The Association of Serum Levels of stromolysin-1 and connexin-37 with the severity of coronary artery disease

Melika Naebian
Tabriz Medical University: Tabriz University of Medical Sciences
Naser Aslanabadi
Tabriz University of Medical Sciences
Alireza Nourazarian
Tabriz University of Medical Sciences
Reza Rahbarghazi
Tabriz University of Medical Sciences
behrouz shademan
Ege University Faculty of Medicine: Ege Universitesi Tip Fakultesi
Amir Mansour Vatankhah
Tabriz Medical University: Tabriz University of Medical Sciences
Fatemeh khaki-khatibi (Khzanfrjy@gmail.com)
Tabriz Medical University: Tabriz University of Medical Sciences

Short Report

Keywords: Connexin-37, Stromelysin-1, Coronary Artery Disease, Biomarkers

Posted Date: September 28th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-933085/v1

Abstract

Background Coronary Artery Disease (CAD) is a common form of heart disease that is considered a serious health problem in society. Atherosclerosis is widely recognized as a chronic inflammatory disease of the vessels and can lead to CAD and myocardial infarction. The aim of the present study was to investigate serum levels of connexin-37 and stromelysin-1 as significant biomarkers of CAD and their correlation with the extent of CAD.

Methods and results Sixty CAD patients with one-vessel (1VD), two-vessel (2VD), and three-vessel (3VD) disease were enrolled in this study. For comparison with the results, 20 healthy control subjects were also included in this study. Serum concentrations of connexin-37 and stromelysin-1 were determined using commercial ELISA kits. Serum connexin-37 concentrations were not significantly different between the patient and control groups ($p < 0.05$). The analysis showed a statistically significant difference between subjects with one-vessel disease, subjects with two-vessel disease, and subjects with three-vessel disease. Serum Stromelysin-1 concentration was significantly higher in the patients than in the control group ($p < 0.05$).

Conclusions The results of our study indicate that serum levels of stromelysin-1, but not connexin-37, may contribute to the prediction of the occurrence and progression of CAD.

Introduction

Coronary artery disease (CAD) is frequently diagnosed in patients with cardiovascular complications [1]. According to recent findings, excessive inflammatory response in the sub arterial space increases the possibility of thrombus formation [2]. Factors such as hypertension, dyslipidemia, atherosclerosis, smoking, and inappropriate lifestyle may increase the risk of CAD [3, 4]. Atherosclerosis is associated with endothelial dysfunction and correlates with the formation of coronary necrotic plaques that may detach in the early stages. A link between atherosclerosis and the possibility of myocardial infarction has already been demonstrated [5].

Connexins are transmembrane proteins and are encoded by 20 genes in various tissues [6, 7]. These proteins connect adjacent cells using gap junctions and channels and facilitate the passive mutual diffusion of small molecules [8, 9]. One connexin protein, namely connexin-37, plays a key role in regulating the inflammatory response. Connexin-37 can control the attachment of monocyte endothelial cells and reduce pathological remodeling [10]. There is evidence that a mutation in the gene allele C1019T predisposes men to cardiovascular disease [11]. Patients who are prone to atherosclerotic plaques may have a single nucleotide polymorphism (SNP) detected in their genome [12]. Seifi et al. have shown that the presence of a polymorphism in the connexin 37 genes can alter the complications of acute myocardial infarction (AMI) [13]. It has been elucidated that suppression of the connexin 37 gene in apolipoprotein (Apo) E deficient mice can lead to the formation of atherosclerotic plaques [14].

Stromelysin-1, also known as matrix metalloproteinase-3 (MMP-3), is involved in the pathological changes during AMI and CAD [15]. Stromelysin-1 digests the extracellular matrix (ECM) and is frequently found in atherosclerotic plaques [16]. The hyperactivity of MMPs such as stromelysin-1 may promote coronary atheroma due to connective tissue remodeling. Along with these changes, atherosclerotic plaques are detached, leading to coronary artery thrombosis [15].

This study aimed to determine the serum levels of connexin-37 and stromolysin-1 in patients with CAD and to find a possible association between these factors and the severity of CAD. For this purpose, we selected CAD patients with different numbers of affected vessels (1, 2, and 3 vessels).

Material – Method

Ethical considerations

The protocol used in this study was approved by the local ethics committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1398.1181). All patients and healthy volunteers were asked to complete an informed consent form before participating in this study.

