Evaluation of serum cysteine-rich protein 61 levels in patients with coronary artery disease

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Aim: The aim is to evaluate serum cysteine-rich protein 61 (Cyr61) levels in patients with coronary artery disease (CAD). **Patients & methods:** Serum Cyr61 levels were measured in 180 patients with CAD and 74 participants without CAD. **Results:** Serum Cyr61 levels were significantly higher in CAD patients. Patients with acute coronary syndrome showed significantly higher Cyr61 than those with stable angina pectoris. Serum Cyr61 levels in complex lesion group were significantly higher. Serum Cyr61 was positively correlated with Gensini score and C-reactive protein. Multivariable logistic regression analyses demonstrated that serum Cyr61 levels were independently correlated with the existence of CAD ($p = 0.01$). **Conclusion:** Our study suggested Cyr61 as a potential biomarker in characterizing CAD and therapeutic target for CAD.

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Keywords: atherosclerosis • coronary artery disease • C-reactive protein • Cyr61 • inflammation

Matricellular proteins are a group of secreted extracellular matrix-associated proteins which play a wide range of functions and roles including the dynamic changes in extracellular matrix and tissue remodeling at the sites of inflammation, tissue injury and repair as well as atherosclerosis [1–3]. Such proteins as osteopontin, thrombospondin, periostin and secreted protein acidic and rich in cysteine have been demonstrated in an important role in inflammation and pathogenesis or progression of atherosclerosis [3–8]. In recent years, a novel matricellular protein Cysteine-rich protein 61 (Cyr61, CCN1), identified as a product of immediate early gene in response to serum growth factors and high dynamically expressed and secreted matricellular protein, has emerged as multifunctional and inflammatory modulator in cellular signaling and behaviors [3,9,10]. The function of Cyr61 is mediated primarily via specific integrins binding and requiring heparin sulfate proteoglycans co-receptors on the cell surface, which regulate a wide range of cell type-dependent functions and processes including cell growth, adhesion, migration, differentiation and survival or death and participate in inflammation, neovascularization, wound healing and tissue remodeling as well as thrombosis and hemostasis [9–11].

The relationship of Cyr61 with vascular diseases was recently explored. In a rat carotid artery injury model, Cyr61 protein level was rapidly increased in the media and neointima of the injured vessels by day 4 postinjury and lasts for 4 weeks, while the knockdown of Cyr61 by siRNA significantly inhibited neointimal formation [12]. Furthermore, Cyr61 expression in aortic arteriosclerotic plaques of ApoE-deficient mice and in human arteriosclerotic lesions was significantly elevated [13,14]. Immunohistochemical analysis of cardiac tissue specimens from individuals died of sudden cardiac death revealed that Cyr61 immunoreactivity was significantly associated with myocardial ischemia, interstitial edema and atheromatosis of coronary arteries [16]. However, serum level of Cyr61 and its significance in patients with coronary artery disease (CAD) has not been investigated until a recent study by Klingenberg *et al.* [49] published online after our first draft was submitted. Klingenberg *et al.* demonstrated Cyr61 as a novel early biomarker in reflecting myocardial injury that improves risk stratification in patients with acute coronary syndrome (ACS), but not evaluated serum Cyr61 levels and its significance in CAD and its subgroups compared with health participants as control. Based on these studies of Cyr61 in atherosclerosis, the present study aimed to evaluate the serum Cyr61 level and its association with severity of CAD in patients with CAD.

Methods

Study subjects

This study was approved by the ethic committee of the authors' hospitals. Informed consent was obtained from each participant. A total of 180 patients (male 126 and female 54) with CAD at an average age of 62.25 ± 10.36 years were recruited. The clinical diagnoses of CAD in the patients were confirmed by coronary angiographic finding of any coronary arterial luminal stenosis at or more than 50% in diameter. All the patients were classified as stable angina pectoris (SAP, 78 patients), unstable angina pectoris (UAP; class II or III of Braunwald's classification, 62 patients) and acute myocardial infarction (AMI, 40 patients) according to their clinical diagnoses. Age- and gender-matched healthy volunteers ($n = 74$, male 48 and female 26; age 62.39 ± 9.48 years) were randomly recruited in this study as the control (Ctrl) group. All the subjects in the Ctrl group did not show any CAD symptoms and history with normal ECG and ECG treadmill testing. Patients with other heart diseases, acute or chronic infection, tumors, autoimmune diseases, renal and hepatic diseases, anemia, peripheral or cerebrovascular diseases, surgery or trauma within 3 months were excluded.

