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Increased serum cystatin C levels and responses of pancreatic α - and β -cells in type 2 diabetes

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Abstract

Background: Increased serum cystatin C (CysC) can predict the onset of type 2 diabetes (T2D). Meanwhile, impaired pancreatic α - and β -cell functions get involved in the pathophysiological processes of T2D. So this study was to explore the relationships between serum CysC levels and pancreatic α - and β -cell functions in T2D.

Methods: In this cross-sectional observational study, a total of 2634 patients with T2D were consecutively recruited. Each recruited patient received a serum CysC test and oral glucose tolerance test for synchronous detection of serum C-peptide and plasma glucagon. As components of pancreatic β -cell function, insulin secretion and sensitivity indices were evaluated by C-peptide area under the curve (AUC-CP) and C-peptide-substituted Matsuda's index (Matsuda-CP), respectively. Fasting glucagon (F-GLA) and post-challenge glucagon calculated by glucagon area under the curve (AUC-GLA) were used to assess pancreatic α -cell function. These skewed indices and were further natural log-transformed (ln).

Results: With quartiles of serum CysC levels ascending, AUC-CP, F-GLA and AUC-GLA were increased, while Matsuda-CP was decreased (P for trend <0.001). Moreover, serum CysC levels were positively related to lnAUC-CP, lnF-GLA and lnAUC-GLA ($r = 0.241, 0.131$ and 0.208 , respectively, $P < 0.001$), and inversely related to lnMatsuda-CP ($r = -0.195$, $P < 0.001$). Furthermore, after controlling for other relevant variables via multivariable linear regression analysis, serum CysC levels were identified to account for lnAUC-CP ($\beta = 0.178$, $t = 10.518$, $P < 0.001$), lnMatsuda-CP ($\beta = -0.137$, $t = -7.118$, $P < 0.001$), lnF-GLA ($\beta = 0.049$, $t = 2.263$, $P = 0.024$) and lnAUC-GLA ($\beta = 0.121$, $t = 5.730$, $P < 0.001$).

Conclusions: Increased serum CysC levels may be partly responsible for increased insulin secretion from β -cells, decreased systemic insulin sensitivity, and elevated fasting and postprandial glucagon secretion from α -cells in T2D.

Key Words

- ▶ cystatin C
- ▶ C-peptide
- ▶ glucagon
- ▶ type 2 diabetes

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Introduction

In the last few decades, type 2 diabetes (T2D) is increasingly recognized as a serious, worldwide public health concern, and this has attracted a surge of interests in the pathogenesis of T2D. The critical pathogenesis is mainly due to inadequate compensatory insulin secretion from pancreatic β -cells when they counteract insulin resistance (1, 2). In addition, numerous findings highlighted that abnormal α -cell secretion may take part in the progression and exacerbation of glycemic disturbances, which is featured by fasting hyperglucagonemia and reduced suppression of glucagon after food intake (3, 4). Thus, both pancreatic α - and β -cell dysfunctions get involved in the pathophysiological processes of T2D (5). Currently, much effort is being made to seek modifiable risk factors of pancreatic α - and β -cell dysfunctions, which may help orientate the formulation of appropriate and effective treatment strategies to ameliorate diabetes and subsequent diabetes-related prognosis.

Cystatin C (CysC) is small molecular weight protein of approximately 13.3 kDa that is synthesized and secreted by all nucleated cells in every human tissue, including kidney, liver, pancreas, intestine, etc. (6, 7). Due to its free filtration through the glomerulus and then complete reabsorption and degradation by proximal tubular cells without secretion (8, 9), serum CysC is considered as a sensitive biomarker for early kidney dysfunction. In addition to be an ubiquitously expressed measuring substance, CysC has been recognized as a functional protein that directly linked to many pathophysiological processes through multiple mechanisms. It is involved in immunological regulation (antigen procession, cytokines synthesis and apoptosis), autophagy, bone remodeling, atherosclerosis, tumor metastasis, as well as roles in inflammation and cerebral amyloid angiopathy (10, 11).

