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Insulin resistance and inflammation may have an additional role in the link between cystatin C and cardiovascular disease in type 2 diabetes mellitus patients

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Abstract

Recent studies suggest that serum cystatin C level is not only a sensitive marker for renal dysfunction but also a predictive marker for cardiovascular disease (CVD). However, the mechanism of this connection is not fully understood. We aimed to determine whether insulin resistance or various biomarkers of cardiovascular risk have a role in the link between cystatin C and CVD in type 2 diabetes mellitus patients. Anthropometric measurements and biochemical studies including inflammatory biomarkers were performed in 478 patients with type 2 diabetes mellitus. The degree of insulin resistance was assessed by homeostasis model assessment (HOMA-IR) and indicators of metabolic syndrome. Estimated glomerular filtration rate (eGFR) was derived from the Modification of Diet in Renal Disease study equation. After adjusting for age, sex, body mass index, and eGFR, the cystatin C level increased significantly in proportion to the number of metabolic syndrome components present (1.08 ± 0.06, 1.19 ± 0.04, 1.20 ± 0.04, 1.23 ± 0.04, and 1.37 ± 0.06 mg/L; $P < .0001$); and HOMA-IR increased significantly in proportion to cystatin C quartiles (1.16 ± 0.15, 1.40 ± 0.13, 1.49 ± 0.13, and 2.00 ± 0.17; $P < .0001$) (means ± SE). Albumin-creatinine ratio, fibrinogen, uric acid, homocysteine, high-sensitivity C-reactive protein, and lipoprotein(a) all showed significant correlations with cystatin C that were generally higher than those with eGFR. Cystatin C level was independently associated with HOMA-IR ($\beta = 0.0380$, $P = .0082$), albumin-creatinine ratio ($\beta = 0.0004$, $P < .0001$), uric acid ($\beta = 0.0666$, $P < .0001$), and homocysteine ($\beta = 0.0087$, $P = .0004$). In conclusion, cystatin C level was significantly associated with insulin resistance and biomarkers reflecting inflammation independent of renal function. These components may have a role in addition to that of eGFR in explaining the link between cystatin C and CVD in type 2 diabetes mellitus patients.

1. Introduction

Recent data suggest that cystatin C, a cysteine protease inhibitor that is freely filtered at the glomerulus and almost completely reabsorbed and degraded by proximal tubular cells [1], is a more sensitive marker for renal dysfunction than serum creatinine. A meta-analysis incorporating nearly 4500 subjects clearly showed its superiority as a marker of glomerular filtration rate [2], and this result is in line with studies involving type 2 diabetes mellitus patients [3-5]. Furthermore, cystatin C was proven to be a strong predictive marker of the mortality risk and of cardiovascular events in older persons [6,7], in patients with coronary artery disease [8,9], and in patients with heart failure [10]. We also demonstrated a positive association of cystatin C with calculated risks for cardiovascular disease (CVD) in type 2 diabetes mellitus patients [11], proposing its role as a marker of both micro- and macrovascular diabetic complications.

However, the mechanism of the link between cystatin C and CVD is unclear. Possible hypotheses are as follows. First, as renal insufficiency is independently associated with

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the risk of CVD and death [12,13], the relationship between cystatin C and CVD could be explained merely by the higher sensitivity of cystatin C in detecting renal dysfunction. Second, from the data that cystatin C participates in the process of apoptotic neuronal cell death [14], it could be presumed to have deleterious effects on other cell types by direct toxicity. Third, as insulin resistance and metabolic syndrome (MetS) are involved in the development of both CVD and renal dysfunction [15,16], they may mediate the link between cystatin C and CVD. Fourth, close association of cystatin C with biomarkers representing inflammatory and procoagulatory processes leads to the proposal that these pathways could have a role in the connection between cystatin C and CVD [11,17-20].

Although linking cystatin C to CVD within its relationship to renal function seems evident, it is not sufficient to explain their close connection. In this study, we aimed to determine whether an additional explanation of the link between cystatin C and CVD in type 2 diabetes mellitus patients could be explained by its link with insulin resistance and other biomarkers.

