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Correlation between serum Dickkopf-1 (DKK1) levels and coronary artery stenosis

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KEYWORDS
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Abstract *Background and aims:* To study the correlation between the level of serum Dickkopf-1 (DKK1) and the degree of coronary artery stenosis in patients with coronary atherosclerotic heart disease.

Methods and results: In 2018, general data and biochemical indexes of 311 patients who underwent coronary angiography were recorded. Before procedure, arterial blood was drawn and the concentrations of DKK1, retinol binding protein 4 (RBP4), plasminogen activator inhibitor (PAI-1) were measured. Based on coronary angiography results, subjects were divided into a coronary heart disease (CHD) group; and a non-coronary heart disease (non-CHD)group. The CHD group was divided into three subgroups: the low Gensini score; the middle Gensini score; and the high Gensini score subgroups. Compared with those of the non-CHD group, DKK1, RBP4 and PAI-1 of the CHD group were significantly higher, while the OC was lower.

DKK1,RBP4 and PAI-1 levels of the middle and high Gensini subgroups were significantly higher, compared with that of the low Gensini subgroup. Differences between osteocalcin (OC), beta-isomerized C-terminal telopeptidase (β-CTX), and $25(OH)_2D_3$ of the three subgroups were not significant.

Correlation between DKK1 and the inflammatory factors, RBP4 and PAI-1, was positive. Correlation between DKK1 and β - CTX, $25(OH)_2D_3$ and OC was not significant. DKK1 was a risk factor for CHD. The degree of coronary artery stenosis was related to DKK1 concentration.

Conclusions: Serum DKK1 levels in coronary heart disease patients were significantly higher, and positively correlated with the degree of coronary artery stenosis. DKK1 level is an independent risk factor for coronary heart disease.

Abbreviations: Dickkopf-1, (DKK1); Coronary heart disease, (CHD); Non-coronary heart disease, (non-CHD); Wingless/Beta Catenin, (Wnt/β-Catenin); Vascular smooth muscle cells, (VSMCs); Human umbilical vein endothelial cells, (HUVECs); Body mass index, (BMI); β-isomerized C-terminal telopeptides, (β-CTX); Osteocalcin, (OC); retinol binding protein 4, (RBP4); Plasminogen activator inhibitor, (PAI-1); Enzyme-linked immunosorbent assay, (ELISA); Analysis of variance, (ANOVA); Triglycerides, (TG); uric acid, (UA); High density lipoprotein cholesterol, (HDL-C); Low density lipoprotein cholesterol, (LDL-C); Fasting blood glucose, (FBG); Lipoprotein receptor related protein, (LRP); Glycogen synthase kinase-3β, (GSK3); Oscillatory shear stress, (OSS); cAMP response element binding protein, (CREB); Asymmetric dimethylarginine, (ADMA); Carotid intima-media thickness, (cIMT); Endothelial progenitor cells, (EPC).

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1. Introduction

Atherosclerosis is a chronic inflammatory condition. It is the main pathologic basis of cardiovascular diseases. Plaque rupture and thrombosis may induce acute clinical events such as acute coronary syndrome. Wingless/beta catenin (Wnt/β-Catenin) is involved in the progression of vascular lesions, associated with endothelial dysfunction, macrophage activation, proliferation, and vascular smooth muscle cell migration [1–3]. Dickkopf-1 (DKK1), which is an inhibitor of the Wnt/β-Catenin pathway, blocks the Wnt/β-catenin pathway by forming a complex with LRP5/6 [4]. *In vivo* and *in vitro* studies have confirmed that serum DKK1 play an important role in the development of coronary atherosclerosis and acute ischemic stroke by regulating Wnt/β-Catenin [2,5,6].

DKK1 facilitates inflammation, platelet activation, and endothelial dysfunction, and plays an important role during the early stages of atherosclerosis [7–9]. It is secreted by activated platelets in an inflammatory microenvironment of atherosclerotic plaque, inhibits the Wnt/β-catenin pathway in endothelial cells, and contributes to the atherosclerotic process. Di M et al. found overexpression of DKK1 resulted in enlarged and destabilized atherosclerotic lesions and increased apoptosis in aortic as well as carotid plaque. The relative content of vascular smooth muscle cells (VSMCs) and collagen fibers were lowered and plaque size and vulnerability were increased in a group overexpressing DKK1, while the content of VSMCs and collagen fibers were higher in a DKK1-silenced group. The mRNA and protein levels of DKK1 were increased in a time-dependent manner during the reversal of ox-LDL-induced apoptosis by DKK1 in Human umbilical vein endothelial cells (HUVECs), indicating that DKK1 induces apoptosis in HUVECs by activating endoplasmic reticulum stress via the JNK pathway and canonical Wnt signaling [7].

