Clinical Utility of Serum Cystatin C in Predicting Coronary Artery Disease in Patients Without Chronic Kidney Disease

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INTRODUCTION

Renal function has been identified as a risk factor for the onset and prognosis of coronary artery disease (CAD, (1)). Consequently, subjects with chronic renal failure are exposed to increased morbidity and mortality as a result of cardiovascular events. Further, accelerated cardiovascular disease (CVD) is a frequent complication of renal disease (2).

Glomerular filtration rate (GFR) is an important indicator of renal function. However, in practice, since GFR is usually not directly measured in routine clinical practice, markers such as creatinine are used to estimate GFR. Estimating equations such as the Cockcroft-Gault equation (3) or the modification of diet in renal disease (MDRD, (4)) study equation are widely used for this purpose.

In recent years, cystatin C has been proposed as a more reliable marker of renal function than serum creatinine, in particular for the detection of small reductions in GFR (5). Cystatin C is a 122 amino acid, 13-kDa protein that is a member of the family of competitive inhibitors of lysosomal cysteine protease. It is encoded by the housekeeping type CST3 gene, and produced by all nucleated cells at a constant rate. The protein is located extracellularly and detected mainly in biological fluids. Because of its smaller size and cationic nature, it is freely filtered by the glomerulus. It is not secreted, but reabsorbed by tubular cells and subsequently catabolized so that it does not return to the blood (2).

It is more sensitive and specific for the estimation of GFR and less influenced by age, gender, race, muscle...
mass, and medication as compared to serum creatinine (6–8). Therefore, cystatin C is a promising marker of renal function. Prospective studies have shown in various scenarios that patients with increased cystatin C are at a higher risk of developing both CVDs and chronic kidney disease (CKD, (5)).

However, in the recent years, cystatin C has emerged as a potential marker for cardiovascular risk. In fact, previous studies have shown that a high level of cystatin C has been related to suspected or confirmed acute coronary syndrome. The results of studies investigating the value of cystatin C in suspected stable CAD patients are controversial (9–11). But further studies have shown that high concentrations of cystatin C were independently associated with cardiovascular risk factors, such as hypertension, dyslipidemia, smoking, diabetes, and oxidative stress, in individuals without CKD or microalbuminuria (5).

Therefore, the clinical utility of cystatin C as a new predictive marker of CAD merits further investigations. The aim of this study was to investigate the role of cystatin C level as a predictor of cardiovascular events and the association between this protein, oxidative stress markers, and other biochemical risk factors for atherosclerosis in patients with CAD.

MATERIALS AND METHODS

Study Group

We studied 305 consecutive coronary patients (234 men and 71 women) for coronary angiography recruited from Department of Cardiology, CHU–Fattouma Bourguiba, Monastir, Tunisia. The mean age of this group is 61.15 ± 10.64 years.

All patients admitted to our study had a history of stable angina defined by the presence of chest pain or unstable angina and were found to have CAD at angiography. Two experienced cardiologists, unaware of the patients clinical history and biochemical results, visually assessed all angiograms to assess the extent of the CAD.

Patients with severe renal dysfunction, life-threatening arrhythmias, acute and chronic liver disease, infectious and inflammatory disease, and symptomatic heart failure were excluded. The detailed histories of patients included demographics data, and cardiovascular risk factors were recorded. All participants were interviewed, and data on dyslipidemia, diabetes mellitus, hypertension, and smoking habits were recorded. For coronary risk factors, the following definitions were used: individuals were defined as hypertensive if their blood pressure was >140/90 mmHg or if they were receiving any antihypertensive treatment; individuals with a history of diabetes mellitus or those receiving any antidiabetic medication were considered to be diabetic; individuals were deemed dyslipidemic if their total cholesterol (TC) concentration was ≥5.68 mmol/l, or their triglyceride (TG) concentration was ≥2.28 mmol/l, or they were receiving lipid-lowering drugs. Smoking history was coded as never and current smoker.

