

Extracellular matrix turnover in coronary artery ectasia patients

Ruifeng Liu · Lianfeng Chen · Wei Wu · Houzao Chen · Shuyang Zhang

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Abstract Dysregulation of the metabolism of the extracellular matrix (ECM) may contribute to coronary artery ectasia (CAE). This study evaluated the turnover of main ECM components and related proteolytic enzymes activities. In this study, thirty patients with CAE, 30 patients with coronary artery disease (CAD) and 30 subjects with normal coronary arteries (Control) were selected. The following circulating ECM metabolism markers were measured: soluble elastin (sElastin), collagen type I cross-linked telopeptides (ICTP), procollagen type I carboxy terminal peptide (PICP), procollagen III N-terminal propeptide (PIIINP), and procollagen $\alpha 1(\text{III})$ C-terminal propeptide (PIIICP). Serum total elastase activity and total matrix metalloproteinase (MMP) activity were also determined. The level of sElastin was higher in the CAE group than in the CAD and Control groups ($P_1 = 0.009$, $P_2 = 0.000$). There was no difference in ICTP ($P = 0.168$) or PIIICP ($P = 0.079$) among the three groups. PICP was significantly elevated in CAE ($P_1 = 0.001$, $P_2 = 0.002$). PIIINP was also significantly increased in CAE ($P_1 = 0.002$, $P_2 = 0.007$). Total elastase activity was higher in the CAE group than in the other two groups ($P_1 = 0.006$, $P_2 = 0.022$). Total MMP activity was significantly higher in the CAE group than the Control group ($P_2 = 0.013$) but not higher than the CAD

group ($P_1 = 0.477$). In conclusion, within CAE patients the main changes in ECM metabolism were increased degradation of elastin fibres and the transition of collagen from type III to type I. Elastase and MMPs appear to be associated with this kind of ECM turnover.

Keywords Coronary artery ectasia (CAE) · Extracellular matrix (ECM) · Soluble elastin (sElastin)

Introduction

Coronary artery ectasia (CAE) has been defined as the inappropriate dilatation of a coronary artery, with the luminal diameter 1.5 or more times wider than that of adjacent normal segments [1]. Its prevalence has been reported to range from 0.3 to 4.9 % according to previous autopsy and angiography studies [1, 2]. Clinically, it predisposes patients to adverse coronary events, such as vasospasm, thrombosis and dissection [3]. The pathogenesis of CAE remains elusive. More than 50 % of CAE patients have obstructive coronary artery disease (CAD), indicating that CAD could be the main underlying aetiology of CAE [4]. Characteristic changes in CAE are extensive destruction of musculoelastic elements, including damage of elastin fibres and a decreased number of smooth muscle cells [4–6]. Makis noted that CAE patients showed confirmed evidence of cystic medial necrosis [6], and previous studies showed that proteolytic enzymes may play vital roles in the pathogenesis of CAE [7–9].

Normally, the coronary media contains many layers of lamellar units, which are composed of vascular smooth muscle cells lying between two layers of elastin fibres and surrounded by type I and type III collagen and other extracellular matrix (ECM) proteins [10]. Elastin fibres are the

R. Liu · L. Chen · W. Wu · S. Zhang (✉)
Cardiac Department, Peking Union Medical College Hospital (PUMCH), Chinese Academy of Medical Sciences and Peking Union Medical College, No.1 Shuai Fu Yuan, Dongcheng District, Beijing 100730, China
e-mail: zhangshuyang103@163.com

H. Chen
National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

dominant ECM proteins in the coronary wall, constituting up to 50 % of the dry weight [11, 12], and supporting the elasticity and tensile strength of the vessels [13]. Due to a lack of de novo synthesis of elastin after reaching adulthood, its degradation is irreversible and can lead to various symptoms and diseases [13]. Increased levels of proteolytic enzymes, including serine, thiol, aspartic and metallo enzymes, can promote fragmentation of the elastin fibre network in the media. The most widely studied enzymes are elastases, such as neutrophil elastase and pancreatic elastase, which belong to the serine family, and matrix metalloproteinases (MMPs) [13]. After destruction, soluble elastin (sElastin) and its fragments are released into the circulating blood. Thus, the degradation of elastin fibres can be detected by evaluating blood sElastin [11].

Type I and type III collagen are the most abundant collagen fibres in the artery wall. Collagen type I consists of thick and strong fibres, and it mainly provides rigidity to the vessels. In contrast, type III collagen is thin and interweaved into a fine reticular network, which gives elasticity to tissue and organs [14, 15]. Various circulating markers can be used to indicate the turnover of collagens. Collagen type I cross-linked telopeptides (ICTP) and the procollagen III N-terminal propeptide (PIIINP) are cleavage products of degraded type I [16] and type III collagen [17], respectively. Thus, ICTP and PIIINP can be used as collagen degradation makers. The procollagen type I carboxy terminal peptide (PICP) and procollagen a1(III) C-terminal propeptide (PIIICP) are fragments produced during the biosynthesis of type I [18] and type III [19] collagen and act as markers of collagen synthesis. As it is not easy to gather human coronary artery autopsy specimens, levels of ICTP, PIIINP, PICP and PIIICP in serum can be used as an alternative method to assess coronary ECM metabolism in CAE patients.

