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# Correlation of Serum Cystatin C level with Coronary Artery Disease and Its Severity

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## Abstract

**Background**: Atherosclerosis is an important cause of cardiovascular mortality and morbidity in the world and its progression might be slowed in many people with appropriate lifestyle and drug interventions. Hence a lot of researches are targeting the atherosclerotic process and its mediators. Cystatin C is considered to be an active cysteine protease inhibitor found in all body fluids and expressed in all nucleated cells in the body and is a better marker of renal function when compared to creatinine. Elevated plasma levels of cystatin C is thought to be associated with increased risk of cardiovascular disease (CVD) and mortality in different populations. This may be due to the fact that it represents occult impaired renal function, which is associated with increased risk of CVD. However, in several studies, cystatin C has been associated with CVD even within normal ranges of eGFR. **Aim**: To evaluate the relation of the serum cystatin C level and atherosclerotic burden in coronary arteries **Methods:** Our study included 80 patients of both sexes with known or suspected ischemic heart disease who were candidates for coronary angiography. Their serum Cystatin C level was measured using ELISA technique and correlated with coronary atherosclerosis using Gensini score. **Results:** No association was found between coronary atherosclerosis severity and serum cystatin C level. There was also no difference in serum cystatin C level between patients presenting with acute coronary syndrome and those presenting with stable ischaemic heart disease. **Conclusion:** The relation between serum cystatin C level and coronary atherosclerosis is still unclear.

**Keywords:** Cystatin C, Cardiovascular disease, Coronary atherosclerosis, Renal function.

## INTRODUCTION

Atherosclerotic cardiovascular disease is a worldwide health epidemic. It is the leading cause of death in both men and women, with an estimated 17.5 million deaths globally in 2012 [1]. Deaths are mainly caused by acute coronary syndrome (ACS) [2]. Despite this catastrophic burden of disease, evidence over the last decade suggests that the progression of atherosclerosis can often be slowed or maybe reversed in many people with lifestyle and drug interventions. So lots of research has been directed at the atherosclerotic process and its mediators [3].

In atherosclerosis, proteolysis of the extracellular matrix of the arterial wall occurs by matrix metallo-proteinases, serine and lysosomal cysteine proteases, resulting in extensive extracellular matrix degradation and vascular wall remodeling. Atherosclerotic lesions in humans have shown an overexpression of elastolytic and collagenolytic cysteine proteases and a decreased expression of cystatin C, their most abundant extracellular inhibitor [4, 5].

Human γ-trace, also called post-γ-globulin, was first described in 1961 and the name Cystatin C was proposed in 1984 [6]. Cystatin C (Cys C) is a low molecular weight protein produced by all nucleated cells at a constant rate regardless of variations of the intracellular and extracellular environment, and acts as a cysteine protease inhibitor. This protein is eliminated by glomerular filtration, reabsorbed and catabolized in proximal renal tubular cells without tubular secretion and thus has the characteristics of an ideal endogenous GFR marker [7]. The plasma concentration of Cystatin C does not depend on patient size, shows a strong negative correlation with GFR, and is a better marker of especially mild renal dysfunction than serum creatinine [8].

Elevated cystatin C is associated with increased risk of cardiovascular diseases because it represents occult impaired renal function, which contributes to this increased risk. However, in several studies, cystatin C has been associated with CVD even within normal ranges of eGFR suggesting existence of GFR independent cystatin C mediated CVD risk [9]. Several studies have demonstrated the superiority of Cystatin C compared with serum creatinine or eGFR in predicting cardiovascular events and all-cause mortality [10, 11, 12]. Elevated cystatin C may promote progression towards a dysmetabolic state [13]. Cystatin C is considered a protective protein that has an anti-atherogenic role. It prevents breakdown of the extracellular matrix in the vasculature by preventing enzymatic cleavage of connective tissues by inhibiting pro-inflammatory cathepsins [10].

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The aim of the current study was to assess the relation between serum cystatin C level and atherosclerotic burden in coronary arteries and investigate the possibility that cystatin C measurement can improve the early risk stratification of patients with suspected ACS.