Actually, CysC is a disease-associated protein, and alteration in CysC levels may suggest important clinical implications. Serum CysC levels were reported to be associated, in a dose-dependent manner, with an increased risk of coronary artery diseases, cerebrovascular accidents and mortality from all causes in the general population (12, 13, 14). Moreover, increased serum CysC levels have been well established to account for common complications in diabetic population, such as diabetic kidney disease (15), diabetic retinopathy (16), diabetic peripheral neuropathy (17), diabetic foot ulceration (18) and cardiovascular diseases (19, 20). Additionally, there is accumulative evidence that elevated serum CysC levels are responsible for incidence of metabolic syndrome and T2D (21, 22, 23).

Those metabolic diseases and their related complications are always accompanied by a background of pancreatic α - and β -cell dysfunctions. Therefore, it is reasonable to speculate that CysC overexpression may be central to the T2D pathogenesis. However, no relevant literature has systematically investigated the associations of serum CysC levels with pancreatic α - and β -cell functions in T2D.

Therefore, the present study is performed to explore the relationship between increased serum CysC levels and responses of pancreatic α - and β -cells in T2D.

Methods

Study design and patient recruitment

The present study is a part of the Diabetes Clinical Research Center Project that authorized and funded by the Nantong Science and Technology Bureau. We used a cross-sectional observational design to conduct this study. The study design was reviewed and approved by the Human Study Review Committee of Affiliated Hospital 2 of Nantong University. At the recruitment stage, we placed a notification at the Endocrinology Department of our hospital to recruit patients for this study from January 2016 to February 2021. Eligible patients were between 25 and 75 years of age, diagnosed with T2D according to the reference published by American Diabetes Association in 2015 (24). Patients would be excluded if they had the following conditions: (i) presence of diabetes-associated autoantibodies; (ii) previous malignancies; (iii) severe cardiovascular diseases, such as myocardial infarction; (iv) ischemic and hemorrhagic stroke; (v) chronic liver diseases, such as viral hepatitis and alcoholic hepatitis; (vi) chronic kidney diseases, and estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m²; (vii) hyperthyroidism or hypothyroidism; (viii) current treatment with systemic corticosteroids; (ix) recent use of glucose cotransporter 2 inhibitors (SGLT-2Is); (x) connective tissue diseases. At last, complete data from 2634 eligible patients were qualified for this cross-sectional study. The study conduction was adhered to the Declaration of Helsinki involving research of human subjects, and all patients signed an informed consent when admitted to the study.

Clinical data collection

Experienced physicians were trained to collect clinical data from all patients. These data included demographic data (such as age, sex and blood pressure), medical history (such

as diabetes duration, history of hypertension and smoking), prescription information (such as glucose-lowering therapies and statins treatments), and biochemical measurements. Glucose-lowering therapies were acquired by searching the electronic medical record system and then were categorized into subclasses, which included insulin injections, sulfonylureas (SUs), metformin, thiazolidinediones (TZDs), α -glucosidase inhibitors (AGIs), dipeptidyl peptidase-4 inhibitors (DPP-4Is), SGLT-2Is and glucagon-like peptide-1 receptor agonists (GLP-1RAs).

Fasting venous blood samples were taken to detect biochemical indices, such as serum CysC levels, hepatic function index, creatinine, uric acid (UA), lipid profiles, whole blood glycosylated hemoglobin A1c (HbA1c), etc. The serum CysC was measured by latex-enhanced immunoturbidimetry in an automated biochemical analyzer (Model 7600, Hitachi). The renal function index, eGFR, was assessed by the equation from the Modification of Diet in Renal Disease study (25), that is eGFR_M.

Assessment of pancreatic α - and β -cell functions

Each patient was undergone an oral glucose tolerance test using 75 g anhydrous glucose early in the morning under fasting status. Venous blood samples were drawn at 0, 30, 60, 120, and 180 min for synchronous detection of serum glucose, serum C-peptide and plasma glucagon. Insulin was substituted by C-peptide in the β -cell function indices to avoid interference by exogenous insulin. As components of pancreatic β -cell functions, β -cell secretion and insulin sensitivity indices were evaluated by C-peptide area under curve (AUC-CP) (26) and C-peptide-substituted Matsuda's index (Matsuda-CP) (27), respectively. Fasting glucagon (F-GLA) and post-challenge glucagon calculated by glucagon area under the curve (AUC-GLA) were applied to evaluate pancreatic α -cell function. C-peptide was measured with the chemiluminescence in an immunoassay analyzer (DxI 800, Beckman Coulter), and glucagon was measured with the RIA in an automated γ -counter (GC-1200, USTC Zonkia).