To the best of our knowledge, the turnover of ECM, including elastin fibres and type I and type III collagens, has not been studied before in patients with CAE. Thus, the present study evaluated the main components of ECM metabolism in CAE patients by measuring levels of circulating sElastin, ICTP, PIIINP, PICP and PIIICP. We also determined the main ECM proteolytic enzyme activities, including total elastase activity and total MMP activity in the same patients [20, 21]. Those results would be useful clues for the further in-depth mechanism researches for CAE.

Patients and methods

Patient population

In total, 1640 patients' serum samples were collected after coronary angiograms immediately from September 2009

to March 2013 in the cardiac catheterisation centre of Peking Union Hospital. The indications of angiographic examination for these patients were stable angina, acute coronary syndrome and atypical chest discomfort. Among these patients, 51 (3.11 %) of them had CAE and 30 of them without conditions listing in exclusion criteria were included in the CAE group. During the same operation period, we randomly selected 30 patients with angiographically documented CAD and 30 subjects with relatively normal coronary arteries (named Control or CON). The three groups were balanced in age, sex and other baseline characteristics. This study was approved by the local ethics committee and was in accord with the Declaration of Helsinki. Informed consent was obtained from all the study participants.

Inclusion criteria

Coronary angiograms were performed via a femoral approach using the Judkins technique, without the use of nitroglycerin, adenosine or a calcium channel blocker. Each angiogram was interpreted by two independent cardiologists. CAE was defined as an ectatic artery diameter exceeding 1.5 times that of adjacent normal segments [6]. CAD was defined as 50 % or more stenosis in one or more major coronary arteries [9]. Subjects without abnormal coronary arteries were used as controls.

Exclusion criteria

The exclusion criteria were acute coronary syndrome, cardiomyopathy, valvular heart disease, heart failure, collagen tissue diseases, vasculitis, syphilis, chronic obstructive lung disease, pulmonary hypertension, early menopause, organic hepatic diseases, chronic alcoholism, use of steroid or anti-inflammatory drugs within the last 3 months, renal failure and cancer.

Medical records and blood samples

The hospital medical records were detailed and intact. Most of the data in this research were extracted from the medical records. The blood samples were collected immediately after the coronary angiography, and serum samples were separated within 6 h and kept at -80°C for the future experiments.

ELISA for the detection of circulating sElastin, ICTP, PIIINP, PICP and PIIICP

Quantitative determination of sElastin, ICTP, PIIINP, PICP and PIIICP was performed using sandwich ELISA kits according to the manufacturer's instructions (Elabscience

Biotechnology Co.,Ltd, Wuhan, China). Briefly, the serum samples were first incubated in microtitre wells precoated with anti-sElastin, anti-ICTP, anti-PIIINP, anti-PICP and anti-PIIICP antibodies. The microtitre wells were then washed, and peroxidase-labelled FabV antibody was added to each well. The amount of peroxidase bound to each well was determined by the addition of 3,3',5,5'-tetramethylbenzidine substrate. The reaction was stopped with acid solution, and the resultant colours were read at 450 nm in a microtitre plate spectrophotometer. The final concentrations were calculated by interpolation from a standard curve.

Total elastase activity assay

Total elastase activity was measured using the method employed by Bizbiz [20] and Harris [21]. Briefly, serum samples (50 μ L) from different groups were incubated with 150 μ L of polypeptide substrate N-succinyl-(L-alanine)3-p-nitroanilide (1 mmol/L, Sigma-Aldrich [Shanghai] Trading Co., Ltd) dissolved in Tris-HCl (200 mmol/L; pH 8.0) for 2 h at 37 °C in a 96-well plate. Then, 5 μ L of glacial acetic acid was added to stop the reaction. On cleavage by elastase, this substrate is absorbed at 405 nm. The absorption value at 405 nm of each sample was determined and compared to a calibration curve prepared with standard elastase solution and its dilutions (Sigma-Aldrich [Shanghai] Trading Co., Ltd). Elastase activity was expressed as the activity percent compared to that of standard elastase solution.

Total MMP activity assay

The total activity of MMPs was determined using a GenMed MMP Fluorimetric Assay Kit (GenMed Scientifics Inc., Shanghai, China) according to the manufacturer's instructions. Briefly, 50 μ L of serum samples was added to a reaction mixture containing MMP fluorescence resonance energy transfer peptide substrate, Mca-PLGL-Dap-(Dnp)-AR-OH [22]. After 40 min of incubation at 37 °C, the fluorescence intensity was measured at wavelengths of 330 nm/400 nm (excitation/emission) with a fluorescence microplate reader, using 7-methoxycoumarin (MCA) as the standard. One unit of MMP activity was defined as the amount of enzyme that hydrolysed 1 nmol of peptide substrate per min at 37 °C at pH 7.5.

Statistical analysis

Statistical analyses were performed with SPSS 17.00 package software. General descriptive characteristics were assessed as mean \pm standard deviation (SD).

Nonparametric data characteristics were assessed as percent (%). Sex and risk factors were compared between groups using the Chi square test. For parametric variables, statistical differences among the groups were tested with a one-way analysis of variance or Kruskal–Wallis test. The LSD method or Nemenyi test was used for multiple comparisons between the groups. The correlations between sElastin and total elastase activity and total MMP activity were evaluated with Pearson's correlation test. The lowest level of significance was accepted as $P < 0.05$.