One study detected DKK-1 levels were significantly higher, compared to those of the controls. Elevated DKK-1 levels were significantly associated with premature coronary artery disease during the stable phase, which occurs after one year of the disease [10]. A prospective, population-based Bruneck Study confirmed that the elevation of baseline DKKI levels was independently associated with the incidence of cardiovascular events after a median follow up period of 15.6 years [11]. Further, almost all clinical studies that have been conducted so far suggest that serum levels of DKKI are associated with stable cardiovascular disease.

Coronary artery stenosis is a marker of the severity of coronary heart disease during the stable phase. Therefore, the current study investigated the correlation between serum DKK1 and the degree of coronary artery stenosis in patients with coronary atherosclerotic heart disease. It will provide a new clue for prevention and treatment of coronary atery diseases.

2. Methods

2.1. Subjects

A total of 311 patients who underwent coronary angiography in the Department of Cardiology at The Affiliated Hospital of ChengDe Medicine University from March to August 2018 were enrolled. These patients included 185 males and 126 females, aged 28–81 years (average age, 59.24 years). Coronary angiography was performed on all the patients and their Gensini score was calculated using the modified scoring schema (Table 1). Based on their coronary angiography results, patients were divided into a non-coronary heart disease group (non-CHD group; n = 105) and a coronary heart disease group (CHD group; n = 206). The CHD group was further divided into three subgroups based on the severity of coronary artery stenosis according to their Gensini score. (i) a low Gensini score group, S1 (2–21 points; n = 70), (ii) a middle Gensini score group, S2 (22–36 points; n = 69), and (iii) a high Gensini score group, S3 (≥37 points; n = 67).

Diagnostic criteria for coronary heart disease were based on the guidelines of the American Heart Association as follows: at least one stenosis ≥50% of left main trunk, anterior descending artery, circumflex artery, right coronary artery and its main branches amounts to a diagnosis of coronary heart disease. All patients underwent coronary angiography through the radial or femoral artery, and two specialists jointly assessed the extent of vascular disease.

Table 1 Gensini score rule.

Degree of coronary artery stenosis	Score	Coronary lesion site	Score
≤25%	1	LM	5
26%–50%	2	Proximal LAD or LCX	2.5
51%–75%	4	Middle LAD	1.5
76%–90%	8	Distal LAD	1
91%–99%	16	Middle or distal LAD	1
100%	32	RCA	1
		Subbranch	0.5

LM: left main coronary artery; LAD: left anterior descending; LCX: left circumflex coronary; RCA: right coronary artery.

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Inclusion criteria: (1) more than 18 years old; (2)male and female; (3)underwent coronary angiography in the Department of Cardiology at The Affiliated Hospital of ChengDe Medicine University from March in 2018 for the diagnosis of CHD.

Exclusion criteria of the study included: (1) Severe infection, trauma, anemia (hemoglobin<120 g/L for adult male and<110 g/L for adult female) [12]; (2) Recent (within six months) use of anti-osteoporosis drugs, thyroid hormones, thiazolidinedione, thiazide or cortisol, or other drugs that affect bone metabolism. (3)chronic kidney disease (CKD) Stages III,IV and V,Transaminase level increase for more than three times of the upper normal range (4) Pregnant females;

2.2. Clinical variables and laboratory procedure

General anthropometric data of the patients were collected. The average of two blood pressure readings, each taken after sitting for 5 min in a resting position, was recorded. The height and weight were measured, and the body mass index (BMI) was calculated according to the formula BMI (kg/m^2) = body weight (kg)/height ^{2} (m^2).

Samples of blood drawn from the radial or femoral artery in the morning after overnight fasting were let stand at room temperature for 2 h and then centrifuged at 3000 rpm for 10 min. The upper portion of the serum supernatant was aspirated and stored at −80 °C until measured.

Plasma concentrations of β-isomerized C-terminal telopeptides (β-CTX), osteocalcin (OC), and 25-(OH)-D were detected using an electrochemiluminescence method (Roche cobase e601 electrochemiluminescence Immunoanalyzer, Roche Diagnostic GmbH, Mannheim,Germany; Elecsys β-CrossLaps/serum, Elecsys Vitamin D total, Elecsys N-MID Osteocalcin kits ,Roche Diagnostic GmbH, Mannheim,Germany). Plasma levels of DKK-1, retinol binding protein 4 (RBP4) and plasminogen activator inhibitor (PAI-1) in the patients and controls were determined via a commercially available, enzyme-linked immunosorbent assay (ELISA); (Thermo Scientific Multiskan FC,Thermo Fisher, Shanghai,China; ELISA Kit for Dickkopf Related Protein 1,Plasminogen Activator Inhibitor 1 and Retinol Binding Protein 4,Cloud Clone Corp, Shanghai,China) according to the manufacturer's instructions. The differences between intra- and inter-assays of DKK1 were less than 10% and 12%, respectively. Informed consent was obtained from all the participants. Ethics approval was granted.

