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Original Article

Correlation Between Cystatin C and Coronary Artery Disease

Hamidreza Nasri[1*], MD; Yahya Eftekhari[1], MD

ABSTRACT

Background: Coronary artery disease (CAD) is one of the most common complicated cardiovascular diseases causing morbidity and mortality. Early detection of CAD can help us choose the best therapeutic procedures and gain optimal results. This study aimed to evaluate cystatin C serum levels in patients with CAD.

Methods: This cross-sectional study was performed on 95 cases (60 in the CAD group and 35 in the control group). Cystatin C was measured via the enzymatic method and the sensitivity and specificity of cystatin C for diagnosing CAD were measured by ROC curve.

Results: The mean serum level of cystatin C was 2.21 ± 0.97 mg/L; it was higher among the patients with CAD (3.57 ± 0.58 mg/L). The area under the ROC curve was calculated to be 0.702, with sensitivity of 88.5% and specificity of 82.4% based on 0.5 as the best cutoff point for serum cystatin C (*P* = 0.001, 95% CI: 0.59 to 0.81).

Conclusions: Our results showed that cystatin C serum levels with a cutoff point of 0.5 had high and acceptable sensitivity and specificity in diagnosing CAD and could, thus, play an important role in the early diagnosis of CAD and prevention of adverse cardiovascular events. ***(Iranian Heart Journal 2019; 20(4): 56-63)***

KEYWORDS: Coronary artery disease, Cystatin C, Sensitivity, Specificity

[1] Cardiovascular Research Center, Institute of Basic and Clinical Physiology Sciences, Kerman University of Medical Sciences, Kerman, IR Iran.

*** Corresponding Author:** Hamidreza Nasri, MD; Cardiology Department, Institute of Basic and Clinical Physiology Sciences, Kerman University of Medical Sciences, Kerman, IR Iran.
Email: Dr_h.nasri@yahoo.com **Tel:** 09131406756

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Coronary artery disease (CAD), one of the most common complicated cardiovascular diseases causing morbidity and mortality (accounting for 11.2% of all deaths), is caused by atherosclerosis, vascular cavity stenosis, and/or occlusion. [1-3] Early treatment of CAD based on reperfusion procedures and drugs limits cardiac dysfunction and subsequently reduces morbidity and mortality rates. [4] In the achievement of early treatment, diagnostic methods play an important role. Studies have demonstrated that the early diagnosis and treatment of patients with CAD are the most important predictive

factors of long-term outcomes. [2] An earlier detection of CAD can help us select the best therapeutic procedures and obtain optimal results. [5] Although the application of high-sensitive troponin has improved the diagnostic accuracy of CAD, the diagnosis still needs other noninvasive, safe, and affordable markers to evaluate the early diagnosis and severity of CAD. [6]

Cystatin C, as one of such markers, is a 13-kDa protein. It is a competitive inhibitor of lysosomal cysteine protease factor and is produced in all nucleated cells. Cystatin C has been used to evaluate the kidney function (better than serum creatinine) and to distinguish small drops in the glomerular filtration rate. [7-9] Recently, different studies have reported that cystatin C is an impending indicator for cardiovascular risk and high levels of it are significantly correlated with cardiovascular outcomes in diverse clinical scenarios. [10-14] Research has also shown that cystatin C can predict mortality in patients with suspected CAD, [15] non–ST-elevation acute coronary syndrome, [16] and ST-elevation myocardial infarction (MI). [17]

To the best of our knowledge, there is a dearth of prospective studies on the diagnostic accuracy of cystatin C in detecting CAD. We, therefore, planned to assess the diagnostic accuracy of cystatin C in predicting CAD severity with the best cutoff point.