Methods

Measurement of biochemical variables

Levels of TC, TG, high-density lipoprotein cholesterol (HDLc), and serum creatinine were measured using standardized enzymatic methods (Randox-Antrim, UK). GFR was estimated by the MDRD formula. Serum high-sensitive C-reactive protein (hs-CRP), fibrinogen, and cystatin C were quantified according to the instructions of the manufacturer using particle-enhanced immunonephelometric assay (Dade Behring, Marburg Allemagne).

Determination of the oxidative state markers

Superoxide dismutase (SOD), glutathione peroxidase (GR), glutathione reductase (GR), glutathione reductase (GPX) activities, and total antioxidant status (TAS) concentration were checked using commercials tests manufactured by Randox Laboratories (Antrim, UK) in a Daytona analyser. The catalase (CAT) activity was determined spectrophotometrically as the rate of substrate decomposition per unit time (12). Serum lipid peroxides were measured by the fluorimetric method of Yagi (13) using thiobarbituric acid reaction (Ex 515 nm, Em 553 nm; (14)).

Coronary angiography

All patients underwent coronary angiography, performed using standard Judkins techniques before the start of the study. Blood samples were collected before coronary artery angiography from the antecubital vein of the patients who were resting in the supine position. The CAD severity was determined by two experienced independent observers, blinded to both the arterial waveform and clinical patient data, according to the Gensini scoring system (15) In short, the stenosis score comprises the maximum stenosis, and four CAD groups were drawn according to their CAD severity score: namely, normal coronary arteries (score 0), mild CAD (score: 1–20), moderately severe (score 20–50), and severe CAD (score > 50).

Statistical Analysis

All analyses were performed using SPSS 15.0 (SPSS Inc, Chicago, IL). Categorical variables of cystatin C groups were compared by the Chi-square test. Continuous
variables were expressed as mean ± SD and compared by one-way analysis of covariance.

Correlations between continuous variables were assessed using Spearman’s correlation analysis. A linear regression analysis was applied for CAD severity score, oxidative markers levels, homocysteine, and hs-CRP.

In order to analyze the separate effect of CAD severity score on serum cystatin C levels and the possible interaction between the severity score and renal impairment, an analysis of covariance (ANCOVA) was used. CAD severity score and estimated GFR (eGFR) were entered as categorical independent variables, and log hs-CRP and homocysteine were entered as covariates in this analysis. A P-value <0.05 was considered as statistically significant.

RESULTS

We studied 305 patients grouped according to the CAD severity score. Cystatin C concentration was significantly elevated in the patients with a CAD severity score >50% compared to the normal coronary artery patients (2.9 ± 2.2 mg/l vs. 0.7 ± 0.2 mg/l).

The variables measured in relation with the CAD severity score are shown in Table 1. The patients with CAD severity score >1 were slightly older than those with normal arteries (P < 0.01). Creatinine and cystatin C were positively correlated to the increase in the CAD severity score using ANCOVA analysis (P < 0.01), as was the decrease in eGFR (<0.01).

The concentration of lipid peroxides measured as malondialdehyde is significantly correlated to the increase in the CAD severity score. On the other hand, our results showed a decrease in the activities of antioxidant enzyme in the patients with CAD severity score >50 (Table 1).

Subject characteristics and clinical laboratory variables of cystatin C quartile groups are presented in Table 2. The number of CAD patients increased as the quartile of cystatin C with a remarkable statistical difference between cystatin C groups (P < 0.001). Overall, subjects with higher serum cystatin C were older. Also, body mass index (BMI), hs-CRP, and fibrinogen were higher at higher serum cystatin C quartile. Similarly, the concentration of lipid peroxides measured as malondialdehyde, homocysteine, and CAD severity score were higher at higher serum cystatin C quartile. But HDLc and antioxidant markers (SOD, GPX, GR, CAT, and TAS) decreased progressively with higher serum cystatin C quartile. Cigarette smoking, diabetes, HTA (Arterial hypertension), and obesity are not associated with serum cystatin C.