Sample preparation

This study was a cross-sectional analysis of 80 cases (aged 40–70 years), 60 CAD patients and 20 controls. In this study, patients with symptoms of heart disease were studied. The possibility of heart disease was confirmed by angiography. The exclusion criteria were hyperlipidemia, diabetes mellitus, respiratory disease, liver dysfunction, renal disease, and cancer. Healthy volunteers with no history of heart disease or other conditions were asked to participate in the current study to compare results. Blood samples were collected from CAD patients referred to the Cardiology Unit of Shahid Madani Hospital, affiliated with Tabriz University of Medical Science (Tabriz, Iran). Based on the results of angiography, CAD patients were classified into three categories as follows: patients with one-vessel disease (1VD), patients with two-vessel disease (2VD), and patients with three-vessel disease (3VD). All information, such as blood pressure, height, weight, family history, hyperlipidemia, age, and sex, was collected by appropriate questionnaires.

Blood Sampling

For this purpose, 10 ml of fasting venous blood was drawn from each patient and collected in tubes containing EDTA. The samples were centrifuged at 3000 g for 20 minutes. After completion of the centrifugation procedure, the samples were immediately placed in the − 80˚C freezer and stored until use.

Biochemical assays

Serum levels of triglycerides (TG), total cholesterol, and high-density lipoprotein (HDL) were measured with spectrophotometry and commercially available kits (Pars Azmoun, Iran). To calculate low-density lipoprotein cholesterol (LDL-C), we used the formula of Fried Wald (17). LDL-C was measured directly in

the samples with a higher value of TG (400 mg/dl). An enzyme-linked immunosorbent assay (ELISA) was used for the determination of connexin-37 (Cat. No.: E67777Hu; Bioassay Technology) and stromelysin-1 (Cat. No.: E1722Hu; Bioassay Technology).

Statistical analysis

Statistical analysis was performed using SPSS version 16. Differences between the patient and control groups were analyzed by t-test and ANOVA. All variables were expressed as mean ± SD. $P < 0.05$ was considered statistically significant.

Results

Demographic characteristics

The present study included 80 subjects (60 patients and 20 healthy controls). According to the demographic data, the mean age was 58.4 ± 9.5 and 58 ± 9.8 years in the patient and control groups, respectively. The data showed no statistically significant differences between the two groups in age, sex, and family history. A significant difference was found in blood pressure levels and smoking in the patients with CAD compared with the control group (Table 1).

Table 1
Demographic characteristics

Variable/Groups	1VD	2VD	3VD	Control	P-value
Gender (Male/Female)	9.11	8.12	9.11	10.10	**0.5**
Age (Mean ± SD)	59 ± 9.5	59 ± 9.5	57 ± 9.6	58 ± 9.8	**0.9**
History of blood pressure number (%)	11 (55%)	12 (60%)	12 (60%)	3 (15%)	**0.03**
Smoking history number (%)	8 (40%)	11 (55%)	10 (50%)	0 (0%)	**0.01**
Family history number (%)	6 (30%)	11 (55%)	12 (60%)	4 (20%)	**0.4**
The case group with one blocked artery**) 1VD);** two blocked arteries **(2VD);** three blocked arteries **(3VD)**					

Laboratory findings

The data showed that the mean concentrations of cholesterol (TC), triglycerides (TG), and LDL-C were significantly higher in the patients with CAD compared with the control group. In contrast, serum levels of HDL-C were significantly lower in the patient group (Table 2). Statistical analysis revealed no significant difference in connexin-37 levels between the two groups ($p > 0.05$; Table 3). We found that the mean connexin-37 level in the patient group CAD reached 7.3 ± 1.63 ng/ml, while this value in the control group was 7.28 ± 2.47 ng/ml. Of note, these changes were statistically significant between the patient groups with CAD (patients with one-vessel disease, patients with two-vessel disease, and patients with three-vessel disease) (Tables 3 and 4). According to our data, the serum concentration of connexin-37 was

higher in the 2VD group than in the 3VD and 1VD groups. The 1VD group had the lowest levels of connexin-37.