2. Methods

2.1. Subjects

Four hundred seventy-eight consecutive type 2 diabetes mellitus patients aged at least 20 years who were admitted to Kangnam St Mary's hospital between 2006 and 2007 for the purpose of glucose control were recruited. Patients on renal replacement therapy or having accompanying infectious diseases were excluded. The Institutional Review Board of the Clinical Research Coordinating Center in Kangnam St Mary's Hospital approved the study protocol.

2.2. Study of anthropometry, biochemistry, and diabetic complications

Body mass index (BMI) and waist-hip ratio were calculated after anthropometric measurements. Blood samples were obtained on the day after admission after at least a 10-hour fast. The clotting method with Dade Thrombin reagent (Dade Behring, Marburg, Germany) was used for measurement of fibrinogen, and the intraassay coefficient of variation (CV) was 5.4%. Lipoprotein(a) was measured by immunoturbidimetry with the TAC-3 test kit (MBL, Nagoya, Japan), and its CV was 9.85%. Immunoturbidimetry with the LT CRP-HS II kit (Wako, Osaka, Japan) was used for high-sensitivity C-reactive protein (hs-CRP) assay, and its CV was 2.42%. An enzymatic method using the HiSense Homocysteine kit (HBI, Anyang, Korea) was used for the homocysteine assay, and its CV was 11%. Serum cystatin C concentration was measured by latex-agglutination immunoturbidimetry using the HiSense Cystatin C kit (HBI, Anyang, Korea) and an automatic chemistry analyzer. The range of detection for the assay was 0.4 to 14 mg/L, and the intraassay CV was 4.49%. The National Cholesterol Education Program–Adult Treatment Panel III criteria with the abdominal obesity cutoff for the Asia-Pacific region were used to define MetS. Because all of the subjects had diabetes, MetS was defined if 2 or more of the following criteria were satisfied: (1) waist circumference greater than 90 cm in men and greater than 80 cm in women, (2) triglyceride of at least 150 mg/dL, (3) high-density lipoprotein (HDL) cholesterol less than 40 mg/dL in men and less than 50 mg/dL in women, and (4) blood pressure (BP) of at least 130/85 mm Hg. The degree of insulin resistance was assessed by homeostasis model assessment (HOMA-IR) using the HOMA2 calculator (www.dtu.ox.ac.uk). The presence of nonproliferative or proliferative diabetic retinopathy was defined by an ophthalmologist, and peripheral neuropathy was diagnosed when a patient had typical symptoms or abnormalities on neurologic examination (10-g monofilament test, vibration test, pinprick test, and ankle reflex). Albumin-creatinine ratio (ACR) was calculated with first-voided spot urine samples, and 30 to 299 mg/g was defined as the microalbuminuric range. Estimated glomerular filtration rate (eGFR) was derived from the Modification of Diet in Renal Disease (MDRD) study equation. The patients were regarded as having coronary heart disease (CHD) when they had a history of angina pectoris or myocardial infarction or when significant stenosis (≥50%) in the coronary artery was observed by multidetector computed tomography or conventional angiography.

2.3. Statistical analysis

All statistical analyses were performed using the SAS 8.0 package (SAS institute, Cary, NC). Parameters showing skewed distribution (C-peptide, ACR, hs-CRP) were logarithmically transformed to achieve a normal distribution. The differences in cystatin C levels between subgroups were compared by *t* tests. Analysis of covariance was used to compare the cystatin C levels according to the number of MetS components present and to compare the levels of HOMA-IR and other biomarkers according to cystatin C quartile. Pearson and Spearman correlation analyses were performed to examine the relationship between various biomarkers. Multiple regression analysis revealed the factors independently associated with cystatin C; and at this time, the selection of variables was made based on a forward selection method. Data are expressed as means ± SD or percentage except where noted. A P value < .05 was considered significant.

3. Results

3.1. Baseline characteristics

The mean age of subjects and duration of diabetes were 61.2 ± 14.9 and 13.0 ± 10.3 years, respectively. The mean BMI was 24.2 ± 3.7 kg/m^2, and 43.7% of subjects were male. Of all subjects, 68.4% (n = 327) had MetS and 17.8% (n = 85) had a previous history of CHD. The mean serum creatinine, MDRD-GFR, and cystatin C levels were 1.11 ±