Statistical analysis

Clinical variables of the patients are presented for the total and four subgroups of first, second, third and fourth quartile (Q1, Q2, Q3 and Q4) of serum CysC levels (Table 1). Descriptive statistics for the data, including mean with s.d., median with 25–75% interquartile range, and frequency with percentage, were performed according to the data type and distribution. Islet α - and β -cell

function indices were non-normally distributed data, and were natural-logarithm transformed, such as lnAUC-CP, lnMatsuda-CP, lnF-GLA and lnAUC-GLA. One-way ANOVA with linear polynomial contrasts, Jonckheere-Terpstra test and chi-squared test with linear-by-linear association were performed to assess the trends of corresponding data type in four subgroups.

Moreover, we applied Pearson's correlation analysis to assess the correlation of serum CysC levels with pancreatic α - and β -cell function indices (Fig. 1). Considering that HbA1c, eGFR_M and glucose-lowering therapies may have impacts on these correlations, the partial correlation analysis was applied to achieve the actual associations of serum CysC levels with lnAUC-CP, lnMatsuda-CP, lnF-GLA and lnAUC-GLA by adjusting for HbA1c, eGFR_M and glucose-lowering therapies (Fig. 2). Furthermore, we applied multivariable linear regression analysis to determine whether serum CysC levels had an independent effect on pancreatic α - and β -cell function indices (lnAUC-CP, lnMatsuda-CP, lnF-GLA and lnAUC-GLA) by gradually adjusting effects of other clinically relevant variables in Model 1, Model 2 and Model 3 (Table 2).

We used standard version of SPSS 19.0 for Windows (IBM Co.) to input and analyze the clinical variables. During statistical analysis, statistical significance was identified if *P* value less than 0.05.

Results

Clinical characteristics of patients

Table 1 has displayed the clinical characteristics of the patients with T2D. The serum CysC levels of all recruited patients were 0.93 ± 0.36 mg/L, with a range of 0.1–4.5 mg/L. The ranges of the serum CysC quartiles were 0.1–0.7 mg/L (Q1), 0.8–0.9 mg/L (Q2), 1.0–1.1 mg/L (Q3) and 1.2–4.5 mg/L (Q4), respectively. From Q1, Q2, Q3 to Q4 of serum CysC levels, AUC-CP, F-GLA and AUC-GLA were increased, while Matsuda-CP was decreased (*P* for trend <0.001). Moreover, with ascending quartiles of serum CysC levels, age, BMI, systolic blood pressure, diabetic duration, aspartate aminotransferase, triglycerides, UA, hypertension prevalence and statins treatments were significantly increased, while the ratio of female, total cholesterol, HDL, eGFR_M and HbA1c were decreased, but diastolic blood pressure, alanine aminotransferase and LDL did not exhibit any difference between the quartiles of CysC levels. As to the glucose-lowering therapies, lifestyle intervention alone and frequency of AGIs taken were

Table 1 Clinical characteristics of the recruited patients.