**METHODS**

The study included 80 patients of both sexes and different ages with known or suspected ischemic heart disease who were candidates for urgent or elective coronary angiography in the cardiology department cath lab in Fayoum University Hospital. The study was performed from November 2016 to June 2018. Patients with renal dysfunction or known hyper- or hypothyroidism were excluded.

**Exclusion criteria**

1. Patients with renal dysfunction.
2. Known hyper or hypothyroidism.
3. Life-threatening arrhythmias.
4. Acute and chronic liver disease.
5. Infectious and inflammatory disease.
6. Decompensated heart failure.

**Study design**

All patients were subjected to the following:

1. Detailed history for angina pectoris, diabetes mellitus, thyroid disorders, chronic kidney or liver disease.

2. Full clinical examination.

3. Twelve lead ECG: A 12-lead surface ECG was obtained from all patients while in the supine position. All ECGs were recorded at a paper speed of 25 mm/s with 1 mV/cm standardization.

4. Transthoracic echocardiography: Using Siemens Acuson CV 70 and Siemens Acuson X 300 machines in the traditional views (parasternal long, parasternal short, apical 4 chambers and apical 2 chambers). Estimation of the LV systolic function was done using the EF% (ejection fraction) by M-mode in the parasternal long or parasternal short views together with 2D (Simpson's method) and eyeball estimation.

5. Cystatin C was measured using ELISA technique. The kit was provided by MyBiosource ® USA. Blood samples were collected before coronary artery angiography from the antecubital vein of the patients who were resting in the supine position.

6. Coronary angiography: The diagnostic procedure was performed via right femoral or radial arteries using Seldinger's technique after giving xylocaine for local anesthesia, with 6 French (6F) JL (curve 3.5 or 4) catheters to visualize the left system and 6F JR (curve 3.5 or 4) catheters to visualize the right system. Images were taken in standard views and recorded digitally.

Assessment of the severity of coronary artery disease was done using Gensini score, which grades narrowing of the lumen of the coronary artery and scores it with numerical values with the following method:

- 1 for 1–25% narrowing
- 2 for 26–50% narrowing
- 4 for 51–75%
- 8 for 76–90%
- 16 for 91–99%
- 32 for totally occluded artery.

- This score is then multiplied by a factor according to the importance of the coronary artery. The multiplication factor is 5 for a left main (LM) lesion, 2.5 for proximal left anterior descending artery (LAD) and proximal left circumflex artery (LCx) lesions, 1.5 for a mid-LAD lesion, 1 for distal LAD, proximal/mid/distal OM and right coronary artery lesions, and 0.5 for any other branch.

- The severity of coronary artery disease is then classified into: Normal coronary arteries (score 0), mild CAD (score: 1–20), moderate (score 20–50), and severe CAD (score >50).

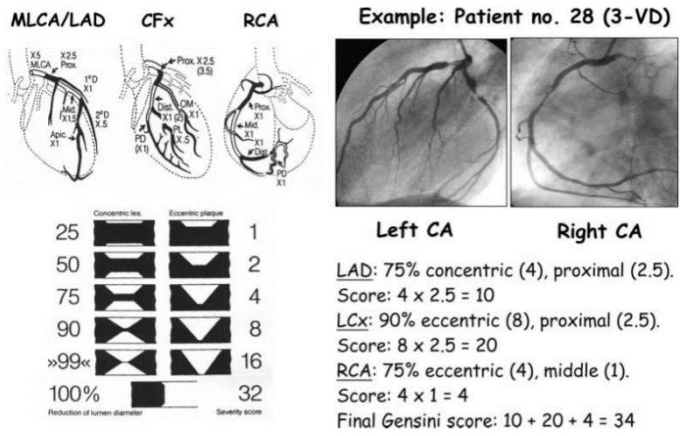


**Figure 1:** Schematic drawing of the GENSINI score (Left). The method assigns a different severity score depending on the degree of stenosis, its location (proximal, middle or distal tract) along the target vessel and the type of coronary vessel involved (LAD, LCX or RCA). An example of Gensini score calculation is shown on the right part of the figure [38].