Results

The baseline characteristics of the CAE, CAD and Control are given in Table 1. The three groups were similar with regard to age, sex, presence of hypertension, fasting glucose, lipid profile, renal function, and most other cardiovascular risk factors ($P > 0.05$). The diastolic blood pressure, body mass index, hs-CRP, and blood monocyte number were higher in the CAE and CAD groups. The main results were the following:

1. Increased circulating sElastin in patients with CAE

As shown in Table 2 and Fig. 1, the level of sElastin was significantly higher in the CAE group than in the CAD ($P_1 = 0.009$) and Control groups ($P_2 = 0.000$). It was also higher in the CAD group than the Control group ($P_3 = 0.015$). The data indicate that the rate of elastin fibre degradation was higher in the CAE group than in the other two groups.

2. Turnover of type I collagen in CAE patients

As shown in Table 2 and Fig. 2, there was no significant difference in the serum ICTP concentrations among the three groups ($P = 0.168$). However, the serum PICP concentration was significantly higher in the CAE group compared to the other two groups ($P_1 = 0.001$, $P_2 = 0.002$). The data reveal that the synthesis, but not the degradation of type I collagen, was greater in the CAE group.

3. Turnover of type III collagen in CAE group.

According to the data in Table 2 and Fig. 2, the concentration of PIIINP increased significantly in the CAE group compared to that of the other two groups ($P_1 = 0.002$, $P_2 = 0.007$). In contrast, there was no statistically significant difference in the concentration of PIIICP among the three groups ($P = 0.079$). The results demonstrate that the degradation, but not the synthesis of type III collagen, increased in the CAE group.

Table 1 Clinical characteristics of the collected subjects

	CAE (<i>n</i> = 30)	CAD (<i>n</i> = 30)	CON (<i>n</i> = 30)	<i>P</i> value	Comparison between groups		
					<i>P</i> 1	<i>P</i> 2	<i>P</i> 3
Mean age, years	59.10 ± 11.09	63.03 ± 10.06	61.50 ± 8.30	0.233			
Gender, male, %	19 (63.3 %)	20 (66.6 %)	15 (50.0 %)	0.103			
Diabetes mellitus	10 (33.3 %)	12 (40.0 %)	7 (23.3 %)	0.373			
Glucose	5.73 ± 1.11	6.53 ± 1.62	6.47 ± 1.72	0.057			
Hypertension	19 (63.3 %)	20 (66.6 %)	19 (63.3 %)	0.205			
Systolic pressure (mmHg)	126.72 ± 18.03	131.04 ± 17.39	129.83 ± 20.58	0.659			
Diastolic pressure (mmHg)	79.03 ± 10.49	79.39 ± 11.58	73.07 ± 9.90	0.004	0.970	0.015	0.004
Family history of CAD	6 (20.0 %)	10 (33.3 %)	5 (16.7 %)	0.230			
Family history of type 2 diabetes mellitus	6 (20.0 %)	10 (33.3 %)	11 (36.7)	0.145			
Smoking	10 (33.3.0 %)	12 (40.0 %)	7 (23.3 %)	0.062			
Alcohol consumption	6 (20.0 %)	9 (30.0 %)	5 (16.7 %)	0.086			
TG (mmol/L)	4.43 ± 1.11	4.11 ± 1.02	4.02 ± 0.81	0.072			
HDL-c (mmol/L)	1.18 ± 0.47	1.14 ± 0.40	1.13 ± 0.34	0.090			
LDL-c (mmol/L)	2.59 ± 1.08	2.39 ± 0.86	2.32 ± 0.72	0.745			
TC (mmol/L)	1.18 ± .047	1.15 ± 0.345	1.14 ± 0.34	0.342			
Body mass index (kg/m ²)	27.38 ± 3.86	26.58 ± 2.98	24.30 ± 3.26	0.006	0.303	0.000	0.000
Hs-CRP (mg/L)	4.14 ± 4.35	3.71 ± 3.60	2.40 ± 3.00	0.018	0.934	0.055	0.011
Leukocytes (10 ³ /μL)	6.68 ± 1.79	6.44 ± 1.72	6.21 ± 1.41	0.051			
Neutrophil	4.24 ± 1.41	3.99 ± 1.44	4.00 ± 1.08	0.867			
Lymphocyte	1.83 ± 0.54	1.91 ± 0.62	1.80 ± 0.54	0.640			
Monocyte	0.36 ± 0.13	0.32 ± 0.10	0.27 ± 0.11	0.002	0.217	0.002	0.015
ALT (U/L)	24.54 ± 10.50	24.18 ± 14.47	19.07 ± 7.20	0.064			
BUN (mmol/L)	5.86 ± 2.07	5.46 ± 1.11	5.79 ± 1.45	0.446			
Creatinine (μmol/L)	78.90 ± 14.38	72.60 ± 14.68	75.80 ± 18.55	0.174			
Prevalence of CAD	27 (90 %)	30 (100 %)	0 (0 %)				
Ectatic vessel							
LM	5 (16.7 %)						
LAD	15 (50 %)						
LCX	17 (56.7 %)						
RCA	20 (66.7 %)						

P values less than 0.05 are in bold. *P* values for comparison among groups and between groups: *P*1, CAE group vs. CAD group; *P*2, CAE group vs. CON group; *P*3, CAD group vs. CON group. Significance level was 0.05. CAE coronary artery ectasia, CAD coronary atherosclerosis disease, CON Control group, TC total cholesterol, TG triglyceride, HDL-c high-density lipoprotein cholesterol, LDL-c low-density lipoprotein cholesterol, LM left main coronary artery, LAD left anterior descending coronary artery, LCX left circumflex coronary artery, RCA right coronary artery

4. Increased serum proteolytic enzyme activities in the CAE group.

The total elastase activity was significantly higher in the CAE group than in the CAD and Control groups (*P*1 = 0.006, *P*2 = 0.022). The data imply that elastase activity was higher in the CAE group than in the other two groups (Table 2; Fig. 3).