Variables	Total	Quartiles of serum cystatin C				Test statistic	P for trend
		Q1	Q2	Q3	Q4		
Serum cystatin C (mg/L) (range)	0.93 ± 0.36 (0.1–4.5)	0.58 ± 0.13 (0.1–0.7)	0.85 ± 0.05 (0.8–0.9)	1.05 ± 0.50 (1.0–1.1)	1.45 ± 0.39 (1.2–4.5)	–	–
n	2634	746	810	560	518	–	–
Age (year)	53.3 ± 9.7	49.3 ± 8.9	52.2 ± 9.1	55.1 ± 9.3	59.0 ± 9.0	128.3 ^a	<0.001
Female, n (%)	1457 (44.7)	387 (51.9)	334 (41.2)	224 (40.0)	232 (44.8)	8.095 ^c	0.004
BMI (kg/m ²)	25.4 ± 3.7	25.0 ± 3.7	25.3 ± 3.6	25.6 ± 3.7	25.9 ± 4.0	6.336 ^a	<0.001
SBP (mmHg)	135.7 ± 18.0	133.1 ± 16.5	135.0 ± 18.1	137.0 ± 17.9	139.1 ± 19.0	13.17 ^a	<0.001
DBP (mmHg)	77.5 ± 11.4	81.2 ± 33.2	79.3 ± 10.7	80.4 ± 31.3	77.5 ± 11.4	2.566 ^a	0.053
Diabetic duration (years)	5 (1–10)	3 (3–8)	4 (1–4)	5 (1–10)	8 (2–10)	8.509 ^b	<0.001
Glucose-lowering therapies							
Lifestyle alone, n (%)	273 (10.4)	66 (8.8)	70 (8.6)	67 (12.0)	70 (13.5)	9.702 ^c	0.002
Insulin injections, n (%)	1427 (54.2)	415 (55.6)	432 (53.3)	298 (53.2)	282 (54.4)	0.236 ^c	0.627
SUs, n (%)	1285 (48.8)	355 (47.6)	420 (51.9)	281 (50.2)	229 (44.2)	1.213 ^c	0.271
MET, n (%)	1397 (53.0)	432 (57.9)	441 (54.4)	293 (52.3)	231 (44.6)	21.23 ^c	<0.001
TZDs, n (%)	787 (29.9)	245 (32.8)	248 (30.6)	164 (29.3)	130 (25.1)	8.640 ^c	0.003
AGIs, n (%)	617 (23.4)	162 (21.7)	179 (22.1)	134 (23.9)	142 (27.4)	5.812 ^c	0.016
DPP-4Is, n (%)	686 (26.0)	211 (28.3)	221 (27.3)	133 (23.8)	121 (23.4)	5.504 ^c	0.019
GLP-1RAs, n (%)	73 (2.8)	29 (3.9)	8 (1.0)	23 (4.1)	13 (2.5)	0.151 ^c	0.697
Hypertension, n (%)	1121 (42.6)	224 (30.0)	326 (40.2)	262 (46.8)	309 (59.7)	114.1 ^c	<0.001
Statins treatments, n (%)	1011 (38.4)	201 (26.9)	294 (36.3)	230 (41.1)	286 (55.2)	17.65 ^c	<0.001