METHODS

Study Design

This cross-sectional study was conducted in the Cardiology Department of Kerman Shafa Hospital, from March to August 2018. The inclusion criteria consisted of referral to the cardiology department with a diagnosis of CAD and singing a consent form to participate in the study. The exclusion criteria were comprised of an estimated glomerular filtration rate < 35 mL/min or a serum creatinine level > 1.5 mg/dL, having left ventricular dysfunction (ejection fraction < 50%), undergoing coronary interventions, a history of acute coronary syndrome in the preceding 3 months, MI in the preceding 6 months, surgery within the preceding 3 months, cancer and infections within the preceding month, chronic inflammatory diseases, high C-reactive protein (CRP) levels, high white blood cell counts, liver dysfunction, cardiac events after angiography, uncontrolled diabetes, brain damage in the preceding 3 months, a body mass index > 30 and < 15, pulmonary edema, tachyarrhythmias, heart valve diseases, hyperthyroidism, intracranial hemorrhage, acute trauma, all ischemic events over the preceding 3 months, pregnancy, having pacemaker devices, and dissatisfaction to continue participation in the study. Also excluded were patients with uncompleted data.

Participants

The study flowchart is depicted in Figure 1. Sixty-seven patients with a diagnosis of CAD diagnosed by cardiologists based on clinical and paraclinical findings and the inclusion and exclusion criteria were included. Moreover, 41 subjects without CAD were included as the control group.

Ninety-five individuals completed the study: 60 in the CAD group and 35 in the control group. The study received approval from the Ethics Committee of Kerman University of Medical Sciences (IR.KMU.REC.1397.181. Code Number: IR.KMU.REC.1397.181.), and all the participants gave written informed consent.

Coronary angiography was executed via the standard Judkins technique. CAD was defined as coronary artery stenosis > 50% in at least 1 coronary artery.

On the angiography day, blood samples were taken from the patients in fasting state. An EDTA anticoagulant blood sample was used to measure biomarkers. After preparation, the plasma samples were immediately frozen in separate parts (aliquots) at −70 ° C until the time of measurement. Cystatin C was measured via the enzymatic method (DIAZYME

Laboratories, Poway model, USA), with sensitivity of 0.22 mg/dL and the coefficient of variation of 3.6%.

Data Analysis

The data were analyzed and reported only for the patients with completed data. The statistical analyses of the data were performed using SPSS, version 22 (SPSS Inc, Chicago, IL, USA). The Mann–Whitney *U* test was used to compare the levels of cystatin C between the patients with and without CAD as long as the Kolmogorov–Smirnov test implied a non-normal distribution for serum cystatin C. Logistic regression was employed to evaluate the effects of the variables on CAD, and the measurement of the area under the receiver operating characteristic (ROC) curve was calculated to determine the best cutoff point for serum cystatin C. The quantitative results were expressed as the mean ± standard deviation. The level of significance was set at a *P* level ≤ 0.05.