The data showed that the total MMP activity was significantly higher in the CAE group than in the Control group

(*P*2 = 0.013) but not significantly higher than in the CAD group (*P*1 = 0.477). The data point to a tendency of higher MMP activity in the CAE group (Table 2; Fig. 3).

As shown in Fig. 4, in all selected subjects, correlation coefficients between sElastin and total elastase activity had significant meaning (*r* = 0.390, *P* = 0.000), and the correlation coefficients between sElastin and total MMP activity also had significant meaning (*r* = 0.251, *P* = 0.003). But there were no such correlations within the CAE group (data not provided).

Table 2 Circulating sElastin, ICTP, PIIINP, PICP and PIIICP and activities of proteolytic enzymes

	CAE (<i>n</i> = 30)	CAD (<i>n</i> = 30)	CON (<i>n</i> = 30)	<i>P</i> value	Comparison between groups		
					<i>P</i> 1	<i>P</i> 2	<i>P</i> 3
sElastin (ng/mL)	19.56 ± 7.21	15.76 ± 8.39	13.12 ± 7.57	0.000	0.009	0.000	0.015
ICTP (ng/mL)	1.93 ± 0.74	1.98 ± 1.54	1.87 ± 1.17	0.168	0.341	0.051	0.403
PICP (ng/mL)	4.47 ± 2.80	3.24 ± 1.48	3.47 ± 1.99	0.002	0.001	0.002	0.972
PIIINP (ng/mL)	2.20 ± 1.26	1.38 ± 0.54	1.52 ± 0.66	0.005	0.002	0.007	0.235
PIIICP (pg/mL)	119.79 ± 51.13	105.60 ± 13.45	123.19 ± 44.26	0.079	0.078	0.908	0.010
Total elastase activity (% standard elastase activity)	20.47 ± 7.75	15.76 ± 5.34	16.89 ± 4.75	0.007	0.006	0.022	0.064
Total MMP activity (U/mL)	0.823 ± 0.539	0.723 ± 0.352	0.572 ± 0.363	0.007	0.477	0.013	0.010

P values for comparison among groups: *P*1, CAE group vs. CAD group; *P*2, CAE group vs. CON group; *P*3, CAD group vs. CON group. Significance level was 0.05. CAE coronary artery ectasia, CAD coronary atherosclerosis disease, CON Control group, sElastin soluble elastin, ICTP collagen type I cross-linked telopeptides, PICP procollagen type I carboxy terminal peptide, PIIINP procollagen III N-terminal propeptide, PIIICP procollagen a1(III) C-terminal propeptide, MMP matrix metalloproteinase

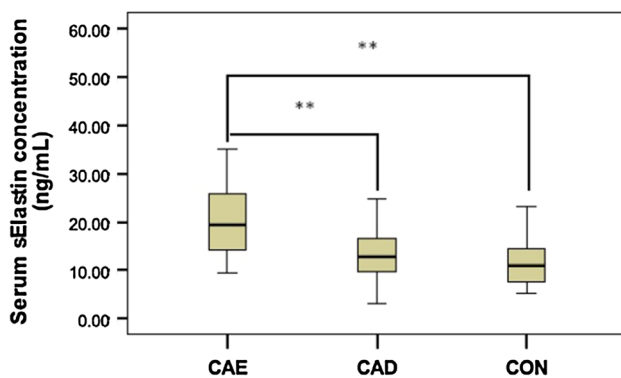


Fig. 1 Serum sElastin concentration. **P* < 0.05, ***P* < 0.01. CAE coronary artery ectasia, CAD coronary atherosclerosis disease, CON Control group, sElastin soluble elastin, a marker of elastin fibre degradation

Discussion

This study mainly evaluated the ECM turnover in CAE populations. CAD group was enrolled in this study because most of the CAE patients have obstructive coronary artery disease [4], and 90 % CAE subjects were associated with CAD in this study. As the baseline characteristics showed, the diastolic blood pressure, body mass index, and blood monocyte number were higher in the CAE and CAD groups. These findings are similar to those observed in other CAE studied by previous researchers [23–25], and are considered characteristics of CAE. The main findings in the present study were as follows: (1) Elastin fibre degradation was significantly increased in the CAE patients according to elevated circulating sElastin. (2) The main change in CAE was overproduction of type I collagen and the excessive degradation of type III collagen. Thus, the ratio of type I collagen to type III collagen was increased

in CAE patients. (3) Elastases and MMPs appear to be associated with the elastin fibre degradation. Total elastase activity was significantly higher in CAE group and total MMP activity has a higher tendency in CAE group. And there were considerable correlations between sElastin and total elastase activity, and between sElastin and total MMP activity. There were no such correlations within the CAE group. We speculate that this is possibly due to the limited sample size in the study.