Table 1
Baseline characteristics of the study subjects

n	478
Age (y)	61.2 ± 14.9
Sex (M/F)	43.7/56.3
Duration of diabetes (y)	13.0 ± 10.3
BMI (kg/m^2)	24.2 ± 3.7
Waist circumference (cm)	89.3 ± 10.7
Waist-hip ratio	0.95 ± 0.08
Systolic BP (mm Hg)	118 ± 16
Diastolic BP (mm Hg)	73 ± 9
Hypertension (no/yes)	49.5/50.5
Smoking (no/yes)	80.9/19.1
CHD (no/yes)	82.2/17.8
MetS (no/yes)	31.6/68.4
Retinopathy (no/yes)	53.0/47.0
Neuropathy (no/yes)	67.7/32.3
Hemoglobin (g/dL)	12.6 ± 2.0
Serum creatinine (mg/dL)	1.11 ± 0.55
MDRD-GFR (mL/min per 1.73 m^2)	72.4 ± 26.3
Uric acid (mg/dL)	5.0 ± 1.8
Total cholesterol (mg/dL)	171.7 ± 43.3
Triglyceride (mg/dL)	160.8 ± 120.8
HDL cholesterol (mg/dL)	44.7 ± 14.2
LDL cholesterol (mg/dL)	98.3 ± 36.8
HbA_{1c} (%)	9.5 ± 2.3
Fasting C-peptide (ng/mL)	1.34 (0.64–2.23)
HOMA-IR	1.51 ± 1.46
ACR (mg/g)	22.6 (8.5–73.7)
Fibrinogen (mg/dL)	310.1 ± 91.9
hs-CRP (mg/dL)	0.13 (0.05–0.46)
Cystatin C (mg/L)	1.22 ± 0.63
Homocysteine (μmol/L)	12.47 ± 7.30
Lipoprotein(a) (mg/dL)	27.1 ± 27.9
Use of antihypertensive agent (no/yes)	56.5/43.5
Use of antidyslipidemic agent (no/yes)	62.4/37.6
Use of antidiabetic agent (OHA/insulin ± OHA)	55.6/32.3

Data are expressed as means ± SD, percentage, or median (25th-75th percentiles). LDL indicates low-density lipoprotein; OHA, oral hypoglycemic agent.

0.55 mg/dL, 72.4 ± 26.3 mL/min per 1.73m^2, and 1.22 ± 0.63 mg/L, respectively (Table 1). As expected, cystatin C level was highly correlated with MDRD-GFR ($r = -0.751$, $P < .0001$) (Fig. 1). When comparing cystatin C levels,

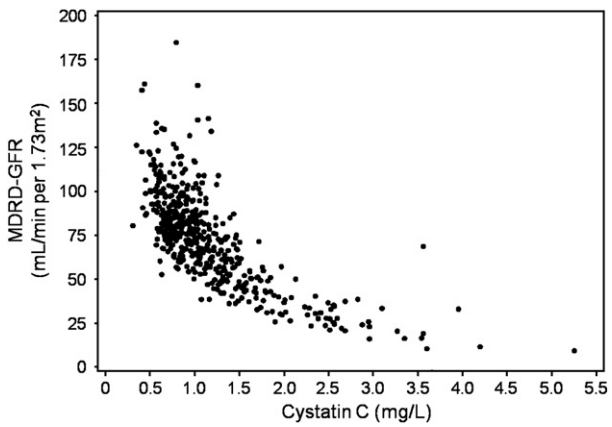

Fig. 1. Scatter plot of MDRD-GFR by cystatin C level.

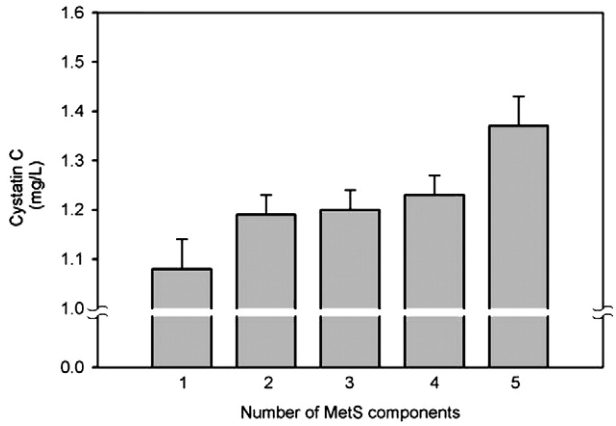

Fig. 2. Cystatin C level according to the number of MetS components present. Data are expressed as means ± SE. Adjusted for age, sex, BMI, and MDRD-GFR. P for trend < .0001.