| TABLE 1. The Relation Between the Variables Measured and CAD Severity Score |
|-------------------|-------------------|-------------------|-------------------|-------------------|
| Number of patients | 91 | 93 | 65 | 56 |
| Gender | 68H/23F | 73H/20F | 49H/16F | 42H/14F |
| Age (years, X ± δ) | 59.8 ± 9.9 | 60.2 ± 11.8 | 61.1 ± 10.1 | 60.5 ± 13.9 |
| BMI (Kg/m², X ± δ) | 26.3 ± 5.1 | 27.6 ± 5.2 | 26.3 ± 3.6 | 24.8 ± 5.5 |
| TC (mmol/l, X ± δ) | 4.0 ± 0.9 | 4.3 ± 1.4 | 4.3 ± 0.8 | 4.4 ± 0.8 |
| HDLc (mmol/l, X ± δ) | 1.08 ± 0.3 | 1.07 ± 0.4 | 1.07 ± 0.2 | 1.05 ± 0.3 |
| TG (mmol/l, X ± δ) | 1.8 ± 1.3 | 1.8 ± 0.9 | 1.8 ± 1.1 | 2.2 ± 0.6 |
| Apo (A1) (g/l, X ± δ) | 1.5 ± 1.2 | 0.9 ± 0.6 | 0.9 ± 0.5 | 0.89 ± 2.2 |
| Apo (A2) (g/l, X ± δ) | 1.3 ± 0.4 | 1.4 ± 0.4 | 1.5 ± 0.6 | 1.7 ± 0.5 |
| Creatinine (mg/l, X ± δ) | 0.7 ± 0.2 | 0.8 ± 0.3 | 1.2 ± 0.9 | 2.9 ± 2.2 |
| eGFR (ml/min/1.73 m², X ± δ) | 71.1 ± 8 | 73.3 ± 8.0 | 83.2 ± 8.2 | 125.6 ± 6.2 |
| Log hs-CRP | 0.62 ± 0.5 | 0.68 ± 0.58 | 0.69 ± 0.46 | 0.7 ± 0.56 |
| Fibrinogen (g/l, X ± δ) | 3.9 ± 1.4 | 3.6 ± 1.1 | 4.2 ± 1.4 | 5.5 ± 1.7 |
| GPX (U/gHb, X ± δ) | 62.1 ± 10.4 | 59.10.5 | 56.6 ± 9.6 | 57 ± 10 |
| GR (U/gHb, X ± δ) | 2.6 ± 0.8 | 2.4 ± 0.8 | 2.29 ± 0.7 | 2.1 ± 0.4 |
| CAT (μmol H₂O₂/min/mg proteins) | 1 ± 0.08 | 1.06 ± 0.04 | 1.02 ± 0.1 | 1 ± 0.05 |
| SOD (U/gHb, X ± δ) | 687.9 ± 454.7 | 662.3 ± 351.4 | 553.7 ± 361.3 | 558.8 ± 464.4 |
| TAS (mmol/l, X ± δ) | 1.1 ± 0.3 | 1.1 ± 0.2 | 1.10 ± 0.3 | 1.3 ± 0.2 |
| Lipid peroxides as malondialdehyde (μmol/l, X ± δ) | 1.8 ± 0.3 | 1.8 ± 0.3 | 1.9 ± 0.2 | 1.95 ± 0.2 |
| homocysteine (μmol/l, X ± δ) | 9.8 ± 5.5 | 12.21 ± 6.1 | 13.36 ± 4.3 | 15.91 ± 6.8 |

Data are expressed as mean ± SD or number.

BMI: body mass index; H: men; F: women; TC: total cholesterol; HDLc: high-density lipoprotein cholesterol; TG: triglycerides; Apo (A1) and Apo (B): apolipoproteins; eGFR: estimated glomerular filtration rate; hs-CRP: high-sensitive C-reactive protein; TAS: total antioxidant status; GPX: glutathione peroxidase; GR: glutathione reductase; CAT: catalase; SOD: superoxide dismutase.