2.3. Statistical analysis

Data for continuous variables are expressed as mean ± standard deviation. Count data are presented as numbers of cases and/or percentages (n/%) or skewed distribution [M(Q25, Q75)]. Continuous variables were tested for normality using the Kolmogorov-Smirnorv test. The Chi-square test, as well as Pearson or Spearman correlation analyses, were utilizied for two group comparisons. Comparisons between the measurement data of groups with skewed distributions were conducted using the rank sum test. Kruskal–Wallis one way analysis of variance (ANOVA) were utilized for multi-group comparisons. Binary Logistic regression was used to detect the correlation between serum DKK1 level and CHD. Multiple ordered logistic regression were used to explore the correlation between serum DKK1 level and the degree of coronary artery stenosis.

All analyses were conducted using the Statistical Package for Social Sciences (IBM SPSSS tatistics for Windows, version 26.0, Armonk, NY). Statistical significance was set at a $p < 0.05$.

3. Results

3.1. Characteristics of the CHD and non-CHD groups

We enrolled 206 CHD patients and 105 non-CHD individuals (controls). Characteristics of the two groups are presented (Table 2). The CHD group was older, with higher triglycerides (TG) and uric acid (UA) levels, as well as lower high density lipoprotein cholesterol (HDL-C) levels, than the control group. There were no significant differences between sex composition, diabetes prevalence, hypertension prevalence, SBP, DBP, and BMI of the CHD group and non-CHD groups.

Serum DKK1 concentration of the CHD group was significantly higher than that of the non-CHD group [5.66 (4.05–7.46) vs 1.14 (0.46–2.03) $P < 0.05$]; [Fig. 1]. Compared with the non-CHD group, RBP4 [11.81 (7.13–17.96) vs 2.18 (1.35–2.75), $P < 0.05$] and PAI-1 [364.85 (259.21–458.73) vs 198.65 (113.10–316.31), $P < 0.05$] were significantly higher in the CHD group, whereas OC [15.03 (10.35–19.28) vs 12.53 (9.98–17.56), $P = 0.041$] was lower in the CHD group. There were no significant differences between β-CTX and 25-(OH)-D of the two groups.

3.2. Elevated serum DKK-1 levels were increased with the degree of coronary artery stenosis

The characteristics of the three subgroups were compared based on the severity of coronary artery stenosis [Table 3]. Compared with the low Gensini score group, the DKK1 level in the middle Gensini score group and the high-Gensini score group were significantly increased [Fig. 2].

The high Gensini score group had higher OC. Correlation analysis between circulating levels of DKK1 and β-CTX, OC and 25-(OH)-D did not reveal any relevant correlations [Table 4]. No significant differences were detected among total cholesterol (TC), TG, low density lipoprotein cholesterol (LDL-C), fasting blood glucose (FBG), and UA of the three groups.

3.3. Serum DKK1 concentration is correlated with serum RBP4 and PAI-1

Serum RBP4 and PAI-1 levels in the CHD group were elevated. Compared with those of the low Gensini score group, the RBP4 and PAI-1 levels were significantly higher in the middle and high Gensini score groups[Table 3].

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Table 2 Comparison of Baseline data in CHD and non-CHD group.

	non-CHD (n = 105)	CHD (n = 206)	P
Age (y)	57.64 ± 10.08	60.06 ± 8.68	0.028
Gender (male/Female)	50/55	135/71	0.102
Diabetes (%)	14 (13%)	60 (29%)	0.134
Hypertension (%)	48 (46%)	140 (68%)	0.243
SBP (mmHg)	135.89 ± 19.52	139.88 ± 19.37	0.087
DBP (mmHg)	79.77 ± 13.20	80.52 ± 12.23	0.62
BMI (kg/m2)	25.06 ± 3.23	25.25 ± 3.43	0.632
TG (mmol/l)	1.38 (1.02–2.0)	1.70 (1.12–2.74)	0.003
TC (mmol/l)	3.92 (3.48–4.8)	3.96 (3.29–4.7)	0.365
FBG (mmol/l)	6.34 (5.39–7.7)	6.86 (5.37–9.12)	0.072
LDL-C (mmol/l)	2.11 (1.74–2.59)	2.05 (1.55–2.61)	0.326
HDL-C (mmol/l)	1.18 (1.01–1.41)	1.10 (0.95–1.29)	0.004
LPa (mmol/l)	89.3 (18.2,141.6)	106.65 (48.4,332.0)	0.105
APO-A (mmol/l)	1.33 (1.06,1.57)	1.18 (1.05,1.39)	0.165
APO-B (mmol/l)	0.84 (0.66,1.01)	0.60 (0.51,0.78)	<0.05
UA (μmol/l)	296 (228–350)	313 (262–380)	0.033
Ca (mmol/l)	2.3 (2.23–2.36)	2.29 (2.23–2.25)	0.346
ALP (U/l)	73 (59–89)	74 (61–86)	0.96
DKK1 (ng/ml)	1.14 (0.46–2.03)	5.66 (4.05–7.46)	<0.05
RBP4 (ng/ml)	2.18 (1.35–2.75)	11.81 (7.13–17.96)	<0.05
PAI1 (ng/ml)	198.65 (113.10–316.31)	364.85 (259.21–458.73)	<0.05
β-CTX (ng/ml)	0.24 (0.16–0.42)	0.23 (0.16–0.36)	0.394
OC (ng/ml)	15.03 (10.35–19.28)	12.53 (9.98–17.56)	0.041
25- (OH)-D (nmol/l)	52.09 (41.57–62.75)	51.18 (42.89–61.80)	0.808
Smoker number (%)	33 (31.4%)	111 (53.9%)	<0.05
Family history of premature cardiovascular and cerebrovascular disease (%)	19 (18.1%)	41 (19.9%)	0.413
History of cerebrovascular disease (%)	7 (6.67%)	42 (20.4%)	0.01
History of depression (%)	2 (1.92%)	0 (0)	0.113
subjects treated with antiplatelet agents	99 (94.0%)	206 (100%)	0.01
subjects treated with beta-blockers	40 (38.1%)	114 (55.3%)	0.03
subjects treated with RAS modulators	21 (20%)	84 (40.7%)	<0.05
subjects treated with statins	98 (93.1%)	206 (100%)	<0.05

