model 4) = 83.1%, Calibrability (Model 4) = 0.58; Difference of AUC (Model 4¹ vs. Model 1¹) = 1.4%, P < 0.05) in male T2DM patients, as shown in Table 7 and Fig. 1. This suggests that
combining osteocalcin with CVAI maximizes the predictive accuracy of the model for identifying arterial stiffness in male T2DM patients. For females, Model 4’s AUC was notably elevated compared to the other models, while the difference being not statistically significant (Difference in AUC (Model 4²vs. Model 1²) = 1.44%), as shown in Supplementary Fig. 1.

4. Discussion

Increasing clinical evidence suggests that osteocalcin and CVAI are closely correlated with the occurrence and progression of cardiovascular disease, T2DM and its associated complications. Our study primarily examines the relationship between osteocalcin, CVAI, and ASCVD risk, emphasizing the predictive performance for arterial stiffness in the context of sex differences.

T2DM patients with long duration, poor glycemic control, visceral obesity, and normal renal function were the main subjects of this study. Men in the medium-high-risk of the ASCVD group had lower osteocalcin levels than those in the low-risk of ASCVD group, which is consistent with previous studies [7]. Compared to the low-risk ASCVD group, women in the medium-high-risk of ASCVD group had higher CVAI levels [10], especially after menopause. However, no significant difference was observed in CVAI levels among men across the two ASCVD risk categories. Similarly, there was no notable variation in osteocalcin levels among women across the ASCVD risk categories or between pre- and post-menopausal female T2DM patients, which contrasts with prior research [13].

Osteocalcin is a hormone with pleiotropic effects. An increasing number of studies have found that osteocalcin regulates glucose [5] and lipid [6] metabolism, vascular calcification, and atherosclerotic formation. This influences the frequency of CVD events and mortality, similar to the major active form of serum osteocalcin, undercarboxylated osteocalcin (ucOC) [15]. However, owing to the limitations of the existing detection technology for ucOC, clinical studies usually detect serum total osteocalcin levels. Osteocalcin regulates insulin secretion, and the function of β-cells, and inhibits the release of glucagon from β-cells after meals to maintain blood glucose homeostasis in the pancreas [16]. It has been observed to negatively correlate with adverse outcomes in glucose metabolism [17]. This study demonstrated a negative correlation between diabetes duration and FBG and osteocalcin levels in men, which is consistent with other studies [18, 19]. However, this study found a positive relationship between osteocalcin level and HOMA-IR, contradicting the results of other studies [20, 21]. This may be a consequence of the prolonged duration of diabetes and increased use of insulin treatment among most subjects in this study. Osteocalcin is involved in lipid metabolism, linked to fat distribution patterns [22], and is closely related to visceral obesity in all obesity types [23]. Osteocalcin mechanically regulates the secretion of adiponectin by adipocytes [24], and an animal study has shown that injection of recombinant osteocalcin can decrease the expression of adiponectin in white and brown adipocytes in wild-type mice [25]. This study indicated a positive relationship between HDL and osteocalcin in women, a negative association between the TyG index and VFA with osteocalcin in women, and a negative association between osteocalcin and CVAI in men; however, this association was no longer evident after accounting for other confounding factors. Moreover, osteocalcin can decrease inflammatory factor
secretion from hyperglycemia and reduce inflammation through the PI3K/Akt/NF-kB signaling pathway. One study reported an independent negative correlation between ucOC and leukocyte count in patients with T2DM, which supports our study in the results of female patients with T2DM [26]. In this study, creatinine was independently and positively correlated with osteocalcin levels in both male and female T2DM patients in this study. Serum creatinine reflects individual muscle mass, nutrition, and physical activity status as a common byproduct of skeletal muscle metabolism [27]. Studies have shown that the interaction between osteocalcin and muscle also maintains blood sugar levels, and that higher levels of serum osteocalcin are closely related to an elevated risk of impaired grip strength and decreased physical function in patients with T2DM over 40 years of age [28]. We speculate that the lower serum osteocalcin levels may reflect lower skeletal muscle quantity in male and female patients in our study.

Recently, the relationship between innovative indicators and the incidence of ASCVD and T2DM has shown improvement over traditional anthropometric indicators. This has garnered increasing research interest and attention. The CVAI can distinguish between excessive central obesity in Chinese adults and is the most powerful replacement marker for visceral fat dysfunction. Previous studies have shown that visceral fat is closely associated with insulin resistance. Vascular active substances, such as adipose cytokines, inflammatory factors, anticoagulant molecular markers, and growth factors, are secreted by visceral adipocytes, promoting the formation of atherosclerosis and increasing the risk of cardiovascular disease [29]. This study showed that DBP, BMI, FCP, HDL, uric acid, and eGFR are independently and negatively associated with CVAI in male T2DM patients, while WHR, SBP, VFA, and the TyG index are independently and positively linked with CVAI. In women, HDL level was independently negatively associated with CVAI, and age and WHR were independently and positively linked with CVAI. After controlling for age and sex, Wei et al. demonstrated an inverse association between CVAI and HDL levels in healthy adults, which is consistent with our study [30].