P-value is from χ² test.
TABLE 2. Association Between Variables of Study Population Quartiles of Cystatin C

| Plasm e cystatin C (mg/l) | Quartile 1 (≤0.615) | Quartile 2 (0.61–0.84) | Quartile 3 (0.84–1.15) | Quartile 4 (≥1.15) | P (ANCOVA) |
|--------------------------|---------------------|------------------------|------------------------|---------------------|-------------|
| Number of patients       | 74                  | 76                     | 75                     | 80                  | <0.01       |
| Gender                   | 58H/16F             | 59H/17F                | 54H/21F                | 61H/19F             | 0.33        |
| Age (years, X ± s)       | 52 ± 10.6           | 61.5 ± 10.5            | 65 ± 10                | 70 ± 10.3           | <0.01       |
| Diabetes %               | 29                  | 28                     | 27                     | 21                  | <0.01       |
| Hypertension %           | 35                  | 34                     | 34.9                   | 36                  | <0.01       |
| Smoking %                | 14                  | 15                     | 14                     | 16                  | 0.15        |
| Obesity %                | 13                  | 11                     | 15                     | 14                  | 0.45        |
| CAD severity score       | 1.2 ± 4.3           | 16.3 ± 14.8            | 20.5 ± 11.3            | 25.5 ± 18.3         | <0.01       |
| BMI (Kg/m², X ± s)       | 25.2 ± 5            | 27.7 ± 5               | 28 ± 3                 | 31.2 ± 5            | <0.001      |
| TC (mmol/l, X ± s)       | 3.4 ± 1.1           | 4.1 ± 1                | 4.5 ± 0.8              | 4.9 ± 1.1           | 0.031       |
| HDLc (mmol/l, X ± s)     | 1.3 ± 0.3           | 0.9 ± 0.3              | 0.7 ± 0.1              | 0.6 ± 0.3           | <0.05       |
| TG (mmol/l, X ± s)       | 1.08 ± 1.1          | 1.4 ± 1.1              | 1.8 ± 1.01             | 2.1 ± 1.1           | <0.05       |
| ApoA1 (g/l, X ± s)       | 1.3 ± 0.2           | 0.9 ± 0.1              | 0.7 ± 0.09             | 0.6 ± 0.1           | <0.001      |
| ApoB (g/l, X ± s)        | 1.1 ± 0.4           | 1.3 ± 0.4              | 1.5 ± 0.4              | 1.7 ± 0.4           | <0.05       |
| Creatinine (μmol/l, X ± s)| 58 ± 2.8            | 72 ± 2.9               | 83 ± 3.1               | 90.1 ± 3.1          | 0.004       |
| eGFR (ml/min/1.73 m², X ± s)| 86.2 ± 4.8         | 76.3 ± 3.1             | 72.4 ± 2.9             | 66.5 ± 3.01         | <0.001      |

Data are expressed as mean ± SD or number.

BM1: body mass index; TC: total cholesterol; HDLc: high-density lipoprotein cholesterol; TG: triglycerides; Apo (A1) and Apo (B): apolipoproteins; eGFR: estimated glomerular filtration rate; hs-CRP: high-sensitive C-reactive protein; TAS: total antioxidant status; GPX: glutathione peroxidase; GR: glutathione reductase; CAT: catalase; SOD: superoxide dismutase; H: men; F: women.

P-value is from χ² test.

Independent predictors of incident CAD, determined by logistic regression, were as follows: cystatin C, hs-CRP, eGFR, HDLc after adjustments for age, creatinine, eGFR, homocysteine, gender, presence of hypertension, and diabetes (Table 3). Every 0.1 mg/l increase in cystatin C, 2 mg/l increase in hs-CRP, 0.2 mmol/l decrease in HDLc, 13.7 ml/min decrease in eGFR, and 1.51 μmol/l increase in homocysteine caused a 34, 12, 5, and 22% increase in the risk of having CAD, respectively.