Data & blood sample collection

Weight was measured in kilograms and height in meters. BMI was measured as weight (kg)/height (m^2). Blood pressures (BPs) were measured in a supine position. Smoking habits were recorded by questionnaire. Three milliliters venous blood was collected from each participant after a 12-h overnight fast. Blood samples from patients with UAP or AMI were taken immediately after admission. All the blood samples were centrifuged at 3000 rpm at 4°C for 10 min. The serum were separated and stored at -70°C for detection within 3 months.

Coronary artery angiography & Gensini score

Diagnostic coronary angiography was performed in multiple views in all patients using standard Judkins femoral approach and techniques. Coronary stenotic lesion was divided into simple lesion defined as one coronary branch stenosis $\geq 50\%$ and complex lesion defined as >1 coronary branches with stenosis $\geq 50\%$. Gensini score [17], an index to reflect coronary stenotic severity in clinic, was calculated by two experienced cardiologists who were blinded to the clinical and laboratory data to evaluate the severity of coronary atherosclerotic lesions based on the angiographic results according to the standard method.

Laboratory measurements

Serum Cyr61 levels were measured using a commercially available ELISA kit (DRG Diagnostics, Marburg, Germany) according to the manufacturer's instruction. Blood glucose, lipids and other related indicators were also detected simultaneously with a Hitachi Automatic Biochemical Analyzer (Hitachi Co., Tokyo, Japan). Serum high sensitivity C-reactive protein (hs-CRP) levels were measured with the commercial ELISA kit (Dade Behring Diagnostics GmbH, Germany).

Statistical analysis

All the continuous variables with normal distributions are expressed as mean $\pm$ standard deviation and analyzed by Student's t-test or the one-way analysis of variance test, continuous variables with skewed distributions are expressed as median and quartile ranges and analyzed by Mann-Whitney U test. Categorical data were expressed as numbers or percentages and compared with χ^2 test. Spearman coefficients were calculated to determine the correlation between biochemical parameters. Multivariable logistic regression models were used to evaluate the association of cardiovascular risk factors with CAD. Statistical analysis was done with SPSS version 17 except MedCalc software (z-test) used for comparison of receiver operating characteristic (ROC) curves of Cyr61 and hs-CRP. A p-value less than 0.05 was considered significant statistically.

Results

Demographic characteristics of the study subjects

The clinical and biochemical parameters of the study subjects are summarized in Table 1. In the Ctrl group ($n = 74$), 52 were male and 22 were female, the average age was 62.39 ± 9.48 years. In CAD group ($n = 180$), 132 were male and 48 were female, the average age was 65.25 ± 10.36 years. There were no significant differences in age, gender, BMI and history of diabetes, and also no significant differences in high-density lipoprotein-cholesterol, white blood cell, platelets, glucose, blood urea nitrogen and serum creatinine between the Ctrl and CAD groups. However,

Table 1. Demographic characteristics of the study subjects.

Variables	Ctrl (n = 74)	All CAD (n = 180)	SAP (n = 78)	UAP (n = 62)	AMI (n = 40)
Age (years)	62.39 ± 9.48	65.25 ± 10.36	65.42 ± 9.62	62.35 ± 11.33	64.18 ± 11.54
Male (n, %)	52 (70.3)	132 (73.3)	58 (73.1)	44 (70.9)	30 (75)
BMI (kg/m ²)	24.48 ± 3.72	26.28 ± 4.85	25.52 ± 3.78	23.12 ± 2.98	26.75 ± 4.83
Smoking (n)	12	98*	38	36	24
HTN (n)	15	101*	45	34	22
DM	8	40*	18	13	9
TG (mg/dl)	147.65 ± 38.93	168.84 ± 46.67*	162.56 ± 50.49	155.26 ± 43.57	175.48 ± 62.68
TC (mg/dl)	170.38 ± 41.52	210.45 ± 38.72*	196.73 ± 48.86	194.75 ± 37.65	208.45 ± 52.45
HDL-C (mg/dl)	46.45 ± 7.68	43.84 ± 6.86	42.82 ± 8.86	44.23 ± 7.34	42.45 ± 8.98
LDL-C (mg/dl)	113.45 ± 27.55	129.65 ± 29.3*	124.3 ± 26.6	128.5 ± 34.7	130.42 ± 31.46
WBC (10 ³ /μl)	6.78 ± 2.09	7.38 ± 1.88	6.72 ± 1.75	9.24 ± 2.68	8.85 ± 3.22
Platelet (10 ³ /μl)	221.75 ± 65.53	228.75 ± 78.48	216.65 ± 72.45	235.93 ± 93.67	235.05 ± 88.89
Uric acid (mg/dl)	5.34 ± 1.73	6.96 ± 2.65*	6.44 ± 1.57	7.38 ± 2.45	7.78 ± 3.82
Glucose (mg/dl)	97.84 ± 22.46	115.33 ± 24.56	97.63 ± 25.86	113.72 ± 21.86	120.96 ± 28.48
BUN (mg/dl)	11.37 ± 4.72	12.06 ± 3.54	12.64 ± 5.78	10.38 ± 4.77	13.75 ± 6.22
Cr (mg/ml)	1.14 ± 0.36	1.09 ± 0.47	1.13 ± 0.24	0.97 ± 0.59	1.24 ± 0.56
hs-CRP (mg/l)	1.58 ± 0.68*	6.05 ± 3.78	2.75 ± 1.42	7.60 ± 2.37**	10.1 ± 3.2**