Table 2
Serum lipid profile in CAD patients and healthy controls

Variables/Groups	1VD	2VD	3VD	Control	P-value
	Mean ± SD				
Chol (mg/dL)	183 ± 23	181 ± 33	187 ± 26	160 ± 11.5	0.02
TG (mg/dL)	136.5 ± 25	166 ± 39.5	164.5 ± 36	136.525	0.04
HDL-C (mg/dL)	34 ± 3.3	34 ± 3.4	33 2.3	37 ± 3.4	0.02
LDL-C (mg/dL)	117 ± 15	115 ± 20	122 18.5	1019.5	0.02

Cholesterol (**Chol** (; Triglycerides (**TG**); High-density lipoprotein (**HDL**); Low- density lipoprotein (**LDL**). The case group with one blocked artery**) 1VD);** two blocked arteries **(2VD);** three blocked arteries **(3VD)**

Table 3
Mean serum concentration of Connexin-37 in CAD patients and control groups.

Variable/Groups	CAD Patients	Controls	P-value
	Mean ± SD		
Connexin-37 (ng/mL)	7.30 ± 1.63	7.28 ± 2.47	> 0.05

Table 4
Comparison of mean serum concentrations of Connexin-37 in the studied groups

Concentration of Connexin-37 (ng/ml) (mean SD)		**P-value**
2VD	**Control**	**< 0.05**
7.9 ± 2.02	7.28 ± 2.47	
3VD	**2VD**	**< 0.05**
7.5± 1.25	7.9 ± 2.02	
1VD	**3VD**	**< 0.05**
6.5 ± 1.64	7.5 ± 1.25	
The case group with one blocked artery**) 1VD);** two blocked arteries **(2VD);** three blocked arteries **(3VD)**		
The ELISA results showed that the mean serum level of stromelysin-1 in the CAD patients was 15.2 ± 8.65 ng/ml, and this value reached about 9.3 ± 5.93 ng/ml in the control group. Independent-sample t-test analysis showed a statistically significant difference in the stromelysin-1 levels of CAD patients compared with healthy controls (Table 5). In the one-way analysis ANOVA, the mean difference between stromelysin-1 was statistically significant at 1VD, 2VD, and 3VD. Accordingly, the serum concentration of stromelysin-1 was higher in the 2VD group than in the 3VD and 1VD subgroups. According to our data, these levels were higher in the 1VD subgroup than in the 3VD CAD patients (Table 6).		

Table 5
Mean serum concentration of Stromelysin-1 in patient and control groups

Variable	**Controls**	**CAD Patients**	**P value**
	Mean ± SD		
Stromelysin-1 (ng/mL)	**9.3 ± 5.93**	**15.2 ± .8.65**	**< 0.05**

Table 6
Comparison of mean serum concentrations of Stromelysin-1 in the studied groups

Mean serum concentration of Stromelysin-1(ng/mL) (Mean ± SD)		**P-Value**
1VD	**2VD**	**≥ 0.05**
14.7 ± 7.53	18.1 ± 9.97	
3VD	**1VD**	**≥ 0.05**
12.9 ± 8.45	14.7 ± 7.53	
Control	**3VD**	**≤ 0.05**
9.3 ± 5.93	12.9 ± 8.45	
The case group with one blocked artery**) 1VD);** two blocked arteries **(2VD);** three blocked arteries **(3VD)**		

Discussion

Atherosclerosis is a pathological condition characterized by the accumulation of lipids on the luminal surface of vessels, which promotes the recruitment of macrophages and T lymphocytes. It has been suggested that dyslipidemia may sensitize coronary arterioles to atherosclerotic plaques. On this basis, the increase in TC, TG, LDL-C and the decrease in HDL-C are predisposing factors in myocardial infarction patients [18]. Here, we showed that TG, TC, and LDL-C were significantly increased in CAD patients compared with control subjects. These conditions coincided with a significant decrease in HDL-C levels in CAD patients compared with control subjects. Remarkably, we also found that the intensity of CAD and vascular complications (3VD patients) was closely associated with the change in TG, TC, LDL-C, and HDL-C. We found no significant differences in serum levels of connexin-37 between the control group and the CAD group. Connexin-37 is thought to regulate the bioactivity of endothelial cells by participating in various signal transductions and juxtacrine connections between the endothelium and smooth muscle cells [16]. According to previous experiments, connexin-37 is involved in the formation of hemichannels that prevent atherosclerotic plaques by suppressing the interaction between endothelial cells and leukocytes [19]. According to our results, the occurrence of CAD may not affect the serum levels of connexin-37, but the intensity of vascular complications in CAD patients leads to significant differences between 3VD and other subgroups. Stromelysin-1 (MMP3) has been reported to be elevated in CAD patients compared with healthy controls. It has been suggested that elevation of MMPs may lead to the formation of atherosclerotic plaques in the carotid artery [20]. In agreement with our data, Tengiz and coworkers confirmed the elevation of stromelysin-1 in patients with coronary aneurysms compared with the control group [21]. Stromelysin-1 is a specific proteinase released by connective tissue cells. This enzyme can degrade ECM when activated [22]. In support of our data, Wu and colleagues investigated the prognosis of different plasma levels of MMPs in patients with CAD [23]. They found that the intensity of