Correlation was then made between cystatin C level and coronary atherosclerosis degree classified by Gensini score.

The collected data were organized, tabulated and statistically analyzed using SPSS software statistical computer package version 18 (SPSS Inc, USA). For quantitative data, the mean, standard deviation (SD), and range were calculated. Kolmogorov-Smirnov test (KS) test was performed as a test of normality. Mann-Whitney-U test or Kruskal Wallis test was used in comparing between any two groups or three groups, respectively. Qualitative data were presented as number and percentages, Chi square ($\chi 2$) was used as a test of significance. Spearman correlation was run to identify relation of Gensini with study variables. Multiple linear regression analysis was done to identify the different predictors of Gensini. For interpretation of results of tests of significance, significance was adopted at $P \leq 0.05$.

**RESULTS**

The current study included 80 patients with ischemic heart disease who were candidates for coronary angiography, either elective (40 patients) or urgent (40 patients). Table 1 shows patient characteristics. The mean age was 57.6 ± 11.6 years. Males were more than females (71.3% vs 28.7% respectively). Regarding some risk factors for IHD; 38.8% of patients had hypertension and 32.5% had diabetes mellitus. Renal functions of all patients were normal with a mean serum creatinine of 1.16 ± 0.19 mg/dL. Mean cystatin level was 0.36 ± 0.06 mg/L. Severity of coronary affection was estimated by Gensini score with a mean score of 80.74 ± 29.76. The patients were categorized according to this score into three groups; 4 patients with normal coronaries, 6 patients with intermediate affection, while most of the patients [70, (87.5%)] had severe coronary affection. No patients had mild affection.

Correlation of Gensini score to other study parameters (mean age, creatinine, cystatin C and ejection fraction) did not change significantly between patient categories (Table 2). Pearson correlation coefficient showed weak positive correlation with cystatin C, but strong negative correlation with ejection fraction (Table 3).

**Table 1:** Patients characteristics

| Variable | Value |
|---|---|
| Age (years) | 57.6 ± 11.6 |
| Sex (n.) | Males: 57 (71.3%) |
| | Females: 23 (28.7%) |
| Hypertension (n.) | 31 (38.8%) |
| Diabetes mellitus | 26 (32.5%) |
| Serum creatinine (mg/dl) | 1.16 ± 0.19 |
| Blood cystatin C (mg/L) | 0.36 ± 0.06 |
| Ejection fraction (%) | 48.56 ± 10.64 |
| Gensini score | 80.74 ± 29.76 |
| | Normal 4 (5%) |
| | Intermediate 6 (7.5%) |
| | Severe 70 (87.5%) |

**Table 2:** Correlation of Gensini score with other study parameters (mean±SD)

| Variable | Normal | Moderate | Severe | P value |
|---|---|---|---|---|
| Age | 49.8 ± 10.7 | 52.5 ± 15.8 | 58.5 ± 11.2 | 0.205 (NS) |
| Serum creatinine | 1.01 ± 0.26 | 1.18 ± 0.16 | 1.17 ± 0.19 | 0.484 (NS) |
| Blood cystatin C | 0.38 ± 0.05 | 0.39 ± 0.09 | 0.36 ± 0.06 | 0.344 (NS) |
| Ejection fraction | 59.25±14.75 | 53.33±14.33 | 47.54 ± 9.78 | 0.199 (NS) |

**Table 3:** Correlation coefficient between Gensini score and other variables

| | GENSINI SCORE | |
|---|---|---|
| | R | P-value |
| Age | 0.012 | 0.915 |
| Creatinine | -0.111 | 0.329 |
| Cystatin C | 0.048 | 0.674 |
| EF % | -0.337 | 0.002* |

**DISCUSSION**

This study included 80 patients with known or suspected ischemic heart disease who were candidates for coronary angiography. Their serum Cystatin C level was correlated with coronary atherosclerosis using Gensini score. The aim was to find a relationship between coronary atherosclerosis and serum cystatin C level which has emerged as a strong predictor of incident or recurrent cardiovascular events and adverse outcomes in patients without kidney disease. The study did not find strong association between coronary atherosclerosis severity and serum cystatin C level. There was no difference in serum cystatin C level among patients presenting with acute coronary syndrome and those presenting with stable ischaemic heart disease.