*Significance between Ctrl and CAD groups, $p < 0.05$;** $p < 0.001$ versus SAP.

AMI: Acute myocardial infarction; BMI: Body mass index; BUN: Blood urea nitrogen; CAD: Coronary artery disease; Cr: Serum creatinine; Ctrl: Control; DM: Diabetes mellitus; HDL-C: High-density lipoprotein-cholesterol; hs-CRP: High sensitivity C-reactive protein; HTN: Hypertension; LDL-C: Low-density lipoprotein-cholesterol; SAP: Stable angina pectoris; TC: Total cholesterol; TG: Triglyceride; UAP: Unstable angina pectoris; WBC: White blood cell.

Figure 1. Serum Cyr61 levels in each group. Serum Cyr61 levels in patients with CAD were significantly increased compared with those in Ctrl group, and serum Cyr61 levels in patients with UAP or AMI were significantly increased compared with those with SAP. AMI: Acute myocardial infarction; CAD: Coronary artery disease; Ctrl: Control; SAP: Stable angina pectoris; UAP: Unstable angina pectoris.

cigarette smoking, diabetes mellitus (DM) and hypertension (HTN) history, triglyceride, total cholesterol, low-density lipoprotein-cholesterol, hs-CRP and uric acid were higher significantly in CAD group compared with that in the Ctrl group.

Serum Cyr61 levels in CAD & subgroups

The serum Cyr61 levels in the Ctrl and CAD groups were shown in Figure 1. Serum Cyr61 levels in patients with CAD were significantly elevated compared with those in the Ctrl group (621.30 ± 283.72 vs 265.26 ± 116.70 pg/ml; $p < 0.001$). To evaluate serum Cyr61 level changes in CAD types, all patients were grouped into three subgroups clinically: SAP, UAP and AMI. Compared with the serum Cyr61 level in SAP subgroup, serum Cyr61 levels in UAP (729.58 ± 247.15 vs 415.08 ± 143.33 pg/ml; $p < 0.001$) or AMI (855.61 ± 270.88 vs 415.08 ± 143.33 pg/ml; $p < 0.001$) subgroups were significantly higher. Serum Cyr61 in AMI group was higher than in UAP one, but there was no statistic difference in serum Cyr61 levels between UAP and AMI subgroups. As ACS includes UAP and AMI, we also found that serum Cyr61 levels in ACS subgroup were significantly higher (811.44 ± 265.39 vs 415.08 ± 143.33 pg/ml; $p < 0.001$).

Figure 2. Association of serum Cyr61 with the severity of atherosclerotic lesions. (A) Serum Cyr61 levels in patients with complex lesions were significantly increased compared with those with simple lesions. **(B)** Serum Cyr61 levels were positively correlated with Gensini score in patients with CAD. CAD: Coronary artery disease; Ctrl: Control.