Data are presented as mean (SD) or number (%) or mediam. SBP, Systolic blood pressure; DBP, Diastolic blood pressure; BMI, Body mass index; TG, Triglycerides; TC, Total cholesterol; FBG, Fasting blood glucose; LDL-C, Low-density lipoprotein cholesterol; UA, Uric acid; ALP, Alkaline phosphatase; DKK1, dickkopf-1; RBP4, Retinol-binding protein 4; PAI1, plasminogen activator inhibitor 1.

Serum DKK1 concentration was inversely correlated with serum PAI-1 (Rs = 0.083; P = 0.005) and RBP4 (Rs = 0.494; P < 0.001) concentrations [Table 4].

3.4. DKK1 level was associated with degree of coronary artery stenosis

When adjusted for age, gender, BMI, FBG and biochemical indexes, such as TG, TC, LDL-C, HDL-C, diabetes mellitus and hypertension history, logistic regression analysis showed that DKK1 was a risk factor for coronary heart disease (OR = 2.309; 95% CI 1.866–2.858; P < 0.001); [Table 5].

Multiple ordered logistic regression analysis, which was performed with age, gender, BMI, FBG, TG, TC, LDL-C, HDL-C, β-CTX, OC, 25- (OH)-D, and DKK1, diabetes and hypertension as independent variables, indicated that the levels of DKK1 (OR:1.163 [1.035, 1.309]; P = 0.012), and OC (OR:1.114 [1.046, 1.186]; P = 0.001) were associated with the degree of coronary artery stenosis. Further, the higher the level of DKK1, the more serious the coronary artery stenosis, and the coefficient was 0.151. When the level of DKK1 was increased by 1 ng/ml, the degree of increase in coronary artery stenosis was 1.163 [Table 5].

4. Discussion

The results of our study indicated that serum DKK1 concentration in the CHD group was significantly higher than that in the non-CHD group. Binary logistic regression indicated that increased serum DKK1 levels were associated with coronary heart disease. We further investigated the association between DKK1 and coronary artery stenosis. Our results demonstrated that the DKK1 concentration in the high Gensini score group was significantly higher than that in the low Gensini score group, which indicated that the correlation between DKK1 concentration and coronary artery stenosis was a positive one. DKK1 concentration increased with the severity of coronary stenosis.

DKK1 is highly expressed in macrophages and endothelial cells present in atherosclerotic lesions. *In vivo* and *in vitro* studies have investigated the role played by DKK-1 in the inflammatory interaction between platelets and endothelial cells as a major modulator of WNT signaling, in atherogenesis and plaque destabilization [6]. WNTs are secreted glycoproteins which are present in virtually all animals. WNT signaling includes the “canonical” WNT/β-catenin signaling pathway and the “non-canonical” planar