significant differences were noted between subjects with and without MetS (1.29 ± 0.65 vs 1.07 ± 0.57 mg/L, $P < .0004$), with and without a history of CHD (1.35 ± 0.54 vs 1.20 ± 0.65 mg/L, $P = .0215$), with and without diabetic retinopathy (1.34 ± 0.71 vs 1.11 ± 0.53 mg/L, $P < .0001$), and with and without peripheral neuropathy (1.35 ± 0.73 vs 1.16 ± 0.57 mg/L, $P = .0053$). Cystatin C level was significantly higher in subjects with longer duration of diabetes (1.48 ± 0.75 mg/L, ≥20 years [n = 134]) than in subjects with shorter duration of diabetes (1.12 ± 0.45 mg/L, 10-20 years [n = 153]; 1.12 ± 0.62 mg/L, <10 years [n = 191]) (P for trend < .0001). However, no significant differences were observed based on sex, obesity (by BMI cutoff of 25 kg/m^2 or as a continuous variable), or use of medications.

3.2. The association between cystatin C level and insulin resistance

After adjusting for age, sex, BMI, and eGFR, cystatin C level increased significantly in proportion to the number of

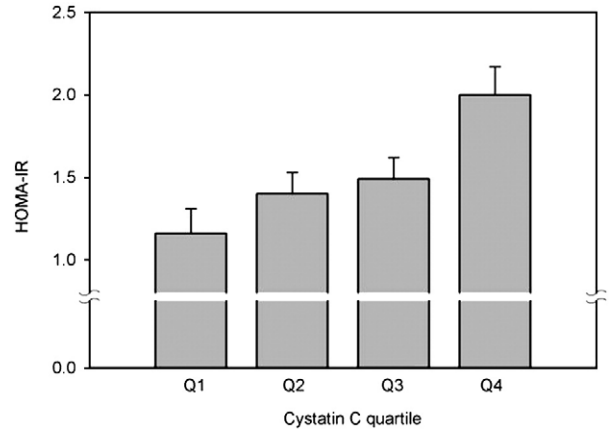

Fig. 3. The HOMA-IR according to the cystatin C quartile. Data are expressed as means ± SE. Adjusted for age, sex, BMI, and MDRD-GFR. Cut points for cystatin C quartiles are less than 0.82, 0.82 to 1.05, 1.05 to 1.40, and at least 1.40 mg/L. P for trend < .0001.

Table 2
Correlations between various biomarkers associated with inflammation or CVDs

	Fibrinogen	Uric acid	hs-CRP[a]	Homocysteine	Lipoprotein(a)	Cystatin C	MDRD-GFR
ACR[a]	0.317	0.276	0.243	0.248	0.181	0.324	−0.322
	<.0001	<.0001	<.0001	<.0001	.0002	<.0001	<.0001
Fibrinogen		0.197	0.512	0.044	0.216	0.381	−0.265
		<.0001	<.0001	.357	<.0001	<.0001	<.0001
Uric acid			0.089	0.280	0.076	0.556	−0.511
			.058	<.0001	.108	<.0001	<.0001
hs-CRP[a]				0.011	0.105	0.258	−0.173
				.822	.026	<.0001	.0002
Homocysteine					0.001	0.315	−0.227
					.980	<.0001	<.0001
Lipoprotein(a)						0.180	−0.167
						.0001	.0003

Upper row indicates r, lower row indicates P value.
[a] Data from Spearman correlation analysis.

MetS components present (1.08 ± 0.06, 1.19 ± 0.04, 1.20 ± 0.04, 1.23 ± 0.04, and 1.37 ± 0.06 mg/L; P for trend < .0001) (Fig. 2); and HOMA-IR increased significantly in proportion to cystatin C quartiles (1.16 ± 0.15, 1.40 ± 0.13, 1.49 ± 0.13, and 2.00 ± 0.17; P for trend < .0001) (Fig. 3) (means ± SE).