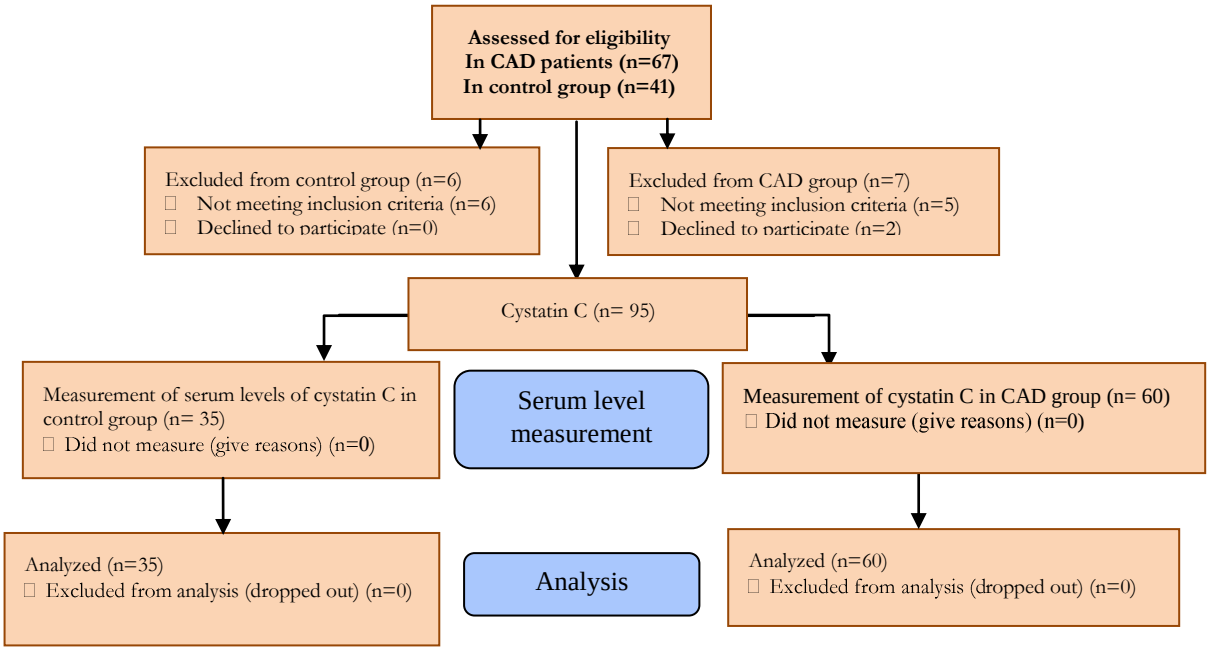

Figure 1. Study flowchart

RESULTS

The final analysis was conducted on 95 male and female patients with and without CAD. From this total, 63.2% of the patients were diagnosed to have CAD and 36.8% were diagnosed to be normal. The mean age at the time of visit for the total study population was 58.29 ± 10.45 years old (32–77 y), with the minimum of 32 for the non-CAD and 39 for the CAD patients. The mean overall body mass index was 24.37 ± 4.93; it was higher in the patients without CAD than in those with CAD (24.94 ± 4.43 vs 27.91 ± 9.32, respectively). The mean serum level of cystatin C was 2.21 ± 0.97 mg/L; it was higher among the patients with CAD (3.57 ± 0.58 mg/L) (Table 1) and it was significantly different between the CAD and non-CAD groups based on the Mann–Whitney *U* test ($P < 0.001$). According to the normality test, the distribution of cystatin C was not normal ($P = 0.030$) and based on the Kruskal–Wallis test, there was a significant difference in the serum level of cystatin C between the different groups of CAD patients ($P < 0.0001$). Most of the patients were male

(50.5%), with a higher frequency in the CAD group (60% vs 40%, respectively). Overall, most of the study population had no family history of coronary heart disease (70.5%), smoking (69.5%), opium addiction (53.7%), blood pressure at the time of study > 140/90 mm Hg (60%), hyperlipidemia (54.7%), and diabetes (77.9%); nonetheless, the majority had hypertension (50.5%). These quantities were all similar for the CAD and non-CAD groups except for smoking, which was lower in frequency in the patients without CAD (46.4%), and opium addiction (39.3%) and also hyperlipidemia, which was higher in frequency among the patients with CAD (42.9%) (Table 2). According to the results of the logistic regression, age, smoking status, hyperlipidemia, and the level of serum cystatin C were significantly correlated with CAD (Table 3). Additionally, the area under the ROC curve was calculated to be 0.702 (Fig. 1), with sensitivity of 88.5% and specificity of 82.4% based on 0.5 as the best cutoff point for serum cystatin C (P = 0.001, 95% CI: 0.59 to 0.81), which implied that serum cystatin C was a good indicator to discriminate patients with CAD from those without CAD. The accuracy of the test was calculated to be 86.31%. Positive and negative predictive values were 88.52% and 82.35%, respectively.