Association of serum Cyr61 with coronary lesion severity

To exam the association of Cyr61 levels with the severity of coronary atherosclerosis, patients were classified into two groups: simple lesion and complex lesion. As shown in Figure 2A, serum Cyr61 levels were significantly higher both in the simple lesion (531.1 ± 238.92 pg/ml) and complex lesion (662.04 ± 293.68 pg/ml) groups compared with those in Ctrl group (265.26 ± 116.70 pg/ml), and serum Cyr61 levels in patients with complex lesion were significantly higher compared with those with simple lesion (662.04 ± 293.68 vs 531.1 ± 238.92 pg/ml; $p = 0.003$). Spearman correlation analyses showed a positive correlation between serum Cyr61 levels and Gensini score ($r = 0.45$; $p < 0.001$) (Figure 2B).

Association of serum Cyr61 with hs-CRP & ROC curve analysis

CRP is not only a useful biomarker, but also an independent risk factor for CAD pathogenesis. Consistent with other studies, we found hs-CRP levels were significantly elevated in CAD group than that in Ctrl group ($p = 0.01$) and hs-CRP levels in ACS (including UAP and AMI) group were significantly higher than that in SAP group ($p < 0.001$) (Table 1). Since both serum levels of Cyr61 and hs-CRP were elevated significantly in CAD group in our study, we analyzed the correlation of serum Cyr61 with hs-CRP levels using Spearman's correlation analysis. As shown in Figure 3A, a significant correlation between these two parameters was demonstrated in CAD group ($r = 0.59$; $p < 0.001$), but no correlation in the Ctrl group ($r = 0.19$; $p = 0.11$; data not shown).

ROC curve analyses were carried out to evaluate the diagnostic value of serum Cyr61 as biomarker to differentiate patients with or without CAD. ROC curve analyses showed the area under the curve (AUC) for serum Cyr61 was 0.90 (95% CI: 0.861–0.937; $p < 0.001$) and AUC for hs-CRP was 0.91 (95% CI: 0.888–0.951; $p < 0.001$) (Figure 3B), indicating excellent accuracy as hs-CRP had. The ability of the area under the ROC curve based on serum Cyr61 levels to predict the presence of CAD was 0.90. The optimal cutoff values of serum Cyr61 level were 365.7 pg/ml (84.44% sensitivity and 81.08% specificity) to detect CAD. There was no significant difference in comparison of ROC curves of Cyr61 with hs-CRP by z-test.

Figure 3. Relationship of serum Cyr61 with CRP. (A) Serum Cyr61 levels were positively correlated with hs-CRP in patients with CAD. (B) ROC curves of Cyr61 and hs-CRP for the identification of CAD. AUC for Cyr61 0.90 (95% CI: 0.861–0.937; $p < 0.001$) and for hs-CRP 0.91 (95% CI: 0.888–0.951; $p < 0.001$). AUC: Area under the curve; CAD: Coronary artery disease; hs-CRP: High sensitivity C-reactive protein; ROC: Receiver operating characteristic.

Table 2. Serum levels of Cyr61 in each groups and subgroups.

Characteristic	Ctrl group		CAD group	
	No.	Cyr61 (pg/ml)	No.	Cyr61 (pg/ml)
Male	52	292.48 ± 148.67	132	513.53 ± 211.45
Female	22	273.37 ± 170.45	48	485.68 ± 227.36
HTN	15	338.45 ± 163.67 [†]	101	586.25 ± 270.33 [‡]
Normal BP	59	244.33 ± 127.45	79	470.67 ± 190.56
DM	8	288.75 ± 157.33	40	514.44 ± 220.95
Non-DM	66	266.78 ± 163.56	140	478.36 ± 193.65
Smoking	12	288.35 ± 149.89	98	496.15 ± 206.88
Nonsmoking	62	265.87 ± 170.45	82	478.48 ± 222.75
Dyslipidemia	14	288.76 ± 170.34	102	506.98 ± 247.67
Normolipidemia	60	279.57 ± 155.56	78	518.45 ± 207.52

[†] $p = 0.045$.

[‡] $p = 0.028$ versus normal BP.

BP: Blood pressure; CAD: Coronary artery disease; Ctrl: Control; DM: Diabetes mellitus; HTN: Hypertension.