It has been suggested that cystatin C level is closely related to both inflammation and atherosclerosis, but the relation between inflammation, atherosclerosis, cardiovascular risk, and cystatin C is not fully explained. This is supported by a long list of studies, as shown below.

According to some authors, elevated plasma level of cystatin C was supposed to cause increased risk of cardiovascular disease (CVD) and mortality in the elderly [11, 14, 15] and in different patient populations [16-18]. Association of elevated cystatin C with increased cardiovascular risk may be explained by the fact that it represents impaired renal function, which is responsible for increased cardiovascular disease risk. However, several studies [9, 19] showed that cystatin C has been associated with CVD even within normal ranges of eGFR suggesting cystatin C mediated CVD risk, irrespective of renal dysfunction. Serum cystatin C in patients with CAD has been correlated with total mortality, cardiovascular events and heart failure [9], and was also found to be correlated with total mortality in a follow up study of patients with acute coronary syndrome [20]. Moreover, a study in elderly patients without chronic renal disease showed that increased serum cystatin C was associated with the risk of total mortality, cardiovascular disease mortality, myocardial infarction and stroke [14]. In a study of an adult population without proteinuria and with GFR > 60 ml/min/1.73 m², serum cystatin C level was associated with increased incidence of cardiovascular disease [21]. Depending on the association of serum cystatin C and high sensitivity CRP (hsCRP) levels, cystatin C level was suggested to be associated with inflammation [11, 22]. It was also proposed that cystatin C has a direct effect on atherosclerosis and inflammation [23]. It inhibits polymorphonuclear cell chemotaxis, $O_2$-release and phagocytosis [24].

Salgado *et al*., [10] have found a significant correlation between high serum cystatin C levels and cardiovascular risk factors in primary hypertensive patients. Likewise, Dent [25] reported a positive and graded association between higher serum cystatin C levels and increased cardiovascular disease prevalence in individuals with GFR ≥60 ml/min/1.73 m².

Another study conducted by Urbonaviciene *et al*., [26] demonstrated that higher serum cystatin C levels independently predicted 5-year all-cause and cardiovascular mortality in symptomatic peripheral arterial disease patients with normal renal function.

Batra *et al*., [27] carried out a prospective study on 150 Indian patients undergoing coronary angiography. The authors found that higher plasma cystatin C levels were associated with higher carotid intimal medial thickness, diffuse CAD, and more frequent occurrence of triple vessel disease and documented that the association of high cystatin C levels with CAD remained robust even in those patients with normal or mildly impaired renal function.

In addition, Imai *et al*., [28] demonstrated that higher serum cystatin C concentrations correlated with early stage coronary atherosclerosis plaques among patients without established chronic kidney dysfunction. The results further suggested that non-calcified plaques are more strongly associated with serum cystatin C levels than calcified plaques. In addition, the risk for presence of non-calcified plaques increased with the increasing quartiles of cystatin C, independent of GFR and other cardiovascular risk factors. The authors also assumed that adverse cardiovascular events may derive from involvement of cystatin C in early stage coronary plaque formation even in those patients with estimated GFR ≥60 ml/min/1.73 m².

In agreement with previous authors, Niccoli *et al*., 2009 [29] demonstrated that in patients with CAD and with normal creatinine-derived GFR, increased serum cystatin C levels are associated with a stable lesion phenotype and independently predict extent of coronary atherosclerotic burden. This study suggests that the association between cystatin C and risk of cardiovascular events is mediated by an increased coronary atherosclerotic burden.

Nine studies composed of 38,854 participants were analyzed by Luo J *et al*. [30], who found that elevated serum cystatin C level was associated with excessive risk of all-cause mortality and cardiovascular mortality comparing the highest to lowest category of cystatin C. Each standard deviation increment in serum cystatin C level increased 32% all-cause and 57% cardiovascular mortality risk. They concluded that elevated serum cystatin C level is independently associated with excessive cardiovascular and all-cause mortality risk in elderly persons.