In native vessels, the ECM is composed mostly of elastin fibres, type I and III collagens and proteoglycans. These proteins play important roles in maintaining vessel structure and functions [26, 27]. Elastin fibre, the dominant ECM protein in the coronary wall [12], is composed of soluble elastin subunits, which are synthesised by vascular smooth muscle cells (VSMCs) in the media in response to mechanical and other stimuli. The elastin fibres mainly provide elasticity and tensile strength to the vessels [13]. As elastin is not produced in adults [11], the destruction of elastin fibres is irreversible and may lead to serious damage of ECM integrity [13] and even coronary ectasia. Thus, the increased sElastin further indicates the over degradation of coronary ECM in CAE patients, and could be a promising candidate marker for CAE. The sElastin and its fragments also could promote the disease by: (1) inducing chemotactic attraction of monocytes or macrophages, (2) triggering the production of free radicals and MMPs, (3) promoting the oxidation of LDL, which is then taken up in the vessel wall intima by phagocytic cells, and (4) altering NO• production [10, 12].

Type I and III collagens are the major fibrillar collagen components in vessels, representing 60 and 30 % of vascular collagens, respectively [26]. Changes in the ratio and volume of the two collagens have been implicated in functional properties of the vessels. A higher ratio of

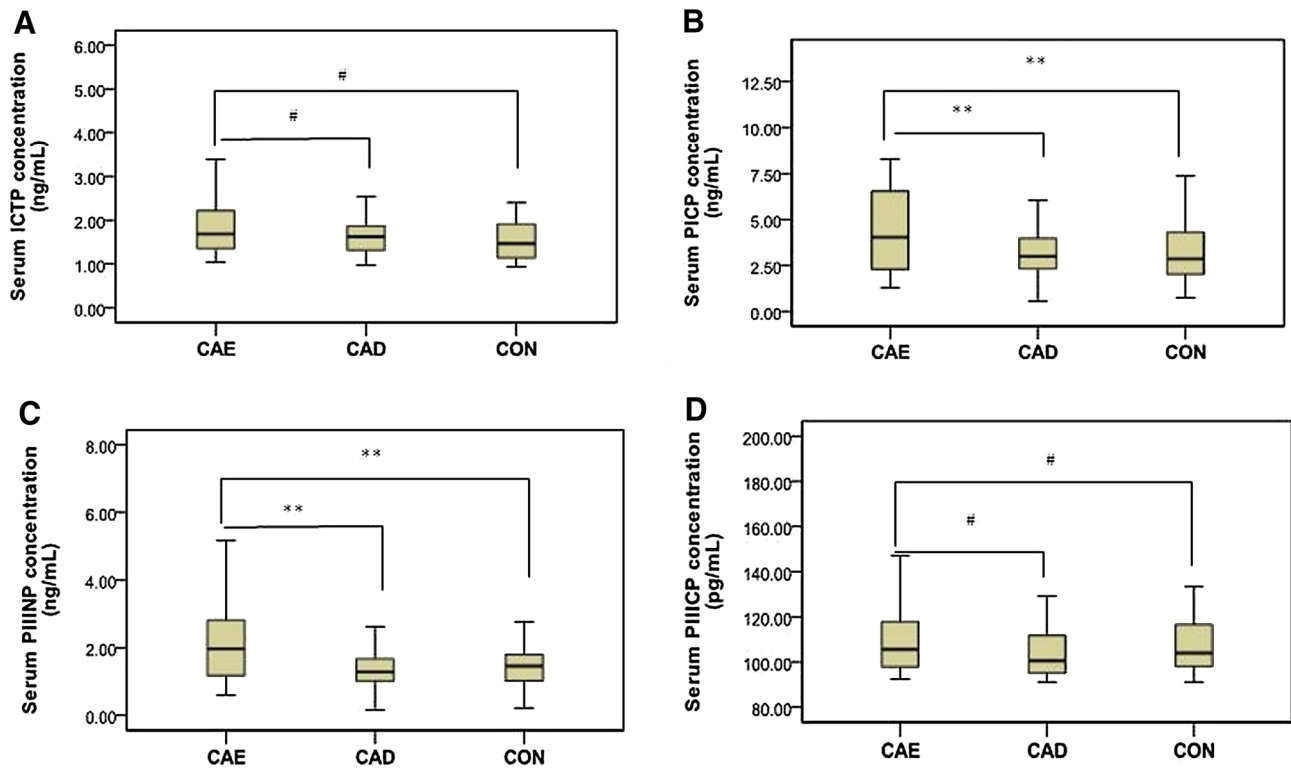


Fig. 2 Serum ICTP, PIIINP, PICP and PIIICP concentration. * $P < 0.05$, ** $P < 0.01$, # $P > 0.05$. ICTP collagen type I cross-linked telopeptides, a marker of type I collagen degradation; PICP procollagen type I carboxy terminal peptide, a marker of type I collagen syn-

thesis; 2) PIIINP procollagen III N-terminal propeptide, a marker of type III collagen degradation; PIIICP procollagen a1(III) C-terminal propeptide, a marker of type III collagen synthesis