ALT (U/L)	17 (12–27)	17 (12–27)	19 (13–28)	18 (12–29)	17 (12–25)	-0.218 ^b	0.828
AST (U/L)	17 (14–22)	16 (13–21)	16 (14–22)	17 (14–23)	17 (14–22)	3.975 ^b	<0.001
TG (mmol/L)	1.68 (1.10–2.73)	1.67 (1.08–2.83)	1.58 (1.04–2.59)	1.74 (1.12–2.72)	1.78 (1.18–2.89)	2.223 ^b	0.026
TC (mmol/L)	4.48 ± 1.11	4.58 ± 1.02	4.48 ± 1.30	4.44 ± 0.94	4.40 ± 1.08	2.908 ^a	0.033
HDLc (mmol/L)	1.08 ± 0.29	1.11 ± 0.30	1.10 ± 0.29	1.06 ± 0.27	1.04 ± 0.30	7.341 ^a	<0.001
LDLc (mmol/L)	2.50 ± 0.83	2.60 ± 0.75	2.57 ± 0.82	2.58 ± 0.74	2.51 ± 0.83	1.462 ^a	0.223
UA (μ mol/L)	348.1 ± 106.7	269.6 ± 88.0	291.9 ± 93.0	311.6 ± 92.4	348.1 ± 106.7	75.37 ^a	<0.001
eGFR _M (mL/min/1.73m ²)	123.8 ± 22.7	136.2 ± 23.7	126.7 ± 19.0	119.0 ± 18.2	106.7 ± 18.7	232.6 ^a	<0.001
HbA1c (%)	9.10 ± 2.31	9.58 ± 2.48	8.96 ± 2.20	8.95 ± 2.20	8.78 ± 2.25	14.88 ^a	<0.001
AUC-CP (ng/mL·h)	8.46 (5.36–12.96)	7.04 (4.28–10.53)	8.34 (5.33–12.31)	9.14 (6.04–13.63)	11.56 (6.83–17.60)	13.18 ^b	<0.001
Matsuda-CP	570.56 (390.0–870.8)	663.2 (457.7–986.4)	581.3 (412.0–875.8)	538.8 (381.5–788.6)	454.8 (316.4–725.9)	-11.36 ^b	<0.001
F-GLA (pg/mL)	123.4 (88.2–154.2)	116.28 (85.0–142.9)	121.9 (88.4–150.7)	125.0 (80.8–154.4)	140.33 (99.8–178.4)	7.694 ^b	<0.001
AUC-GLA (pg/mL·h)	347.3 (284.1–445.1)	315.6 (267.0–397.6)	347.1 (289.7–444.9)	350.6 (386.4–452.1)	392.0 (321.3–506.8)	10.32 ^b	<0.001
In AUC-CP (ng/mL·h)	2.09 ± 0.70	1.86 ± 0.71	2.05 ± 0.66	2.18 ± 0.63	2.38 ± 0.58	66.69 ^a	<0.001
In Matsuda-CP	6.39 ± 0.58	6.55 ± 0.57	6.42 ± 0.56	6.32 ± 0.55	6.18 ± 0.58	47.32 ^a	<0.001
In F-GLA (pg/mL)	4.70 ± 0.57	4.63 ± 0.56	4.68 ± 0.55	4.67 ± 0.61	4.84 ± 0.57	14.79 ^a	<0.001
In AUC-GLA (pg/mL·h)	5.87 ± 0.43	5.77 ± 0.41	5.86 ± 0.41	5.88 ± 0.45	6.01 ± 0.44	32.34 ^a	<0.001