Low serum osteocalcin levels are associated with a higher occurrence of T2DM and its complications, as well as a higher rate of all-cause and cardiovascular death in T2DM [31, 32]. The CVAI is the best obesity parameter for predicting the prevalence of diabetes and has the strongest relationship with the occurrence rate of CVD in T2DM [33, 34]. Furthermore, CVAI has emerged as the most reliable indicator for increased ASCVD risk in adults among various innovative cardiovascular and metabolic indicators. This is supported by prior research that introduced markers such as the TyG index, VFA, and TyG-BMI [10]. This study is the first to investigate the association between osteocalcin level, CVAI, and ASCVD risk in T2DM patients. In this study, we found that in males with T2DM, a lower osteocalcin level is associated with a higher medium-to-high risk of ASCVD than in those with a low risk of ASCVD. CVAI was positively correlated with a medium-high risk of ASCVD in women with T2DM compared to those with a low risk of ASCVD. After adjusting for traditional cardiovascular risk factors, such as current smoking (male), menopausal status (female), hypertension, antihypertensive therapy, ASCVD history, and HDL, and non-traditional risk factors, such as statin therapy, Ca and HOMA-β, both osteocalcin levels and CVAI maintained their independent associations with medium-high ASCVD risk in male and female T2DM patients, in contrast to the low-risk ASCVD group. Specifically, CVAI in men and osteocalcin in women were identified as independent risk factors for ASCVD, though other factors might mask these
relationships. When traditional and non-traditional cardiovascular risk factors, osteocalcin, and CVAI were all incorporated into the model, it was shown that, compared to the low risk of ASCVD, osteocalcin and CVAI were still independently negatively and positively related to the medium-high risk of ASCVD in both men and women with T2DM, respectively. This indicates a potential interaction between osteocalcin and CVAI [24]. Previous study's findings align with our research, their study investigated the relationship between osteocalcin and ASCVD risk in men aged over 40 years [7]. Similarly, Huang et al. revealed that CVAI is an independent risk factor for ASCVD in adults, which is consistent with our conclusion [10]. Osteocalcin and CVAI may be potential biomarkers of cardiovascular risk and offer a fresh understanding of how to reduce residual cardiovascular risk [35]. Moreover, osteocalcin can be obtained directly from the blood [36, 37], and CVAI is based on hematological indicators likely, as well as age, BMI, and WC. Considering that they are all able to objectively show changes in their levels at different stages of the disease, are universally available in all healthcare facilities (including primary healthcare facilities), and are inexpensive, osteocalcin and CVAI may be more favorable for improving ASCVD risk assessment in patients with T2DM [2].

To our knowledge, this study is the first to investigate the link between CVAI and arterial stiffness as well as the predictive ability of CVAI in patients with T2DM. In this study, the incidence of arterial stiffness in female patients with T2DM was positively associated with CVAI, meanwhile osteocalcin was negatively associated with the incidence of arterial stiffness in male T2DM patients. After controlling for confounding factors, osteocalcin level and CVAI were significantly negatively and positively related to the occurrence rate of arterial stiffness in male and female T2DM patients, respectively. Osteocalcin and CVAI offer enhanced predictive values for arterial stiffness in male and female patients with T2DM. Combining these values slightly improves the prediction accuracy for arterial stiffness incidence in males (AUC diff. (%) = 1.4%). However, the AUC in female patients with T2DM group notably increased (AUC diff. (%) = 4.8%), but the difference was not statistically significant. Kanazawa et al. and Yun et al. revealed that higher levels of osteocalcin correlated with lower PWV in men and community residents, which is consistent with our findings [38, 39]. Tacey et al. measured serum ucOC using hydroxyapatite binding and found that the levels of ucOC were negatively related to PWV in community-dwelling men, but were no longer statistically significant after controlling for confounders [16]. Yun et al. reported an inverse correlation between total osteocalcin level and baPWV in a female community population [39]. These findings are consistent with our conclusions. The ongoing debate surrounding the correlation between osteocalcin and arterial stiffness in earlier studies [40, 41], can be attributed to variations in the impact of metabolic factors across different research. Disorders in glucose and lipid metabolism disorders, as well as hypertension, are closely associated with osteocalcin and the progression of atherosclerosis. In addition, confounding factors affecting the relationship between osteocalcin and arteriosclerosis can differ between men and women. Additionally, these studies included female populations of different ages, reflecting different bone turnover rates. Menopausal status is key to determining sex and age differences in osteocalcin (or bone turnover rate).