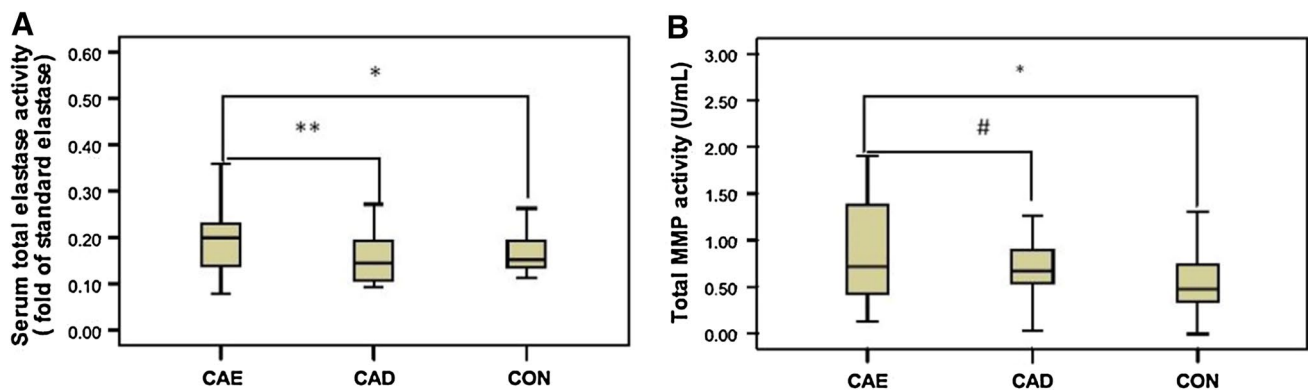


Fig. 3 Serum total elastase activity and total MMP activity. * $P < 0.05$, ** $P < 0.01$, # $P > 0.05$. CAE coronary artery ectasia, CAD coronary atherosclerosis disease, CON Control group

collagen type III to I is associated with more compliant tissue, whereas a lower ratio of collagen type III to I results in stiffer, less compliant tissue [14]. In the current study, the ratio of type I to type III collagen was increased in the CAE patients. This type of collagen transition implies that CAE patients have stiffer coronary vessels. A number of studies found evidence of hardening of the arteries

in CAE populations, such as an increased coronary artery calcification score [28], higher carotid-femoral pulse wave velocity [29] and a thicker carotid intima media [30]. Thus, coronary artery stiffness seems to be the outcome of an imbalance in collagen metabolism. This type of collagen transition might be a compensatory to prohibit coronary over-dilation.

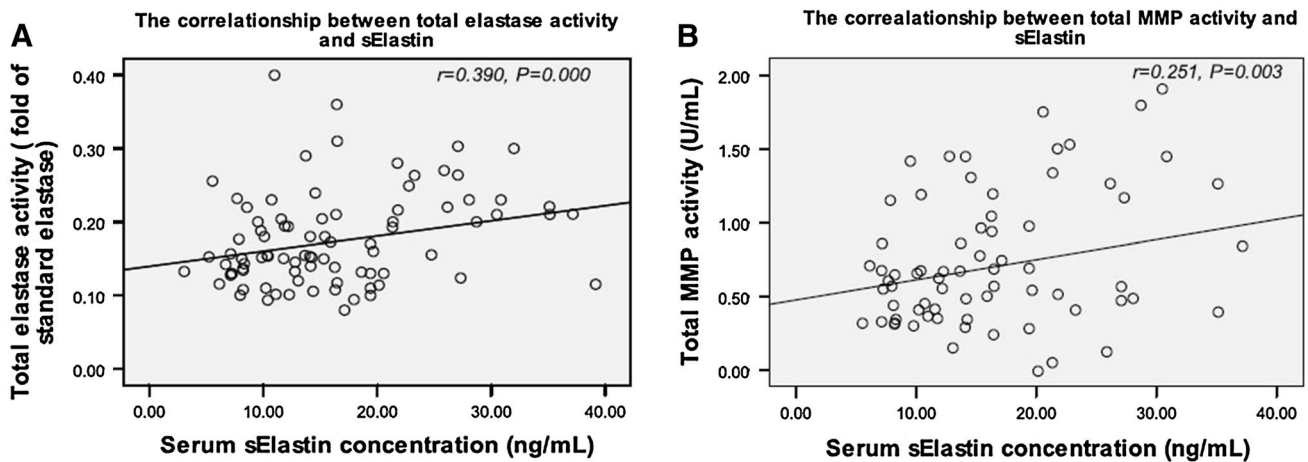


Fig. 4 Correlation between sElastin and total elastase activity and total MMP activity. Pearson's partial correlation between total elastase activity and sElastin: $r = 0.390, P = 0.000$; Pearson's partial correlation between total MMP activity and sElastin: $r = 0.251, P = 0.003$

The total collagen volume appeared to be decreased by comparing the type I collagen synthesis and type III collagen degradation in this study according to the following algorithm: total volume of new synthesis type I collagen = serum PICP concentration \times total serum volume / molecular mass of PICP mass \times molecular mass of type I collagen. Type I collagen is composed of two identical $\alpha 1$ (I) chains (885 aa protein) and one $\alpha 2$ (I) chain (1366 aa protein). PICP was 4.47 ± 2.80 ng/ml in the serum of the CAE patients, had a formula weight of 100,000 g/mol and was cut off from type I procollagen (3136 aa protein) in a ratio of 1:1. The total amount of degraded type III collagen can be calculated with a similar equation. Collagen type III is composed of three identical $\alpha 1$ (III) chains (1466 aa protein). PIIINP was 2.20 ± 1.26 ng/ml in the CAE group, had a formula weight of 42,000 g/mol and was cleaved from type III collagen (4398 aa protein) in a similar ratio of 1:1. Thus, the calculated ratio of type I collagen synthesis to type III collagen degradation was 0.608. As a result, there was 0.608 g of type I collagen synthesis to each 1 g of type III collagen degradation. Therefore, the total collagen volume was decreased in the CAE group, with CAE patients having more fragile vessels, and this change may facilitate the ectasia of coronary artery.