3.3. The association between cystatin C and various biomarkers

Significant correlations between various biomarkers known to be CVD risk factors were noted (Table 2). The correlation coefficients between cystatin C and other biomarkers were generally higher than those between other biomarkers. Cystatin C was strongly correlated with ACR (r = 0.324, P < .0001), fibrinogen (r = 0.381, P < .0001), uric acid (r = 0.556, P < .0001), and homocysteine (r = 0.315, P < .0001), and moderately correlated with hs-CRP (r = 0.258, P < .0001) and lipoprotein(a) (r = 0.180, P = .0001). The MDRD-GFR also showed significant correlations with all of these biomarkers, but the correlation coefficients were generally lower than those between cystatin C and the biomarkers. The MDRD-GFR was strongly correlated with ACR (r = −0.322, P < .0001) and uric acid (r = −0.511, P < .0001), and moderately correlated with fibrinogen (r = −0.265, P < .0001), hs-CRP (r = −0.173, P = .0002), homocysteine (r = −0.227, P < .0001), and lipoprotein(a) (r = −0.167, P = .0003). The levels of these biomarkers significantly increased in proportion to cystatin C quartiles (Table 3). This trend was maintained in the subgroup analysis of subjects with MetS, whereas the positive relationship between hs-CRP, lipoprotein(a), and cystatin C level was lost in the subjects without MetS (data not shown).

3.4. The independent association between biomarkers, insulin resistance, and cystatin C

After adjusting for age, sex, BMI, MDRD-GFR, hemoglobin A_{1c} (HbA_{1c}), duration of diabetes, systolic BP, smoking status, hemoglobin, and HDL cholesterol level, uric acid (β = 0.0666, P < .0001), ACR (β = 0.0004, P < .0001), homocysteine (β = 0.0087, P = .0004), and HOMA-IR (β = 0.0380, P = .0082) showed independent associations with cystatin C level by multiple regression analysis (Table 4). However, MDRD-GFR had the most powerful association with cystatin C (β = −0.0089, P < .0001, partial R^2 = 0.537).

4. Discussion

The known functions of cystatin C are expanding. It is not only regarded as a marker for renal dysfunction, but is also recognized as a marker for MetS [21,22], arterial stiffness [23], subclinical atherosclerosis [24,25], glucose intolerance [26], CVD, and mortality [6-11]. However, whether this is merely a reflection of its sensitivity to renal dysfunction or whether there are other pathways involved is not clear. Because there is ample evidence that renal insufficiency is an

Table 3
The levels of various biomarkers associated with inflammation or CVDs by cystatin C quartile

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P
ACR	17.0 (8.4-42.8)	12.9 (5.3-36.4)	16.4 (7.8-41.0)	96.9 (22.9-378.6)	<.0001
Fibrinogen	288.0 ± 9.5	294.0 ± 8.3	305.4 ± 8.1	348.8 ± 10.6	<.0001
Uric acid	4.59 ± 0.15	4.50 ± 0.13	4.85 ± 0.12	6.03 ± 0.16	<.0001
hs-CRP	0.08 (0.04-0.23)	0.09 (0.05-0.29)	0.11 (0.05-0.42)	0.29 (0.09-1.42)	<.0001
Homocysteine	9.90 ± 0.76	10.43 ± 0.65	13.40 ± 0.64	15.71 ± 0.83	<.0001
Lipoprotein(a)	22.4 ± 3.0	25.4 ± 2.6	28.3 ± 2.5	31.7 ± 3.3	.0006

Data are expressed as means ± SE or median (25th-75th percentiles). Adjusted for age, sex, BMI, and MDRD-GFR. Cut points for cystatin C quartiles are less than 0.82, 0.82 to 1.05, 1.05 to 1.40, and at least 1.40 mg/L.

Table 4
Variables independently associated with cystatin C

	β	P	Partial R^2
ACR	0.0004	<.0001	0.078
Uric acid	0.0666	<.0001	0.043
Homocysteine	0.0087	.0004	0.013
HOMA-IR	0.0380	.0082	0.007

β refers to regression coefficients for a value 1 unit higher in each variable. This model is adjusted for age, sex, BMI, MDRD-GFR, HbA_{1c}, diabetes mellitus duration, systolic BP, smoking, hemoglobin, and HDL cholesterol.

independent risk factor for CVD and mortality [12,13], we investigated whether other factors are also involved in the link between cystatin C and CVD.