A backward stepwise multiple regression analysis was performed in the combined population of normal CAD (score 0) subjects to severe CAD patients (score >50) with cystatin C as a dependent variable (Table 4). In multivariate analysis, the CAD severity score was a significant predictor of the cystatin C concentration, as well as eGFR.

We found that the increased level of cystatin C persisted where either hs-CRP or homocysteine levels were added as covariates in the multivariate model. Linear regression analysis showed that cystatin C, fibrinogen, hs-CRP, TAS, and homocysteine levels were the most significant predictors of CAD severity (r = 0.631, 2019).

TABLE 3. Multivariate Logistic Regression Analysis for Predicting the Presence of CAD

| Variable          | Odd ratio | 95% CI       | P     |
|-------------------|-----------|--------------|-------|
| Cystatin C        | 1.215     | 1.139–1.281  | 0.001 |
| hs-CRP            | 1.123     | 1.117–1.130  | 0.012 |
| Fibrinogen        | 0.989     | 0.952–1.028  | 0.022 |
| eGFR              | 0.945     | 0.919–0.989  | 0.054 |
| HDLc              | 0.920     | 0.903–0.940  | 0.033 |

CI: confidence interval; hs-CRP: high-density lipoprotein cholesterol; eGFR: estimated glomerular filtration rate; HDLc: high-density lipoprotein cholesterol.
TABLE 4. Backward Stepwise Multiple Regression Analysis of Cystatin C As Dependent Variable in the Total Population

| Dependent variable | Independent variables | Standard regression coefficient | Standard error of regression coefficient | Significance |
|--------------------|-----------------------|---------------------------------|-----------------------------------------|--------------|
| Cystatin C         | eGFR                  | −0.014                          | 0.02                                    | P < 0.01     |
|                    | Log hs-CRP            | 0.173                           | 0.013                                   | P < 0.01     |
| F(9.161) = 62.854  | Homocystein           | 0.119                           | 0.017                                   | P < 0.01     |
|                    | CAD severity score    | 0.02                            | 0.01                                    | P < 0.01     |

Values are expressed as number.
hs-CRP: high-density lipoprotein cholesterol; eGFR: estimated glomerular filtration rate.

DISCUSSION

Cystatin C is a protein that belongs to a group of cysteine proteinase inhibitors, one of the four types of proteinases in mammalian cells. These types of proteins are encoded by the so-called housekeeping genes that regulate the factors necessary for global cell function, and all nucleated cells produce them at a stable production rate (16). The protein is located extracellularly and detected mainly in biological fluids. Because of its small size, cystatin C is freely filtered by the glomerulus and is not secreted, reabsorbed, or catabolized in the proximal tubules; it does not return to the blood and is not detected in urine (17). Cystatin C has been reported to provide a more accurate and precise estimate of GFR than serum creatinine (8).

In the recent years, cystatin C has emerged as a marker of cardiovascular events and mortality in different situations. For example, in patients with ischemic heart disease, cystatin C was found to be an independent risk factor together with traditional cardiovascular risk factors, renal function, or the presence of microalbuminuria (18).

The study of Koc et al. (1) assessed a cut value of cystatin C as 0.82 mg/l with a 75.5 and 75% sensitivity and specificity, respectively. However, several studies have demonstrated that CKD patients are known to be at an increased risk of developing CVD and cardiovascular events (5).

In this study, cystatin C was correlated with CAD severity score, BMI, HDLc, creatinine, homocystine, lipid peroxides as malondialdehyde, and GPX. Thus, cystatin C appears to be a marker of cardiovascular risk, and high correlations of circulating cystatin C have been shown to be consistently and strongly associated with the occurrence and the severity of CVD. Our results agree with the previous idea that demonstrates the positive correlation between cystatin C and the increase in the CAD severity score.

Moreover, cystatin C seems to offer more complete prognostic information than other markers of renal disease. Above all, cystatin C appears to be useful for identifying individuals at a higher risk for cardiovascular events among patients classified as belonging to a low category according to both creatinine and eGFR value (5).