Table1. Continuous clinical and biological characteristics of the patients

	Overall				With CAD				Without CAD			
Factor	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**
Age, y	32	77	58.29	10.45	39	77	60.77	9.50	32	77	54.06	10.77
BMI	14	54	24.37	4.93	17	54	24.03	5.20	14	38	24.94	4.43
Cystatin C	0.40	4.86	2.21	0.97	1.04	4.86	2.95	0.74	0.40	2.60	1.40	0.56

CAD, Coronary artery disease, BMI, Body mass index

Table 2. Categorical clinical and biological characteristics of the patients

	Overall		0		1		2		3		
Factor	**No. of Patients**	**(%)**	**No. of Patients**	**(%)**	**No. of Patients**	**(%)**	**No. of Patients**	**(%)**	**No. of Patients**	**(%)**	***P* value**
Sex											
female	47	49.5	23	65.7	8	28.6	4	36.4	12	57.1	0.020*
male	48	50.5	12	34.3	20	71.4	7	63.6	9	42.9	
Family history of coronary heart disease											
yes	28	29.5	14	40	7	25	3	27.3	4	19	0.353
no	67	70.5	21	60	21	75	8	72.7	17	81	
Smoking											
yes	29	30.5	5	14.3	15	53.6	5	45.5	4	19	0.003*
no	66	69.5	30	85.7	13	46.4	6	54.5	17	81	
Opium addiction											
yes	44	46.3	14	40	17	60.7	6	54.5	7	33.3	0.346
no	51	53.7	21	60	11	39.3	5	45.5	14	66.7	
Hypertension											
yes	48	50.5	14	40	12	42.9	8	72.7	14	66.7	0.087
no	47	49.5	21	60	16	57.1	3	27.3	7	33.3	
Blood pressure at the time of study											
≥140/90	34	40	10	28.6	7	25	8	72.7	9	42.9	0.027*
<140/90	61	60	25	71.4	21	75	3	27.3	12	57.1	
Hyperlipidemia											
yes	43	45.3	11	31.4	14	50	6	54.5	12	57.1	0.209
no	52	54.7	24	68.6	14	50	5	45.5	9	42.9	
Diabetes											
yes	21	22.1	8	22.9	4	14.3	3	27.3	6	28.6	0.640
no	74	77.9	27	77.1	24	85.7	8	72.7	15	71.4	

* Significant at the 5% level

Table 3. Effects of the variables based on logistic regression

Factor	OR(95% CI)	*P* value
Age, y	0.921 (0.85,0.99)	0.044*
BMI	0.928 (0.78,1.13)	0.470
Cystatin C	0.039 (0.008,0.195)	<0.0001*
Sex		
female	2.07 (0.21,19.81)	0.525
male**	-	
Family history of coronary heart disease		
yes	3.36 (0.61, 18.46)	0.162
no**	-	
Smoking		
yes	0.067 (0.005,0.93)	0.045*
no**	-	
Opium addiction		
yes	2.43 (0.27,21.77)	0.426
no**	-	
Hypertension		
yes	3.70 (0.63,21.52)	0.145
No**	-	
Blood pressure at the time of study		
≥140/90	0.67 (0.11,3.86)	0.659
<140/90**	-	
Hyperlipidemia		
Yes	0.087 (0.01,0.61)	0.014*
no**	-	
Diabetes		
yes	1.68 (0.14, 19.36)	0.677
no**	-	

* Significant at the 5% level; ** Stands for a control group
BMI, Body mass index