Our data show a close association of cystatin C with insulin resistance in type 2 diabetes mellitus patients. The level of cystatin C was higher in patients with MetS and increased as a function of the number of MetS components even after adjusting for age, sex, BMI, and MDRD-GFR. This result is very similar to the report by Servais et al [21], which included 925 dyslipidemic subjects including 11% diabetic patients. Although the degree of insulin resistance and the number of MetS components do not have an exact proportional relationship, we can presume their intimacy from the information that insulin resistance is the main pathogenic factor of MetS. More directly, HOMA-IR increased in proportion to cystatin C quartiles and showed an independent association with cystatin C by multiple regression analysis. Uric acid, which is known to be closely associated with insulin resistance [27,28], was also independently associated with cystatin C. Because insulin resistance is an important element in the development of CVD, these findings suggest that insulin resistance may have an additional role in the link between cystatin C and CVD, although the cause-and-effect relationship is as yet unclear.

Several studies have elucidated the linear association of cystatin C and inflammatory or procoagulatory biomarkers. In the Cardiovascular Health Study, cystatin C was correlated with CRP and fibrinogen across all levels of renal function, suggesting an association even in persons with mild to moderate renal dysfunction [18]. This indicates that mild renal dysfunction could make for adverse consequences through activating inflammatory pathways. Similar results were shown in the Multi-Ethnic Study of Atherosclerosis, which demonstrated a partial correlation between cystatin C and multiple biomarkers of inflammation and procoagulation including CRP, interleukin-6, tumor necrosis factor–α soluble receptor 1, intercellular adhesion molecule–1, fibrinogen, and factor VIII [29]. This was also relevant to participants both with and without chronic kidney disease. In the Heart and Soul Study, the data from 990 patients with coronary artery disease showed moderate associations between cystatin C and CRP or fibrinogen [19]. However, these associations disappeared after adjusting for 24-hour creatinine clearance. Our data from type 2 diabetes mellitus patients describe the correlation between various biomarkers associated with CVD. In particular, cystatin C was significantly positively correlated with ACR, fibrinogen, uric acid, hs-CRP, homocysteine, and lipoprotein(a). These biomarkers were more strongly correlated with cystatin C than with MDRD-GFR. The main difference in our results from those mentioned above is that the independent association between cystatin C and uric acid, ACR, and homocysteine remained significant even after adjusting for eGFR. These findings suggest that inflammatory pathways may also have a role in mediating the link between cystatin C and CVD. Similar findings were observed by Knight and colleagues [30] who showed an independent association of cystatin C with CRP after adjusting for creatinine clearance. This could be more evidence of cystatin C having a link to inflammatory pathways. However, many researchers prefer to link cystatin C to CVD within its relationship to renal function. Understanding the pathophysiologic role of cystatin C will give us a clue in solving this complex connection.

Analysis of the levels of CVD-associated variables showed that patients within the fourth cystatin C quartile group had significantly increased levels of these biomarkers. This finding indicates the importance of intensive risk management especially in patients with the highest quartile of cystatin C. Previous reports also emphasized the increase in mortality or cardiovascular events, which at least doubled in persons who belonged to the highest quartile or quintile for cystatin C [6,7,9]. In patients with type 2 diabetes mellitus, which is now regarded as a CHD-risk equivalent, serum cystatin C might have an additional role in selecting a group at higher risk of CVD. However, data on the normal range of cystatin C levels in the general population are insufficient; and controversy exists on the factors influencing cystatin C level, although a few articles have discussed these topics [1,30-32]. In basing further research on these arguments, it would be important to set the cutoff value of cystatin C where the risk of CVD starts to rise steeply.

This study has several limitations. Because it is a cross-sectional study, the impact of cystatin C level on actual CVD events could not be observed. Because our subjects were restricted to inpatients with relatively longer duration of diabetes and uncontrolled glycemic status, they may have somewhat different characteristics than the general type 2 diabetes mellitus population. In addition, the methods of measuring insulin resistance and GFR were not the criterion standard. To overcome these limitations and thoroughly understand the role of cystatin C, population-based prospective studies and basic research should be conducted.

In conclusion, cystatin C was significantly associated with insulin resistance and biomarkers reflecting inflammation, independent of renal function. These components may have a role additional to that of eGFR in explaining the link between cystatin C and CVD in type 2 diabetes mellitus patients.

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