For secondary cardiovascular events, cystatin C is one of the strongest risk predictors. The contribution of cystatin C in a multivariate model remains significant, which indicates its added value to established risk factors. Because of the association of renal dysfunction with CVD, it is unclear whether cystatin C is a direct marker of CVD or merely a marker for renal failure, which has implications for therapeutic intervention (19).

Recently, Zethelius et al. (20) assessed whether a combination of biomarkers, including cystatin C, N-terminal pro-brain natriuretic peptide, troponin, and CRP, improved patient risk stratification compared to established cardiovascular risk factors (5). So, a prospective cohort study by Luc et al. (10) showed an association between cystatin C and the incident of CAD. However, inflammation, associated with atherogenic changes, may be one mechanism associated with cystatin C and cardiovascular risk, and high concentrations have been found to be associated with high concentration of CRP (21, 22).
This suggested that the predictive value of cystatin C is not dependent, although, in another study, cystatin C, independently, predicted smooth CAD lesions but not complex lesions or CAD severity (10). Our study is in accordance with the result of Wang et al. (23), Koeing et al. (24), and Koc et al. (1), that among markers of renal function, cystatin C and eGFR are significantly and independently related to the presence of CAD and the CAD severity score.

Some studies found no relationship between cystatin C levels and CRP, and the association between cystatin C and cardiovascular mortality was independent of the CRP. Thus, our result is in agreement with some studies (25, 26) that suggested the presence of a positive correlation between cystatin C and CRP or fibrinogen.

Among all predictors evaluated, cystatin C was the strongest correlate of fibrinogen and the second strongest correlate of CRP. Although it has been suggested that high cystatin C concentrations are directly related to both inflammation and atherosclerosis (27), inflammation, associated with atherogenic changes, may be one mechanism associated with cystatin C and cardiovascular risk, and high cystatin C concentrations have been found to be associated with high concentrations of CRP (21).

Our results are in agreement with the previous idea that demonstrates the correlation between cystatin C and the levels of oxidative parameters, although various studies have demonstrated an association between oxidative stress markers and CKD and CAD events (28). Elevated homocysteine level is a well-known cardiac risk factor for the development of atherosclerosis (29). Likewise, it is the most significant parameter in our study to predict serum cystatin C and the CAD severity score.

Previous studies have demonstrated that high concentrations of cystatin C have been also associated with a hypermetabolic status (30, 31). Given the various possible mechanisms responsible for the changes in cystatin C concentrations, it is conceivable that, depending on the clinical setting considered, increased cystatin C concentrations may variously reflect renal dysfunction, the effects of heart failure as a result of hypertension and/or fluid retention (32), or CAD associated with inflammation and atherosclerosis (33).

There is evidence that both elastolytic cysteine proteases and their inhibitors, an important one being cystatin C, are involved in the pathogenesis of atherosclerosis (34,35). Inflammatory cytokines associated with atherosclerosis stimulate the production of lysosomal cathepsins, and increased plasma concentrations of cystatin C, a cathepsin inhibitor, may reflect, at least in part, an attempt to counterbalance a potentially damaging increased elastolytic activity. Studies have demonstrated that human cathepsins are expressed in endothelial cells, smooth muscle cells, and macrophages, and that they are involved in the progression, composition, and rupture of atherosclerotic plaques (36, 37).

Increased cystatin C is emerging as a marker of both CKD and cardiovascular risk. In patients with CAD, an increase in the plasma concentration of cystatin C indicates severity of coronary lesions. As the presence of CAD is one of the major determinants of the prognosis in patients with reduced renal function, the concentration of serum cystatin C is expected to be useful in detecting patients at risk of CAD.

CONCLUSION

The results of our study indicate that serum cystatin C is as relevant as CRP. There is a considerable association between cystatin C and biochemical cardiovascular risk factors such as homocysteine, low HDL, oxidative stress markers, and CRP. With these results, we suggested that cystatin C could be used as a marker in clinical practice to predict the presence of severity of atherosclerosis in suspected CAD patients.

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Clinical Utility of Serum Cystatin C

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