vasculitis and the levels of high-sensitivity C-reactive protein and stromelysin-1 were closely correlated with the progression of cardiovascular events. However, it appears that plasma stromelysin-1 levels are an independent prognostic factor for cardiovascular disease, suggesting that this factor should be used to classify CAD.

Conclusion

This study shows that stromelysin-1, but not connexin-37, was increased in CAD patients, indicating a close relationship between serum levels of stromelysin-1 and CAD. In patients at risk for atherosclerosis, monitoring serum levels of stromelysin-1 along with other biochemical biomarkers may help predict the possibility of the occurrence of CAD and the intensity of vascular involvement.

Declarations

Compliance with ethical standards

Conflict of interest

The authors declare that there are no conflicts of interest.

Ethical approval

The Ethics Committee of Tabriz University of Medical Sciences approved the study protocols according to the Declaration of Helsinki.

Satisfaction with publication

All authors agree to publish the data of this study.

Acknowledgments

The authors thank Tabriz University of Medical Sciences for financial support. We also thank the staff of the Stem Cell Research Center for their cooperation and advice.

Authorship contribution statement

MN and NA contributed to the study design; **RR** contributed to the analysis and interpretation of the data; **AN, BS, and FKH** contributed to the substantive revision of the manuscript; and **FKH** approved the final version of the manuscript.

Funding

This study was supported by a grant from Tabriz University of Medical Sciences (IR.TBZMED.REC.1396.639).

Availability of data and materials

The data and materials used in this study are available.

References

1. Wang Z, Zhai X, Xue M, Cheng W, Hu H (2019) Prognostic value of lipoprotein (a) level in patients with coronary artery disease: a meta-analysis. Lipids Health Dis 18(1):1–9. https://doi.org/10.1186/s12944-019-1092-6
2. Legarth C, Grimm D, Krüger M, Infanger M, Wehland M (2020) Potential beneficial effects of vitamin d in coronary artery disease. Nutrients 12(1):99. https://doi.org/10.3390/nu12010099
3. Mirzaeipour F, Seyedmazhari M, Pishgooie AH, Hazaryan M (2019) Assessment of risk factors for coronary artery disease in military personnel: A study from Iran. Journal of family medicine primary care 8(4):1347. https://doi.org/10.4103/jfmpc.jfmpc_109_19
4. Collings A, Raitakari OT, Juonala M, Mansikkaniemi K, Kähönen M, Hutri-Kähönen N, … Lehtimäki TJ (2008) The influence of smoking and homocysteine on subclinical atherosclerosis is modified by the connexin37 C1019T polymorphism–The Cardiovascular Risk in Young Finns Study. Clinical chemistry laboratory medicine 46(8):1102–1108. https://doi.org/10.1515/CCLM.2008.216
5. Sara JD, Zhang M, Gharib H, Lerman LO, Lerman A (2015) Hypothyroidism is associated with coronary endothelial dysfunction in women. Journal of the American Heart Association 4(8):e002225. https://doi.org/10.1161/JAHA.115.002225
6. Fang JS, Angelov SN, Simon AM, Burt JM (2011) Cx37 deletion enhances vascular growth and facilitates ischemic limb recovery. Am J Physiol Heart Circ Physiol 301(5):H1872–H1881. https://doi.org/10.1152/ajpheart.00683.2011
7. Kanady JD, Dellinger MT, Munger SJ, Witte MH, Simon AM (2011) Connexin37 and Connexin43 deficiencies in mice disrupt lymphatic valve development and result in lymphatic disorders including lymphedema and chylothorax. Developmental biology 354(2):253–266. https://doi.org/10.1016/j.ydbio.2011.04.004
8. Morel S, Kwak R, B (2012) Roles of connexins in atherosclerosis and ischemia-reperfusion injury. Curr Pharm Biotechnol 13(1):17–26. https://doi.org/10.2174/138920112798868638