Relationship between serum Cyr61 levels and risk factors

The relationship of serum Cyr61 levels with traditional CAD risk factors, such as gender, cigarette smoking, DM, dyslipidemia and HTN were analyzed. As shown in Table 2, all participants with HTN both in the Ctrl and CAD groups exhibited significant higher serum Cyr61 levels compared with those with normal BP (Ctrl group: 338.45 ± 163.67 vs 244.33 ± 127.45 pg/ml; $p = 0.045$; CAD group: 586.25 ± 270.33 vs 470.67 ± 190.56 pg/ml;

Table 3. Multivariable logistic regression analysis for independent factors of coronary artery disease.			
	OR	95% CI	p-value
Age	1.04	0.82–1.26	0.08
Gender	0.95	0.39–2.32	0.16
BMI	1.08	0.80–1.47	0.21
HTN	3.17	1.58–4.72	0.02
DM	1.96	1.55–3.92	0.02
Smoking	1.76	1.11–4.83	0.01
LDL	2.45	1.09–2.54	0.04
HDL	1.05	0.75–1.78	0.09
Uric acid	1.16	0.79–2.06	0.06
hs-CRP	2.06	1.18–5.75	0.02
Cyr61	1.65	1.48–3.92	0.01

BMI: Body mass index; DM: Diabetes mellitus; HDL: High-density lipoprotein; hs-CRP: High sensitivity C-reactive protein; HTN: Hypertension; LDL: Low-density lipoprotein; OR: Odds ratio.

p = 0.028), but there were no significant differences in serum Cyr61 levels in association with gender, smoking, dyslipidemia and DM either in Ctrl or CAD groups.

Multivariable logistic regression analyses

To evaluate the independent predictors of the presence of CAD, multivariable logistic regression analyses were performed to assess their independent contribution to CAD presence. The results (Table 3) show that serum Cyr61 levels were independently correlated with the existence of CAD (odds ratio: 1.85; 95% CI: 1.48–3.92; p = 0.01). In addition, HTN, DM, cigarette smoking, low-density lipoprotein and hs-CRP were also significantly associated with the existence of CAD.

Discussion

In this study, we first demonstrated that serum Cyr61 levels are significantly elevated in patients with CAD, especially with ACS, than in those without CAD. Positive correlations are observed between serum Cyr61 levels and hs-CRP or Gensini score in CAD patients. In addition, multivariable logistic regression analyses demonstrated that elevated serum Cyr61 level is an independent predictor of CAD presence. Those findings suggest that Cyr61 play an important role in pathogenesis and development of CAD and serum Cyr61 level may serve as a biomarker in diagnosis, risk assessment and therapeutic target for CAD.

Cyr61 play important roles in inflammation, wound healing and tissue remodeling including atherosclerosis [3,9–11]. Studies demonstrated that Cyr61 is increased after vascular injury and higher level of Cyr61 is reported in atherosclerotic lesions [12–14]. The expression of Cyr61 is at a very low level in quiescent condition, but is highly expressed within minutes or hours after stimulation by numerous atherogenic factors or extracellular stimuli including VEGF, PDGF, bFGF, TGF-β, growth hormone, angiotensin II, thrombin, inflammatory cytokines, hs-CRP and hypoxia, mechanical stretch [15,18–30]. A variety of cell types including endothelial and epithelial cells, vascular smooth muscle cells, fibroblasts, monocytes, activated platelets and cardiomyocytes have been identified as sources of Cyr61 [11–16,18,28,31]. In addition, disturbed shear stress and mechanical stretch on vessel wall also contributes to Cyr61 secretion into circulation [26,32]. Therefore, it is plausible that these cells activated by numerous atherogenic factors mentioned above express and secrete Cyr61 into circulation resulting in elevated serum Cyr61 in patients with CAD. On the other hand, Cyr61 is recognized as a mediator of inflammation [33,35]. Cyr61 enables monocytes recruitment upon vascular inflammation, and endothelial-bound Cyr61 sustains monocyte patrolling under inflammation condition, while blocking Cyr61, impairs early arrival of monocyte and abolishes neutrophil recruitment [34]. Other studies showed that Cyr61 promotes monocyte chemotaxis and macrophage migration, support macrophage adhesion and activates proinflammatory genetic program by activating NFκB-mediated transcription, and upregulating expression of cytokines and chemokines in macrophages [35,36], indicating an important role of Cyr61 in inflammatory responses and its essential role in atherosclerotic pathogenesis. The initial and key step for atherogenesis is endothelial inflammation and leukocytes recruitment and infiltration [37], thus, elevated serum Cyr61 levels in patients with CAD is consistent with the concept of inflammatory mechanisms in atherosclerosis pathogenesis and reflects atherosclerosis development and progression. In addition, Cyr61 was reported to support