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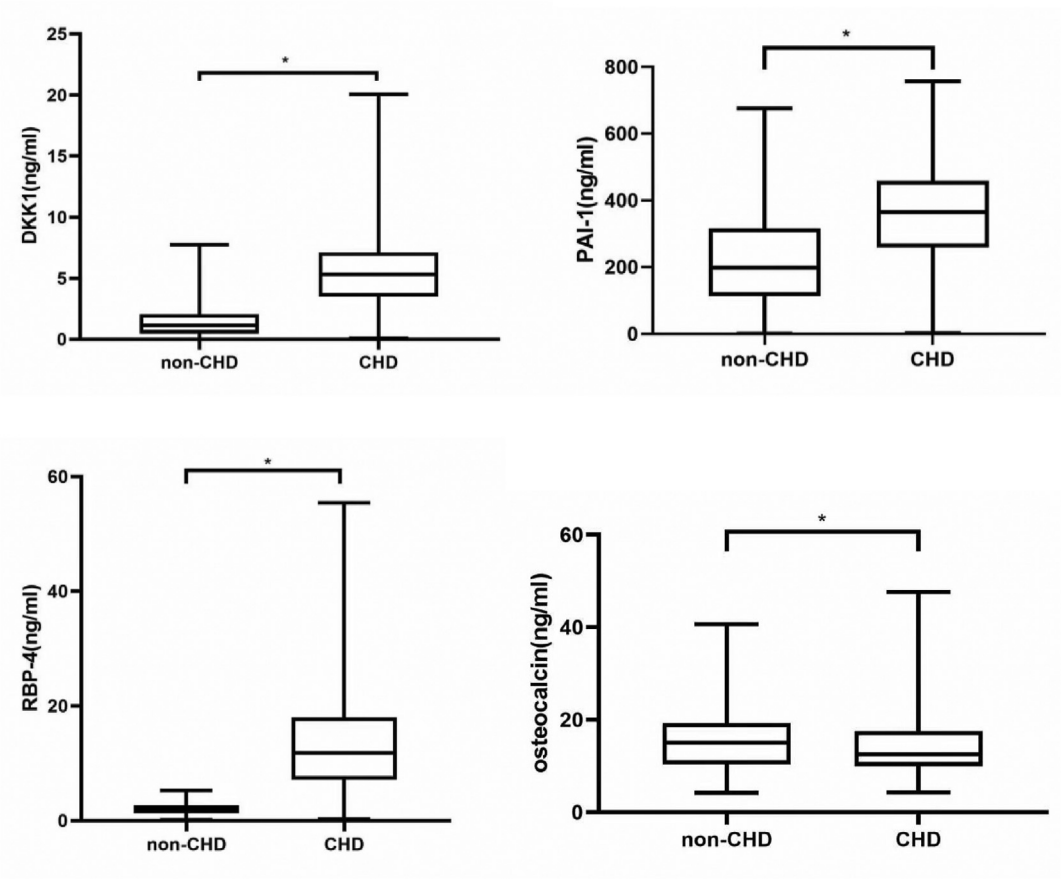

Figure 1 Comparison of DKK1 concentrations between CHD and non-CHD groups. The central line represents the median of the distribution, the boxes span from 25th to 75th percentiles. Outliers (open circles) are defined as a score between 1.5 and 3 box lengths away from the upper edge of the box. Extreme scores (asterisks) are defined as a score that is greater than 3 box lengths away from the upper edge of the box. The concentration of DKK1 in CHD group were higher than that in non-CHD group, $P < 0.05$. DKKI, Dickkopf-1; CHD, Coronary heart disease.

Table 3 Comparison of Baseline data of three subgroups in CHD group.

	S1 (n = 70)	S2 (n = 69)	S3 (n = 67)	P
Age (y)	59 (54～64)	62 (53～65)	61 (55～68)	0.323
Gender (male/Female)	47/23	47/22	41/26	0.658
Diabetes (%)	15 (21%)	25 (36%)	20 (30%)	0.158
Hypertension (%)	47 (67%)	46 (66%)	47 (70%)	0.895
SBP (mmHg)	137 (124～150)	142 (131～157)	137 (123～155)	0.170
DBP (mmHg)	81 (71～88)	84 (73～93)	79 (71～87)	0.182
BMI(kg/m2)	24.2 (23.1～27.1)	24.8 (22.9～27.2)	25.4 (23.5～28.3)	0.393
TG (mmol/l)	1.62 (1.09～2.76)	1.80 (0.99～2.91)	1.75 (1.14～2.56)	0.884
TC (mmol/l)	3.93 (3.34～4.67)	3.98 (3.20～4.48)	3.94 (3.22～4.82)	0.833
FBG (mmol/l)	6.72 (5.42～9.03)	6.57 (5.37～8.86)	7.22 (5.36～9.50)	0.783
HDL-C (mmol/l)	1.11 (0.96～1.25)	1.13 (0.96～1.31)	1.06 (0.93～1.29)	0.333
LDL-C (mmol/l)	1.91 (1.66～2.53)	2.06 (1.45～2.51)	2.06 (1.59～2.72)	0.601
UA (μmol/l)	308.8 (263.6～391.8)	315.4 (255.8～360.5)	310.0 (266.9～387.0)	0.632
Ca (mmol/l)	2.3 (2.24～2.35)	2.27 (2.23～2.34)	2.29 (2.23～2.35)	0.59
ALP(U/l)	70 (59～85)	74 (61～92)	73 (59～89)	0.66
β-CTX (ng/ml)	0.19 (0.12～0.28)	0.26 (0.16～0.38)	0.26 (0.17～0.37)a	0.288
OC (ng/ml)	12.46 (9.35～17.10)	12.09 (10.12～17.26)	13.10 (10.48～18.33)	0.027
25-(OH)-D (nmol/l)	53.85 (46.84～66.10)	49.70 (41.82～58.22)	48.49 (39.80～60.86)a	0.604
DKK1 (ng/ml)	4.61 (3.35～6.38)	6.13 (3.90～7.57)	6.45 (5.09～7.76)	<0.05
RBP4 (ng/ml)	7.51 (4.90～14.62)	15.38 (7.97～21.41)	13.27 (9.14～16.74)	<0.05
PAI1 (ng/ml)	256.63 (198.48～326.9)	398.65 (303.32～465.26)	456.06 (367.68～515.90)	<0.05