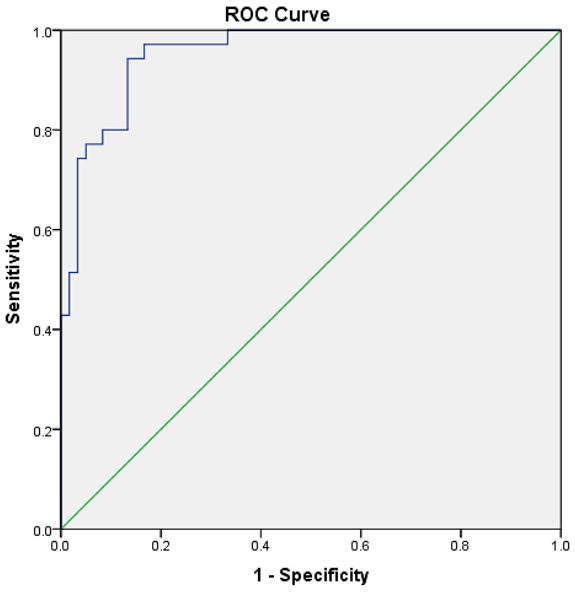

Figure 1. Area under the ROC curve for cystatin C (AUC = 0.954, 95%CI: 0.917 to 0.991; *P* < 0.0001)

DISCUSSION

According to our results, cystatin C serum levels with a cutoff point 0.5 had high and acceptable sensitivity and specificity in diagnosing CAD. Moreover, we found that cystatinC serum levels were significantly higher in our CAD patients than in the control group.

Yan L et al [18] showed that the simultaneous detection of cystatin C in serum might be useful in the detection of CAD, as well as in the clinical classification and assessment of the severity of CAD. Sugiyama et al [19] reported that higher serum cystatin C levels were associated with CAD incidence and greater coronary artery calcification in women without chronic renal disease. They concluded that the measurement of cystatin C might be useful in identifying women at high risk for CAD with high severity. Zhang et al [20] showed that serum cystatin C levels could reflect the severity of vascular lesions in patients with acute coronary syndrome patients and that a high cystatin C level possessed high and acceptable predictive values regarding the severity of vascular lesions in acute coronary syndrome. Wang et al [21] demonstrated that the serum cystatin C level in elderly patients was closely correlated with the degree of blood pressure and coronary artery stenosis and that it was able to predict CAD with high and acceptable sensitivity and specificity. Negrusz-Kawecka et al [22] reported that a high cystatin C level was a major risk factor for acute coronary syndrome and STEMI and that it could play an important role in the early diagnosis of CAD and the prevention of adverse cardiovascular events. Wang et al [23] found that high serum cystatin C levels were significantly correlated with the presence and severity of CAD in patients without chronic renal disease. They suggested that cystatin C might play an important role in the diagnosis of CAD and its severity. All these studies are concordant with the results obtained from our study.

The main functions of serum cystatin C are to inhibit endogenous cysteine proteases activity, regulate the intracellular metabolism of proteins, [24] and play an inflammatory mediator role through the activation of neutrophils. [25] Therefore, its mechanisms involve different organs and vary—including the inhibition of inflammatory factors, the confrontation of plasminogens, the regulation of the activity of pre-hormones, and finally the regulation of proteinases inside and outside cells. [26-28] Moreover, cystatin C can regulate inflammatory processes and participate in atherosclerotic plaque formation. Cystatin C may also have different functions such as inhibiting the activity of cathepsin S and K, reducing the degradation of blood vessels and vascular remodeling, and postponing the development of atherosclerosis. [20] The degradation of the extracellular matrix in the atherosclerotic process occurs in ischemic coronary events; consequently, cystatin C—as a cysteine protease inhibitor—is involved in the atherosclerotic process. [23]

Vakili et al [29] showed that the level of cystatin C was not a suitable predictor for CAD and the severity of the coronary involvement. Svensson-Färbom et al [30] demonstrated that higher levels of plasma cystatin C were not correlated with CAD. All these results are in contrast to our results. These studies reported that plasma cystatin C did not increase in patients suffering from CAD. There are different hypotheses that the expression of cystatin C is reduced before cardiac events and that this reduction activates inflammatory factors and cell factors and induces a lower level of cystatin C. On the other hand, a previous study showed that cystatin C was induced by transforming growth factor β1 (TGF-β1), which is significantly reduced in atherosclerosis. [31]

CONCLUSIONS

Our results showed that cystatin C serum levels with a cutoff point 0.5 had high and acceptable sensitivity and specificity in diagnosing CAD. Accordingly, cystatin C serum levels can play an important role in the early diagnosis of CAD and the prevention of adverse cardiovascular events. We posit that the measurement of cystatin C serum levels, along with other cardiac biomarkers such as high-sensitive troponin application, may enable us to predict cardiac events sooner and decrease the mortality and morbidity of patients.