In addition to evaluating the turnover of ECM within the CAE group, we also attempted to identify the cause of the degradation of elastin fibres and the transition of type III to type I collagen in ECM metabolism. We focused on related proteases not only because of their indisputable importance as mediators of ECM remodelling but also because previous researches detected changes in the activity of these enzymes in autopsy specimens and serum samples of CAE patients [7–9]. Elastase-type enzymes have been found in all four classes of proteinases, serine, thiol and aspartic

proteinases, as well as in several members of the MMP family [11, 26]. Collagenolytic enzymes were mainly MMPs, including at least 26 members [26, 31]. In the present study, the increased activity of both the enzymes could be responsible to some degree, for the degradation of elastin fibres and even the degradation of collagen type III. Proteolytic enzymes seem to play essential roles in the ECM destruction process of coronary media in CAE patients and to be an important aspect of this disease.

In summary, according to results from the detection of circulating markers, the main changes in the ECM metabolism of CAE patients were excessive degradation of elastin fibres, overproduction of type I collagen, increased degradation of type III collagen and a reduction in the total collagen volume. These alterations might contribute to coronary ectasia and stiffness. Proteolytic enzymes possibly took some responsibilities for this kind of abnormal ECM metabolism. Although ECM and proteolytic enzymes were ubiquitous in human organs and tissues, the three groups had distinctive features in the coronary walls, and they were balanced in age, sex and other baseline characteristics. At the same time subjects associated with diseases possibly relating to abnormal ECM metabolism were not included in this study. Thus, those circulating markers could reflect the changes in coronary to some degrees. Further, serum examination was very important in clinical practices due to its convenience and minimal invasion, and human coronary artery autopsy was not practicable, thus optimising the application conditions of those circulating markers (such as combining those markers with untypical chest pain which was the most often symptom of CAE [3]), and giving more comprehensive explanations for the results would be very helpful for evaluating, diagnosing and even monitoring the disease of coronary ectasia.

Limitations

It would have been better to have divided the CAE group into two sub-groups, CAE with CAD, and CAE without other diseases (isolated CAE). However, the incidence of isolated CAE is rare. Thus, we were unable to enroll sufficient numbers of subjects with balanced baseline characteristics and divide the study groups into subgroups with isolated CAE in this study.

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Conflict of interest The authors have no other funding, financial relationships or conflicts of interest to disclose.