Data are presented as mean (SD) or number (%) or median. SBP, Systolic blood pressure; DBP, Diastolic blood pressure; BMI, Body mass index; TG, Triglycerides; TC, Total cholesterol; FBG, Fasting blood glucose; LDL-C, Low-density lipoprotein cholesterol; UA, Uric acid; ALP, Alkaline phosphatase; β-CTX, β collagen specific sequences; 25-(OH)-D,25-hydroxyl vitamin D; OC, Osteocalcin; DKK1, dickkopf-1; RBP4, Retinol-binding protein 4; PAI1, plasminogen activator inhibitor 1.

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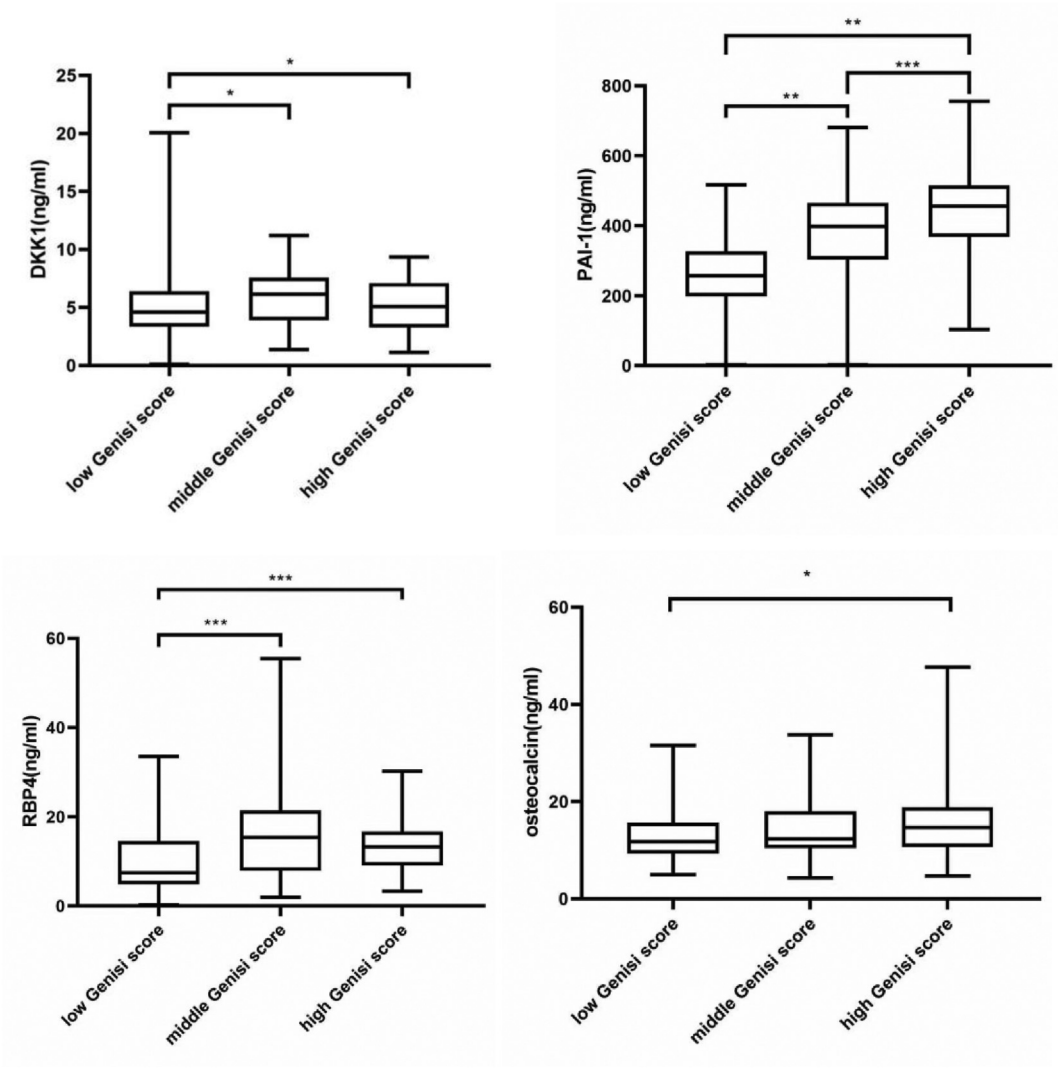

Figure 2 Comparison of the median concentrations of DKK1 in the three subgroups of the CHD group. 1, low Gensini score group; 2, middle Gensini score group, 3, high Gensini score. The central line represents distribution median, the boxes span from 25th to 75th percentiles. Outliers (open circles) are defined as a score that is between 1.5 and 3 box lengths away from the upper edge of the box. Extreme scores (asterisks) are defined as a score that is greater than 3 box lengths away from the upper edge of the box. A, B and C represent the comparison of the median concentrations of DKK1 in three subgroups of CHD group, respectively. In the high Gensini score group, the median concentrations of DKK1 were higher; $P < 0.05$.