Our results are in apparent contrast with those of all the previously mentioned studies. Our study did not find strong association between severity of coronary atherosclerosis and serum cystatin C level in IHD patients without overt kidney disease. In comparison to these studies, our study population was different. The study population were all Egyptian patients, and included both diabetic and non-diabetic patients.

On the other hand, several other studies have reported similar results to our study. In patients without CAD on entry of the 5-year prospective PRIME study [31], serum cystatin C (after adjustment for traditional risk factors as age, diabetes, smoking, hypertension, BMI, triglycerides, LDL- and HDL-cholesterol) was significantly associated with the occurrence of the first ischemic coronary event. However, this association was no longer significant when CRP was included in the analysis. The authors concluded that Cystatin-C is not a more predictive risk marker of CHD than CRP or interleukin-6, but could be useful in detecting moderate chronic renal disease.

In a study of middle-aged subjects by Shimizu *et al*.,, carotid atherosclerosis was found to be associated with microalbuminuria, but not serum cystatin C level [32].

Our results were also similar to those by Kim *et al*., [33], who reported that serum cystatin C level was significantly higher in patients with diabetic nephropathy, both in CAD and non-CAD patients. However, serum cystatin C level did not differ between CAD and non-CAD patients, regardless of diabetic nephropathy. They concluded that serum cystatin C level is a marker of renal dysfunction, but not coronary artery disease, in diabetic patients.

Svensson-Färbom *et al*., [34] reported that genetic elevation of plasma cystatin C is not related to altered risk of CAD, suggesting that there is no causal relationship between plasma cystatin C and CAD. Rather, the association between cystatin C and CAD appears to be due to the association of eGFR and CAD.

Similarly, van der Laan *et al*., [35], through their Mendelian randomization analysis, found no causal role of cystatin C in the etiology of CVD. They concluded that therapeutics targeted at lowering circulating cystatin C are unlikely to be effective in preventing CVD.

Our study showed no difference in serum cystatin C level among patients presenting with acute coronary syndrome and those presenting with stable coronary artery disease, suggesting no association between serum cystatin C level and plaque vulnerability. This comes in agreement with a study by Jin *et al*., [36] which investigated 137 coronary lesions in 82 patients with chest pain by Optical Coherence Tomography (OCT) and analyzed variables of plaque vulnerability, fibrotic cap thickness, and microchannels in plaque. They found no evidence of relationship between Cystatin C level and plaque vulnerability. However, this comes in contrast to the study by Gu *et al*., [37] who found that plasma cystatin C positively correlated with plaque area and plaque burden in the unstable angina group but not in the stable angina group, suggesting the presence of larger atherosclerotic extent in plaques in unstable angina patients.

**CONCLUSION**

Our results suggest that serum cystatin C is not a suitable marker for CAD severity. However, our study has some limitations to be taken into consideration. Firstly, results were obtained from a relatively small number of patients and from a single medical center. Secondly, long term follow up of patients was not included in our study. Thirdly, we did not assess eGFR or microalbuminuria as early parameters of renal dysfunction.

***Ethics:***

*This study was reviewed and approved by the Fayoum University-Faculty of Medicine (Research Ethical Committee). The official approval was obtained from the general director of hospital and the manager of the outpatient clinic and the head of the Cardiology department.*

***Consent to participate:***

*The study was performed after explaining its objectives and confidentiality was expressed to the participants. Written consent was taken from the participants as an agreement to join the study before clinical examination and laboratory investigation. All participants had the right to not participate in the study.*

***Declaration:***

*The authors declare that this manuscript did not previously publish or considered for publication in any other journal. The authors do not hold any stocks or shares, fees, funding or salary from any organization that may in any way gain or lose financially from the publication of this manuscript, either now or in the future.*

*Competing Interests: There is no conflict of interest as there are no commercial or financial relationships from any institution or organization that could be construed as a potential conflict and all the expenses are covered by the authors.*

***Consent to Publish:*** *Not applicable*

***Availability of data and materials:***

*All the data are available on an excel sheet and also SPSS format and if needed the corresponding author is welcome to send it upon request.*

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