Conflict of Interest

The authors hereby declare that they have no conflicts of interest regarding the content of this article.

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REFERENCES

1. Dickens C, Cherrington A, McGowan L. Depression and health-related quality of life in people with coronary heart disease: A systematic review. Eur J Cardiovasc Nurs. 2012;11:265–275.

2. Zwadlo C, Meyer GP, Schieffer B, Westhoff-Bleck M. Anomalous intramural course of coronary arteries in congenital heart disease-three case reports and review of the literature. Congenit Heart Dis. 2012;7:139–144.

3. Mehra M, Somohano T, Choi M. Mandibular fibular graft reconstruction with CAD/CAM technology: A clinical report and literature review. J Prosthet Dent. 2016;115:123–128.

4. WHO Fact sheet N°310. updated July 2013 http://www.who.int/mediacentre/factsheets/fs310/en/index.html.

5. de Hoog VC, Timmers L, Schoneveld AH, Wang JW, van de Weg SM, Sze SK, et al. Serum extracellular vesicle protein levels are associated with acute coronary syndrome. Eur Heart J Acute Cardiovasc Care 2013;2(1):53–60.

6. Reichlin T, Hochholzer W, Bassetti S, Steuer S, Stelzig C, Hartwiger S, et al. Early diagnosis ofmyocardial infarction with sensitive cardiac troponin assays. N Engl JMed 2009;361(9):858–67

7. Dharnidharka VR, Kwon C, Stevens G. Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. Am J Kidney Dis 2002; 40(2): 221-6

8. Grubb A, Bjork J, Lindstrom V, et al. A cystatin C-based formula without anthropometric variables estimates glomerular filtration rate better than creatinine clearance using the Cockcroft-Gault formula. Scand J Clin Lab Invest 2005; 65(2): 153-62.

9. Grubb A, Nyman U, Bjork J, et al. Simple cystatin C-based prediction equations for glomerular filtration rate compared with the modification of diet in renal disease prediction equation for adults and the Schwartz and the Counahan-Barratt prediction equations for children. Clin Chem 2005; 51(8): 1420-31

10. Ix JH, Shlipak MG, Chertow GM, et al. Association of cystatin C with mortality, cardiovascular events, and incident heart failure among persons with coronary heart disease: data from the Heart and Soul Study. Circulation 2007; 115(2): 173-9

11. Kestenbaum B, Rudser KD, de Boer IH, et al. Differences in kidney function and incident hypertension: the multi-ethnic study of atherosclerosis. Ann Intern Med 2008; 148(7): 501-8.

12. Sarnak MJ, Katz R, Stehman-Breen CO, et al. Cystatin C concentration as a risk factor for heart failure in older adults. Ann Intern Med 2005; 142(7): 497-505.

13. Shlipak MG, Sarnak MJ, Katz R, et al. Cystatin C and the risk of death and cardiovascular events among elderly persons. N Engl J Med 2005; 352(20): 2049-60 doi: 10.1056/NEJMoa043161published Online First: Epub Date.

14. Taglieri N, Fernandez-Berges DJ, Koenig W, et al. Plasma cystatin C for prediction of 1-year cardiac events in Mediterranean patients with non-ST elevation acute coronary syndrome. Atherosclerosis 2010; 209(1): 300-5.

15. Koc M, Batur MK, Karaarslan O, Abali G. Clinical utility of serum cystatin C in predicting coronary artery disease. Cardiol J 2010;17(4):374–80.

16. Ristiniemi N, Lund J, Tertti R, Christensson A, Ilva T, Porela P, et al. Cystatin C as a predictor of all-cause mortality and myocardial infarction in patients with non-STelevation acute coronary syndrome. Clin Biochem 2012;45(7–8):535–40.