Table 4 Correlations between DKK1 and RBP4,PAI-1 and bone turnover markers.

	DKK1	
	Rs	P
RBP4	0.494	< 0.001
PAI-1	0.083	0.005
β-CTX	−0.059	0.304
OC	−0.046	0.417
25-(OH)-D	−0.018	0.757

cell polarity and WNT/Ca^{2+} signaling pathways. The WNT/β-catenin signaling pathway plays an important role in many biological processes. When WNT binds with Frizzled receptors and low-density lipoprotein receptor related protein (LRP) 5/6 coreceptors, the gene *Disheveled* (*Dsh/Dvl*, in *Drosophila* and vertebrates, respectively) is activated. Activation of *Dvl* inhibits glycogen synthase kinase-3β (GSK3) activity and disrupts the adenomatous polyposis coil/Axin/GSK3 complex, resulting in β-catenin accumulating in the cytoplasm, and subsequently translocating to the nucleus. In the nucleus, β-catenin binds to LEF/TCF thereby stimulating the expression of WNT target genes [10]. The phosphorylation of LRP5/6 is a key step in the initiation of the WNT/β-catenin signaling. DKK1, which is a soluble glycoprotein, inhibits LRP5/6 and plays a key role in the regulation of the WNT signaling pathway. DKK1 directly inhibits WNT protein activity by competing with it to bind to the LRP receptor, or by indirectly binding to the LRP receptor via the kremen receptor, containing a kringle domain to form trimer, thereby reducing intracellular signal transmission by the WNT protein, which blocks the canonical WNT/β-catenin cytokine transmission pathway as well as the c-jun amino terminal kinase and WNT/plane cell polar transmission pathways [13,14].

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Table 5 The results of logistic regression analysis of factors for CHD and the multiple ordered logistic regression of risk factors for degree of coronary artery stenosis.

	Coronary heart disease			Coronary artery stenosis		
	OR	P	95% CI	OR	P	95%CI
β-CTX	0.878	0.925	0.059−12.982	0.66	0.22	0.005−0.664
25-(OH)-D	1.009	0.822	0.936−1.086	0.995	0.643	0.976−1.015
OC	0.989	0.35	0.966−1.012	1.114	0.001	1.046−1.186
DKK1	2.309	<0.001	1.866−2.858	1.163	0.012	1.035−1.309

Adjustments were made for age, gender, BMI, FBG, TG, TC, LDL-C, HDL-C, history of diabetes mellitus and hypertension. The severity of coronary artery stenosis was evaluated according to their Gensini score. Gensini score was calculated using the modified scoring schema (Table 1).

Atherosclerosis is a common arteriosclerotic vascular disease. It forms the pathological basis of coronary heart disease, cerebrovascular disease and other ischemic cardiovascular and cerebrovascular diseases [15]. It is an important factor associated with morbidity and mortality. Serum DKK1 played a role in the process of atherosclerosis [16,17]. Meanwhile, a large number of studies have shown that serum DKK-1 level is closely associated with atherosclerotic diseases, such as early myocardial infarction [18,19] and ischemic cerebrovascular disease [20].

Endothelial dysfunction involving VSMCs and arterial calcification are the main factors associated with the mechanism underlying atherosclerosis. Many basic studies have confirmed the effect of DKK1 on endothelial cells, VSMCs and vascular calcification [8,9,21]. Disturbed oscillatory flow increases DKK1 expression of HUVECs by generating oscillatory shear stress (OSS). Knockdown or silencing DKK1 via lentiviral genes attenuates OSS-induced increase in monocyte adhesion and endothelial tight junction impairment, thereby attenuating atherogenesis in ApoE−/− mice [22]. DKK-1 is associated with soluble CD40L, a marker reflecting platelet-mediated inflammation and asymmetric dimethylarginine (ADMA), a marker reflecting endothelial dysfunction, and urinary 11-dehydro-thromboxane B2, which is a marker of platelet activation *in vivo* [23]. All of the studies shown that DKK1 facilitates inflammation, platelet activation, and endothelial dysfunction, and plays an important role in the development of atherosclerosis.