vascular smooth muscle cells (VSMCs) adhesion and stimulate proliferation and migration [19,38], this effect may also involve the recruitment of VSMCs from the tunica media into the tunica intima to accelerate atheroma formation and vascular narrowing. Therefore, Cyr61 may be implicated in different stage of atherosclerosis. Indeed, from multivariable logistic regression analyses, we found that serum Cyr61 levels were independently correlated with the existence of CAD suggesting Cyr61 as an atherogenic factor. In this study, we also evaluated the association of elevated serum Cyr61 with clinical severity in patients with CAD, and found that patients with complex atherosclerotic lesions had higher levels of serum Cyr61 compared with those with simple lesions, and serum Cyr61 levels were found to be positively correlated with Gensini score in CAD patients, indicating the clinical significance of serum Cyr61 level to predict atherosclerotic severity and progression.

The observation that patients with ACS have significantly higher levels of serum Cyr61 than that in SAP patients indicates Cyr61 is a hallmark of unstable plaque and implicated in the occurrence or development of ACS. Not only circulating in bloodstream, but Cyr61 also deposits in tissues including vessel wall, participating in inflammation, vasa vasorum angiogenesis and vascular remodeling [9]. It is well documented that inflammation, platelet activation, plaque rupture and mural thrombosis formation are the main events to trigger ACS [44]. Macrophages activation and releasing lytic enzymes contribute significantly to the rupture of plaque. Platelet activation and granule release amplifies thrombus formation at sites of vascular injury and plays critical role in ACS. Hviid *et al.* demonstrated platelets activated by thrombin release Cyr61 [31]. We also found that Cyr61 stimulates matrix metalloproteinase-2 expression through $\alpha_v\beta_3$ integrin in VSMCs [DENG, UNPUBLISHED DATA], which is highly expressed in vulnerable regions of human atherosclerotic plaques and contribute to plaque vulnerability. Jedsadayanmata *et al.* [39] reported that activated platelets adhere to Cyr61 through integrin $\alpha_{IIb}\beta_3$ which may contribute to the stability of the hemostatic plug and the tight adhesion of platelets to the subendothelial matrix suggesting a functional role in hemostasis and thrombosis. The finding by Klingenberg *et al.* [49] that Cyr61 expression is markedly increased in coronary thrombi aspirated from the infarct-related coronary artery in ACS patients also supports a role of Cyr61 in thrombosis. In addition, Cyr61 was demonstrated to bind integrins $\alpha_v\beta_3$ and $\alpha_6\beta_1$ to mediate endothelial cell adhesion, migration, angiogenesis and neovascularization [40], the angiogenic effects of Cyr61 may contribute to the formation of hemorrhagic neovessels in the intima of vulnerable plaques leading to intraplaque hemorrhages and increase in plaque vulnerability to rupture. Considering those above, higher levels of serum Cyr61 may contribute significantly to ACS progression through diverse mechanisms. Although not statistically significant, serum Cyr61 levels were higher in patients with AMI than those with UAP in our study, this suggests a sustained elevation of serum Cyr61 in ACS and a sign of prelude of aggravation of UAP leading to AMI occurrence, implying a role of Cyr61 in the increased cardiovascular events. On the other hand, high levels of serum Cyr61 in ACS patients may also reflect the severity of myocardial ischemia since Cyr61 expression is strongly induced in both ischemic and remote left ventricular myocardium [15,49].

Analyses of the data revealed a positive correlation between serum Cyr61 and hs-CRP levels in patients with CAD. Not only an inflammation biomarker, but also CRP associates with atherosclerosis progression and cardiovascular risk as an atherogenic factor [42]. CRP is reported to increase Cyr61 mRNA in endothelial cells [28], and Cyr61 increases the expression of IL-6 in macrophages through integrin $\alpha M\beta 2$ and in fibroblasts through $\alpha 6\beta 1$ [41], an important cytokine to control CRP synthesis mainly in the liver, therefore, a synergistic effect exists in Cyr61 with CRP of their production, which reinforces the notion of inflammatory effect of Cyr61 on CAD. Moreover, ROC curve analyses showed the AUC of Cyr61 (AUC = 0.90; 95% CI: 0.855–0.945; $p < 0.001$) is similar as hs-CRP (AUC = 0.91; 95% CI: 0.876–0.955; $p < 0.001$) in trends without statistical difference between them, indicating serum Cyr61 level had excellent performance in diagnosis for CAD. CRP is an acute phase reactant and its secretion begins within 4–6 h of the stimulus and peaks at about 36–48 h. CRP is widely used and recognized as valuable biomarker in cardiovascular risk and events assessment. As an inducible immediate early gene, Cyr61 syntheses is increased by numerous atherogenic factors maximally at 1–3 h [13,19,26,30,43], we found Cyr61 is significantly increased maximally at 2 h with lasting more than 8 h in high level after stimulation by angiotensin II and thrombin in human aortic smooth muscle cells [DENG, UNPUBLISHED DATA], indicating the change of Cyr61 precedes CRP in rise and peaking. Therefore, Cyr61 holds promising value as a biomarker candidate in CAD diagnosis, risk stratification and monitoring therapeutic efficacy and prognosis than CRP does.