17. Silva D, Cortez-Dias N, Jorge C, Marques JS, Carrilho-Ferreira P, Magalhães A, et al. Cystatin C as prognostic biomarker in ST-segment elevation acute myocardial infarction. Am J Cardiol 2012;109(10):1431–8.

18. Yan L1, Ding S2, Gu B1,2, Ma P1,2. Clinical application of simultaneous detection of cystatin C, cathepsin S, and IL-1 in classification of coronary artery disease. J Biomed Res. 2017 Jul 13;31(4):315-320.

19. Sugiyama H1, Miyoshi T2, Osawa K1, Miki T1, Koide Y1, Nakamura K1, Morita H3, Ito H1. Serum cystatin C levels are associated with coronary artery calcification in women without chronic kidney disease. J Cardiol. 2017 Dec;70(6):559-564.

20. Zhang J1, Wu X2, Gao P3, Yan P4.Correlations of serum cystatin C and glomerular filtration rate with vascular lesions and severity in acute coronary syndrome. BMC Cardiovasc Disord. 2017 Jan 31;17(1):47.

21. Wang Y1, Su X2, Zhang W2, Yang W2, Wang Y2, He Y3. Correlation between serum cystatin C level and elderly hypertensive patients combined coronaryheart disease. Int J Clin Exp Med. 2015 Apr 15;8(4):6287-90. eCollection 2015.

22. Negrusz-Kawecka M1, Poręba R2, Hulok A1, Sciborski K1, Marczak J3, Bańkowski T4. Evaluation of the significance of cystatin C levels in patients suffering from coronary artery disease. Adv Clin Exp Med. 2014 Jul-Aug;23(4):551-8.

23. Wang GN1, Sun K1, Hu DL1, Wu HH1, Wang XZ2, Zhang JS3. Serum cystatin C levels are associated with coronary artery disease and its severity. Clin Biochem. 2014 Nov;47(16-17):176-81.

24. Abdallah E, Waked E, Al-Helal B, Asad R, Nabil M, Harba T. Novel troponin-like biomarkers of acute kidney injury. Saudi J Kidney Dis Transpl. 2013;24:1111–24.

25. Longenecker CT, Hileman CO, Funderburg NT, McComsey GA. Rosuvastatin preserves renal function and lowers cystatin C in HIV-infected subjects on antiretroviral therapy: the SATURN-HIV trial. Clin Infect Dis. 2014;59:1148–56.

26. Ganda A, Magnusson M, Yvan-Charvet L, Hedblad B, Engström G, Ai D, et al. Mildrenal dysfunction and metabolites tied to low HDL cholesterol are associated with monocytosis and atherosclerosis. Circulation. 2013;127:988–96.

27. Ge C, Ren F, Lu S, Ji F, Chen X, Wu X. Clinical prognostic significance of plasma cystatin C levels among patients with acute coronary syndrome. Clin Cardiol. 2009;32:644–8.

28. 21. Negrusz-Kawecka M, Poręba R, Hulok A, Sciborski K, Marczak J, Bańkowski T. Evaluation of the significance of cystatin C levels in patients suffering from coronary artery disease. Adv Clin Exp Med. 2014;23:551–8.

29. Vakili H1, Mohamadian A2, Naderian M3, Khaheshi I4. Cystatin C may not be a precious predictor for coronary artery disease and its severity: an area of uncertainty. Acta Biomed. 2018 Jun 7;89(2):209-213.

30. Svensson-Färbom P1, Almgren P2, Hedblad B2, Engström G2, Persson M3, Christensson A4, Melander O3. Cystatin C Is Not Causally Related to Coronary Artery Disease. PLoS One. 2015 Jun 9;10(6):e0129269.

31. Shi GP, Sukhova GK, Grubb A, Ducharme A, Rhode LH, Lee RT, et al. Cystatin C deficiency in human atheroscler-osis and aortic aneurysms. J Chin Invest. 1999;104:1191–7.