RBP4 and PAI-1 play an important role in the occurrence and development of coronary heart disease. *RBP4* expression is an important marker of inflammatory response [24,25], which is associated with early endothelial dysfunction. PAI-1 antigen, a single chain glycoprotein produced by endothelial cells and hepatocytes, is an effective inhibitor of plasminogen activation and fibrinolysis. PAI-1 expression was increased in atherosclerotic lesions [26,27]. PAI-1 inhibits the clearance of fibrin mediated by plasminogen,the overexpression and release of PAI-1 promotes the deposition of fibrin on the arterial wall. Studies of pathological processes have indicated the presence of a large number of fibrin related peptides in coronary atherosclerosis related lesions, which promote the growth of plaques by stimulating the adhesion, migration, and proliferation of macrophages and smooth muscle cells as well as plaque growth, by combining with low-density lipoproteins. Therefore, decreased local fibrinolysis reduces the degradation and metastasis of fibrin. Chronic deposition of fibrin for long periods of time causes repetitive damage to blood vessels, facilitates the invasion of the fibrin matrix by fibroblasts, leads to collagen deposition and, together with a large number of accumulated matrix proteins, causes the fibrosis of tissues and rigidity of tube walls, thereby promoting the development of atherosclerosis [28].

Liu et al., found that compared with those of healthy people without coronary heart disease, the serum RBP4 concentrations of patients with coronary heart disease were significantly increased [29]. Our study showed similar results. Our study we also found that the concentrations of RBP4 and PAI-1 in the high Gensini score group were significantly higher than those in the low Gensini score group. One previous study confirmed that DKK1 exerted a beneficial effect by preventing atherosclerosis via inhibition of HMG-CoA reductase and non-steroidal isoprenoid intermediates. DKK-1 mediates the regulation of 21% of statin-modulated proteins, including PAI-1 [30]. We found that serum DKKI concentration was associated with serum PAI-1 and RBP4 concentrations.

Atherosclerosis and osteoporosis are coexisting clinical conditions affected by aging. They share the same pathophysiological mechanism [31,32]. Mineralization of extracellular matrix was observed in both atherosclerosis and osteoporosis. Cellular events that occur during bone metabolism are closely associated with atheromatous plaque [33]. Thus, the hypothesis of "bone-cardiovascular axis" was developed [34]. OC is a marker of osteogenesis which is excreted by osteoblasts. Reportedly, the numbers of endothelial progenitor cells (EPC) co-expressing the osteoblastic marker osteocalcin [OC (+) EPC] were increased in patients with early and late coronary atherosclerosis. OC (+) EPCs were retained in coronary circulation [35], and the number of circulating endothelial progenitor cells were correlated with the degree of calcification [36]. Our study showed that OC levels in the CHD group were decreased compared with those of the non-CHD group. Multiple ordered logistic regression showed that OC was associated with the degree of coronary artery stenosis.

β-CTX is a bone turnover marker, expressing osteoblast activity. It is also a biomarker of collagen turnover. Type I collagen, which is the predominant fibrillar collagen of the myocaridium, plays an important role in heart remodeling. Degradation of myocardial collagen results in ventricular

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dilation and ventricular stiffness reduction. Higher β-CTX levels are associated with a significantly higher risk of cardiovascular death/heart failure in patients with non-ST elevated acute coronary syndrome, over a median follow-up time of 12 months. β-CTX in the top quartile is associated with cardiovascular death and new or worsening heart failure [37]. In our study, no correlation was found between the β-CTX levels with CHD and between the β-CTX levels with the severity of coronary artery stenosis. The results demonstrate,as a part of type I collagen, β-CTX maybe more correlated with myocardial injury than simple vascular disease.

There were some limitations in our study. First,our study was case-controlled, it was be set by certain limitations. Prospective follow-up observation is needed to further clarify the causal relationship between DKK1 and the degree of coronary artery stenosis. The mechanism underlying the effect exerted by DKK1 on atherosclerosis needs further investigation pertaining to the involvement of molecular biological and genetic factors. Second,the patients both with and without ACS were enrolled in the study,for there were only 12 CHD patients without ACS, so we did not do the subgroup analysis according to the subtypes of CHD to further investigate the difference in the relationship of DKK1,ACS and stable angina.

5. Conclusions

The severity of coronary stenosis increased with DKK1 concentration. The DKK1 levels of the middle and high Gensini score groups were significantly increased, compared with that of the low Gensini score group. The concentrations of RBP4 and PAI-1 in the CHD group were significantly higher than those in the non-CHD group. Serum DKKI concentration was associated with serum PAI-1 and RBP4 and PAI-1 concentrations. The levels of OC in the CHD group were decreased, compared with those of the non-CHD group. OC was associated with the degree of coronary artery stenosis. The level of serum DKK1 was increased in patients with coronary heart disease, which was positively correlated with the degree of coronary artery stenosis.

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Data statement

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

Declaration of competing interest

The authors declare no potential conflicts of interests with respect to research, authorship, and/or publication of this article.

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