It is well known that renin-angiotensin system activation and increase in peripheral vascular resistance plays an important role in pathogenesis of HTN. Arterial stiffness due to alterations in extracellular matrix in vascular wall is one of the mechanisms for increased peripheral resistance in HTN [48]. Cyr61 was reported to be induced rapidly by Ang II in vascular cells and tissue [45,46]. BP is largely responsible for mechanical stretch on the blood

vessel wall, while mechanical stretch increases Cyr61 expression in smooth muscle cells [26,47]. In this study, we first observed that participants with HTN both in CAD and Ctrl groups have significant higher levels of serum Cyr61. Such levels could associate with renin-angiotensin system activation and mechanical stretch by high BP. Cyr61 supports VSMC adhesion and stimulates VSMC migration, proliferation [38] and participates in vascular remodeling, thus, high levels of Cyr61 may alter VSMC and arterial stiffness and be implicated in the pathogenesis or progression of HTN. We did not evaluate the correlation between BP reading and serum Cyr61 levels in this study. Further studies are needed to investigate the reciprocal effects of HTN with Cyr61 level change and the mechanism of Cyr61 in HTN pathogenesis.

Conclusion & future perspective

In summary, we reported the first study regarding the serum Cyr61 levels and its clinical significance in CAD patients. Our results showed that serum Cyr61 levels were increased in patients with CAD, especially with complex lesions and ACS, and positively correlated with hs-CRP, Gensini score in patients with CAD. Based on the findings, our study support the notion that serum Cyr61 levels hold the promise as a biomarker in diagnosing and characterizing CAD as well as risk stratification and therapeutic target for CAD.

There are some limitations in the study: this is a single-center study with small size in each group, large scale or multicenter clinical study is recommended to clarify the significance of serum Cyr61 levels in CAD; serum Cyr61 levels were only analyzed at one single time point, and we did not analyze inter- and intra-assay coefficients of variability of Cyr61 in this study. Kinetic analyses with multiple time points and triplicate measurement of serum Cyr61 levels may provide more valuable information. Finally, future studies are warranted to evaluate the association of serum Cyr61 levels with Framingham risk score as well as cardiovascular events.

Executive summary

- This study investigated serum Cysteine-rich protein 61 (Cyr61) levels in patients with coronary artery disease (CAD).
- 180 patients with CAD and 74 participants without CAD were enrolled randomly in this study. Serum Cyr61 levels and high-sensitivity C-reactive protein (hs-CRP) were measured using commercially available enzyme-linked immunosorbent assay kits. Gensini scores were calculated to evaluate the severity of coronary atherosclerotic lesions.
- Results of data analyzed show that serum Cyr61 levels were significantly higher in patients with CAD than in those without CAD.
- Patients with acute coronary syndrome (ACS) had significantly higher Cyr61 levels than those with stable angina pectoris (SAP).
- Serum Cyr61 levels in complex lesion group were significantly higher than in simple one.
- Serum Cyr61 was positively correlated with hs-CRP and Gensini score.
- Area under receiver operation characteristic (ROC) curve analysis showed a sensitivity of 84.44% and specificity of 81.08% at the optimal cutoff point of serum Cyr61 level 365.7 pg/ml.
- Multivariable logistic regression analyses demonstrated that serum Cyr61 levels were independently correlated with the existence of CAD.
- In conclusion, serum Cyr61 levels are increased in patients with CAD, especially in patients with ACS, and show significant correlation with the severity of CAD, suggesting Cyr61 as a potential biomarker in characterizing and stratifying CAD as well as a therapeutic target for CAD.

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Ethical conduct of research

The authors state that this study was approved by the ethics committee of the authors' hospitals, and the authors have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from each participant involved.

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