9. Okamoto T, Suzuki K (2017) The role of gap junction-mediated endothelial cell–cell interaction in the crosstalk between inflammation and blood coagulation. Int J Mol Sci 18(11):2254. https://doi.org/10.3390/ijms18112254
10. Ramadan R, Vromans E, Anang DC, Decrock E, Mysara M, Monsieurs P, … Aerts A (2019) Single and fractionated ionizing radiation induce alterations in endothelial connexin expression and channel function. Scientific reports 9(1):1–16. https://doi.org/10.1038/s41598-019-39317-9
11. Guo SX, Yang ZY, Wang RX, Yang Y, Cao HM, Zhang T (2013) Association between C1019T polymorphism of the connexin37 gene and coronary heart disease in patients with instent restenosis. Experimental therapeutic medicine 5(2):539–544. https://doi.org/10.3892/etm.2012.852
12. Boerma M, Forsberg L, Van Zeijl L, Morgenstern R, De Faire U, Lemne C, … Cotgreave IA (1999) A genetic polymorphism in connexin 37 as a prognostic marker for atherosclerotic plaque development. Journal of internal medicine 246(2):211–218. https://doi.org/10.1046/j.1365-2796.1999.00564.x
13. Seifi M, Fallah S, Ghasemi A, Aghajani H, Razaghi M, Danaei N (2013) Mutations of the connexin 37 and 40 gap-junction genes in patients with acute myocardial infarction. Clinical laboratory 59(3–4):343–348. https://doi.org/10.7754/clin.lab.2012.120305
14. Pfenniger A, Wong C, Sutter E, Cuhlmann S, Dunoyer-Geindre S, Mach F, … Kwak BR (2012) Shear stress modulates the expression of the atheroprotective protein Cx37 in endothelial cells. J Mol Cell Cardiol 53(2):299–309. https://doi.org/10.1016/j.yjmcc.2012.05.011
15. Schwarz A, Haberbosch W, Tillmanns H, Gardemann A (2002) The stromelysin-1 5A/6A promoter polymorphism is a disease marker for the extent of coronary heart disease. Disease markers 18(3):121–128
16. Yeh HI, Lai YJ, Chang HM, Ko YS, Severs NJ, Tsai CH (2000) Multiple connexin expression in regenerating arterial endothelial gap junctions. Arterioscler Thromb Vasc Biol 20(7):1753–1762
17. Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical chemistry 18(6):499–502
18. Mehri H, Aslanabadi N, Nourazarian A, Shademan B, khaki-khatibi F (2021) Evaluation of the serum levels of Mannose binding lectin-2, tenascin-C, and total antioxidant capacity in patients with coronary artery disease. J Clin Lab Anal, e23967. https://doi.org/10.1002/jcla.23967
19. Wong CW, Christen T, Roth I, Chadjichristos CE, Derouette JP, Foglia BF, … Kwak BR (2006) Connexin37 protects against atherosclerosis by regulating monocyte adhesion. Nature medicine 12(8):950–954
20. Beaudeux JL, Giral P, Bruckert E, Bernard M, Foglietti MJ, Chapman MJ (2003) Serum matrix metalloproteinase-3 and tissue inhibitor of metalloproteinases-1 as potential markers of carotid atherosclerosis in infraclinical hyperlipidemia. Atherosclerosis 169(1):139–146
21. Tengiz I, Ercan E, Aliyev E, Sekuri C, Duman C, Altuglu I (2004) Elevated levels of matrix metalloprotein-3 in patients with coronary aneurysm: A case control study. Current controlled trials in

cardiovascular medicine 5(1):1–6

22. Magdalena K, Magdalena K, Grazyna S (2006) The role of matrix metalloproteinase-3 in the development of atherosclerosis and cardiovascular events. EJIFCC 17(1):2
23. Wu TC, Leu HB, Lin WT, Lin CP, Lin SJ, Chen JW (2005) Plasma matrix metalloproteinase-3 level is an independent prognostic factor in stable coronary artery disease. Eur J Clin Invest 35(9):537–545. https://doi.org/10.1111/j.1365-2362.2005.01548.x