In addition to regulating glucose and lipid metabolism and inflammatory responses, future research should focus on determining whether osteocalcin has a direct function in the blood vessel itself in terms
of delaying the development of atherosclerosis and CVD [42]. Mechanically, repeated cyclic shear stress and intracavity pressure not only cause arterial wall elastic fiber loss but also cause endothelial dysfunction that stimulates the production and deposition of excessive collagen in the lesion artery wall, resulting in increased vessel fibrosis and calcification and significantly reduced vascular compliance [43, 44]. In several recent studies, osteocalcin was shown to have a potential protective effect on vascular endothelial cells in atherosclerosis by regulating PI3K/Akt/ eNOS signaling. Similar studies have shown that addition of osteocalcin enhances human umbilical vein endothelial cell function in vitro [39]. However, a New Zealand white rabbit with 4-week diet-induced atherosclerosis underwent perfusion myography to detect carotid vascular activity. After the intervention of ucOC, this study showed that ucOC had no direct impact on the arterial endothelial function of the rabbit in the short term [15]. Therefore, the direct effects of osteocalcin on vascular function should be investigated in the future. A clear relationship between osteocalcin and early atherosclerosis is vital for improving the prevention and treatment of many ASCVDs.

This study has several limitations. First, this cross-sectional study failed to identify a causal relationship between osteocalcin level, CVAI, ASCVD risk, and arterial stiffness. Second, the inflammatory index only included white leukocyte counts and, and other markers such as interleukin 6 and highly sensitive C-reactive protein were not measured. Further, participants included in the study were all inpatients; therefore, the study results may have some selection deviations. Future multicenter prospective cohort studies are necessary to elucidate the causal relationship between osteocalcin level, CVAI, ASCVD risk, and arterial stiffness.

5. Conclusion

Osteocalcin and CVAI are independent risk factors for medium-high ASCVD risk and arterial stiffness in both men and women with type 2 diabetes mellitus, with osteocalcin being a negative factor and CVAI being a positive one. Combining the two indicators enhances the recognition of arterial stiffness in male T2DM patients. This will inject fresh blood into the stratification of ASCVD risk and management of early atherosclerosis in patients with type 2 diabetes.

Abbreviations

ASCVD Atherosclerotic cardiovascular disease

T2DM Type 2 diabetes mellitus

CVAI Chinese visceral adiposity index

CVD Cardiovascular disease

OC Osteocalcin
uc OC Undercarboxylated osteocalcin
VFA Visceral fat area
WHO World Health Organization
HDL-C High-density lipoprotein cholesterol
SBP Systolic blood pressure
FBG Fasting blood glucose
TG Triglycerides
ba PWV Brachial-ankle pulse wave velocity
ABI Ankle-brachial index
TC Total cholesterol
Ca Calcium
P Phosphorus
FT Ferritin
LDL-C Low-density lipoprotein cholesterol
UA Uric acid
HbA1c Hemoglobin
Vit D Vitamin D
FINS Fasting insulin
FCP Fasting c-peptide
CKD Chronic kidney disease
HOMA-IR Insulin resistance of homeostasis model
HOMA-β Insulin sensitivity of homeostasis model
eGFR Glomerular filtration rate
BMI Body mass index
Declarations

Supplementary Information

Name: Additional file 1

Title: Supplementary Table 1. pdf: General characteristics in women with T2DM. Supplementary Table 2. pdf: General characteristics in 10-year ASCVD medium-high-risk women with T2DM. Supplementary Figure1. pdf: Prediction value of osteocalcin and CVAI for arterial stiffness in men with T2DM

Description of data: Additional file 1 contains a supplementary analysis of the population characteristics of female patients with T2DM in the general characteristics section of the study population. This section presents the results grouped according to their menopausal status in all women (Supplementary Table 1) and women in the high-risk group of ASCVD (Supplementary Table 2). In addition, Supplementary Figure1 shown an analysis of the recognition power of osteocalcin and CVAI for arterial stiffness in women with T2DM, but there was no statistical difference in AUC.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the First Hospital of Lanzhou University (Approval No.: LDYYLL-2023-400). All participants provided written informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Authors' contributions

All authors contributed to the conception and design of the study. The first draft of the manuscript was written by CG. All authors devoted themselves to data collection, statistical analysis, and previous manuscript versions commenting.

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Not applicable.

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Tables

Table 1 to 7 are available in the Supplementary Files section.

Figures

Figure 1

Prediction value of osteocalcin and CVAI for arterial stiffness in men with T2DM

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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