References

- Antoniadis AP, Chatzizisis YS, Giannoglou GD (2008) Pathogenetic mechanisms of coronary ectasia. *Int J Cardiol* 130(3):335–343
- Giannoglou GD, Antoniadis AP, Chatzizisis YS, Damvopoulou E, Parcharidis GE, Louridas GE (2006) Prevalence of ectasia in human coronary arteries in patients in northern Greece referred for coronary angiography. *Am J Cardiol* 98(3):314–318
- Zografos TA, Korovesis S, Giazitzoglou E, Kokladi M, Venetsanakis I, Paxinos G, Fragakis N, Katritsis DG (2013) Clinical and angiographic characteristics of patients with coronary artery ectasia. *Int J Cardiol* 167(4):1536–1541
- Virmani R, Robinowitz M, Atkinson JB, Forman MB, Silver MD, McAllister HA (1986) Acquired coronary arterial aneurysms: an autopsy study of 52 patients. *Hum Pathol* 17(6):575–583
- Daoud AS, Pankin D, Tulgan H, Florentin RA (1963) Aneurysms of the coronary artery. Report of ten cases and review of literature. *Am J Cardiol* 11:228–237
- Markis JE, Joffe CD, Cohn PF, Feen DJ, Herman MV, Gorlin R (1976) Clinical significance of coronary arterial ectasia. *Am J Cardiol* 37(2):217–222
- Lamblin N, Bauters C, Hermant X, Lablanche JM, Helbecque N, Amouyel P (2002) Polymorphisms in the promoter regions of MMP-2, MMP-3, MMP-9 and MMP-12 genes as determinants of aneurysmal coronary artery disease. *J Am Coll Cardiol* 40(1):43–48
- Finkelstein A, Michowitz Y, Abashidze A, Miller H, Keren G, George J (2005) Temporal association between circulating proteolytic, inflammatory and neurohormonal markers in patients with coronary ectasia. *Atherosclerosis* 179(2):353–359
- Lee NY, Park HY, Park CK, Ahn MD (2012) Analysis of systemic endothelin-1, matrix metalloproteinase-9, macrophage chemoattractant protein-1, and high-sensitivity C-reactive protein in normal-tension glaucoma. *Curr Eye Res* 37(12):1121–1126
- El-Hamamsy I, Yacoub MH (2009) Cellular and molecular mechanisms of thoracic aortic aneurysms. *Nat Rev Cardiol* 6(12):771–786
- Shinohara T, Suzuki K, Okada M, Shiigai M, Shimizu M, Maebara T, Ohsuzu F (2003) Soluble elastin fragments in serum are elevated in acute aortic dissection. *Arterioscler Thromb Vasc Biol* 23(10):1839–1844
- Robert L, Robert AM, Fülöp T (2008) Rapid increase in human life expectancy: will it soon be limited by the aging of elastin? *Biogerontology* 9(2):119–133
- Du X, Chen NL, Wong A, Craik CS, Brömme D (2013) Elastin degradation by cathepsin V requires two exosites. *J Biol Chem* 288(48):34871–34881
- Kwak HB (2013) Aging, exercise, and extracellular matrix in the heart. *J Exerc Rehabil* 9(3):338–347
- Flevari P, Theodorakis G, Leftheriotis D, Kroupis C, Kolokathis F, Dima K, Anastasiou-Nana M, Kremastinos D (2012) Serum markers of deranged myocardial collagen turnover: their relation to malignant ventricular arrhythmias in cardioverter-defibrillator recipients with heart failure. *Am Heart J* 164(4):530–537
- Okkonen M, Gäddnäs F, Pettilä V, Laurila J, Ohtonen P, Risteli J, Linko R, Ala-Kokko T; Finnali Study Group (2013) Serum markers of collagen synthesis and degradation in acute respiratory failure patients. *Acta Anaesthesiol Scand* 57(9):1193–1200
- Romero JR, Vasani RS, Beiser AS, Polak JF, Benjamin EJ, Wolf PA, Seshadri S (2008) Association of carotid artery atherosclerosis with circulating biomarkers of extracellular matrix remodeling: the Framingham Offspring Study. *J Stroke Cerebrovasc Dis* 17(6):412–417
- Yan CJ, Li SM, Xiao Q, Liu Y, Hou J, Chen AF, Xia LP, Li XC (2013) Influence of serum adiponectin level and SNP +45 polymorphism of adiponectin gene on myocardial fibrosis. *J Zhejiang Univ Sci B* 14(8):721–728
- Salama H, Zekri AR, Medhat E, Al Alim SA, Ahmed OS, Bahnassy AA, Lotfy MM, Ahmed R, Musa S (2014) Peripheral vein infusion of autologous mesenchymal stem cells in Egyptian HCV-positive patients with end-stage liver disease. *Stem Cell Res Ther* 5(3):70
- Bizbiz L, Bonithon-Kopp C, Ducimetière P, Berr C, Alperovitch A, Robert L (1996) Relation of serum elastase activity to ultrasonographically assessed carotid artery wall lesions and cardiovascular risk factors. The EVA study. *Atherosclerosis* 120(1–2):47–55
- Harris LK, Smith SD, Keogh RJ, Jones RL, Baker PN, Knöfler M, Cartwright JE, Whitley GS, Aplin JD (2010) Trophoblast- and vascular smooth muscle cell-derived MMP-12 mediates elastolysis during uterine spiral artery remodeling. *Am J Pathol* 177(4):2103–2115
- Lauer-Fields JL, Kele P, Sui G, Nagase H, Leblanc RM, Fields GB (2001) Kinetic analysis of matrix metalloproteinase activity using fluorogenic triple-helical substrates. *Biochemistry* 40(19):5795–5803
- Işık T, Ayhan E, Uyarel H, Tanboğa IH, Kurt M, Uluganyan M, Ergelen M, Eksik A (2013) Association of neutrophil to lymphocyte ratio with presence of isolated coronary artery ectasia. *Turk Kardiyol Dem Ars* 41(2):123–130
- Kocaman SA, Taçoş G, Sahinarslan A, Cengel A (2008) Relationship between total and differential leukocyte counts and isolated coronary artery ectasia. *Coron Artery Dis* 19(5):307–310
- Li JJ, Nie SP, Qian XW, Zeng HS, Zhang CY (2009) Chronic inflammatory status in patients with coronary artery ectasia. *Cytokine* 46(1):61–64
- Lemarié CA, Tharaux PL, Lehoux S (2010) Extracellular matrix alterations in hypertensive vascular remodeling. *J Mol Cell Cardiol* 48(3):433–439
- Sakamoto N, Hoshino Y, Misaka T, Mizukami H, Suzuki S, Sugimoto K, Yamaki T, Kunii H, Nakazato K, Suzuki H, Saitoh S, Takeishi Y (2014) Serum tenascin-C level is associated with coronary plaque rupture in patients with acute coronary syndrome. *Heart Vessels* 29(2):165–170
- Erkan H, Ağaç MT, Akyol S, Korkmaz L, Kiriş A, Erkan M, Acar Z, Vatan B, Erkuş E, Akyüz AR, Çelik S (2013) Coronary artery calcification score is increased in patients with isolated coronary artery ectasia. *Clin Invest Med* 36(4):E191–E196
- Kiriş A, Bostan M, Korkmaz L, Ağaç MT, Acar Z, Kaplan S, Celik S (2012) Carotid-femoral pulse wave velocity in patients

- with isolated coronary artery ectasia: an observational study. *Anadolu Kardiyol Derg* 12(4):313–319
30. Celik S, Erdoğan T, Kasap H, Kaplan S, Durmuş I, Gedik O, Kiriş A (2007) Carotid intima-media thickness in patients with isolated coronary artery ectasia. *Atherosclerosis* 190(2):385–387
 31. Wang Y, Wu B, Dong L, Wang C, Wang X, Shu X (2014) Circulating matrix metalloproteinase patterns in association with aortic dilatation in bicuspid aortic valve patients with isolated severe aortic stenosis. *Heart Vessels*. doi:[10.1007/s00380-014-0593-5](https://doi.org/10.1007/s00380-014-0593-5)