^aLinear polynomial contrasts of ANOVA; ^bJonckheere-Terpstra test; ^clinear-by-linear association of chi-squared test were performed to detect the trends of corresponding data type in four subgroups. AGIs: α -glucosidase inhibitors; AUC-CP: C-peptide area under curve; AUC-GLA: glucagon area under curve; DPP-4Is: dipeptidyl peptidase-4 inhibitors; F-GLA: Fasting glucagon; GLP-1RAs: glucagon-like peptide-1 receptor agonists; Matsuda-CP: C-peptide-substituted Matsuda's index; MET: metformin; SGLT-2Is: glucose cotransporter-2 inhibitors; SUs: sulfonylureas; TZDs: thiazolidinediones.



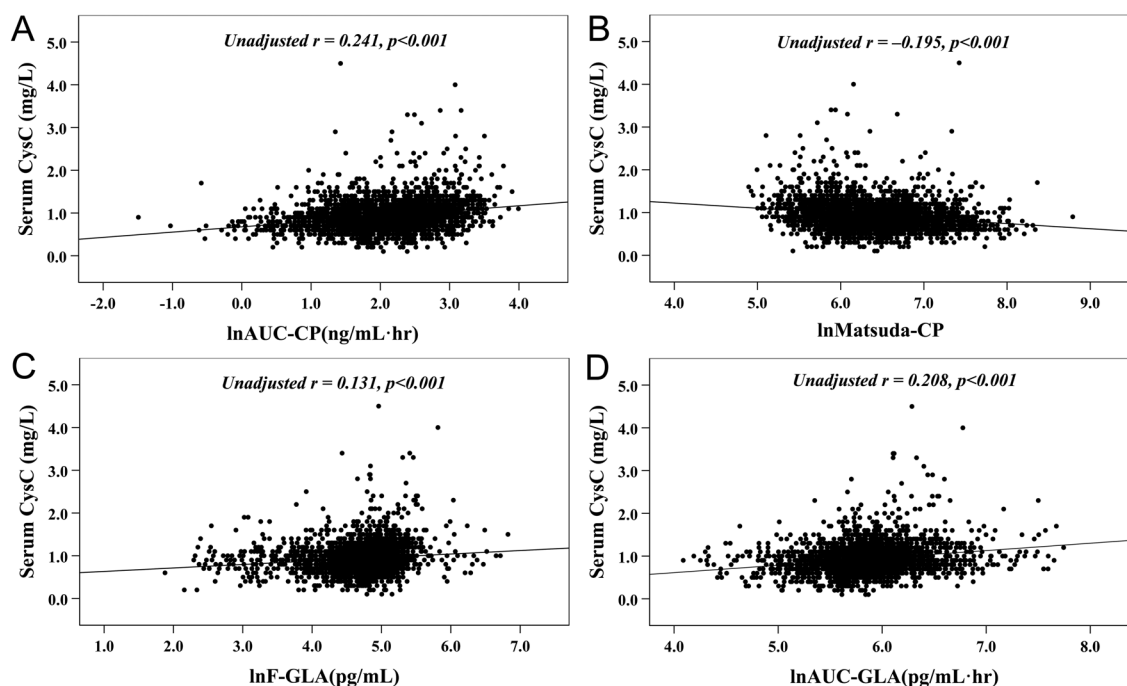


Figure 1

Scatter plots for relationships between serum CysC levels and pancreatic α - and β -cell function indices (A) lnAUC-CP; (B) lnMatsuda-CP; (C) lnF-GLA; (D) lnAUC-GLA.

increased, while MET, TZDs and DPP-4Is were decreased, when the quartiles of serum CysC increased; but insulin injections, SUs and GLP-1RAs were comparable between the quartiles of CysC levels.

Correlations between serum CysC levels and indices of α - and β -cell functions

Pearson's correlation analysis showed that lnAUC-CP, lnF-GLA and lnAUC-GLA had positive correlations with serum CysC levels ($r = 0.241, 0.131$ and 0.208 , respectively, $P < 0.001$), while lnMatsuda-CP was in negative correlation with serum CysC levels ($r = -0.195, P < 0.001$). And graphic representation of the relationships is shown in Fig. 1. Additionally, after controlling for the impacts of HbA1c, eGFR_M and glucose-lowering therapies by the partial correlation analyses, lnAUC-CP, lnF-GLA and lnAUC-GLA still remained positively related to serum CysC levels ($r = 0.247, 0.138$ and 0.183 , respectively, $P < 0.001$), and lnMatsuda-CP still remained negatively related to serum CysC levels ($r = -0.185, P < 0.001$). Graphic representation of the relationships is also shown in Fig. 2.

Considering the close correlation between serum CysC and kidney function, we made a partial correlation analysis to adjust for eGFR only. We found that serum

CysC levels still remained associated with AUC-CP, Matsuda-CP, F-GLA and AUC-GLA ($r = 0.181, -0.147, 0.128$ and 0.177 , respectively, $P < 0.001$) after adjusting for eGFR (Supplementary Fig. 1, see section on [supplementary materials](#) given at the end of this article).

Among the recruited T2D patients, 10.4% ($n = 273$) were lifestyle intervention alone (without antidiabetic agents). When we restricted our analysis in these T2D patients without antidiabetic agents ($n = 273$), we found serum CysC levels were correlated with AUC-CP ($r = 0.273, P < 0.001$), Matsuda-CP ($r = -0.277, P < 0.001$), and AUC-GLA ($r = 0.227, P < 0.001$), but not F-GLA ($r = 0.086, P = 0.156$) (Supplementary Fig. 2). Furthermore, after adjusting for eGFR_M by the partial correlation analysis, we found serum CysC levels were correlated with all α - and β -cell function indices in these T2D patients without antidiabetic agents, that is, AUC-CP ($r = 0.266, P < 0.001$), Matsuda-CP ($r = -0.269, P < 0.001$), F-GLA ($r = 0.129, P = 0.033$) and AUC-GLA ($r = 0.214, P < 0.001$) (Supplementary Fig. 3).

Analyses to explore the effects of serum CysC levels on outcomes of α - and β -cell function indices

Table 2 exhibited the independent effects of serum CysC levels on consequences of pancreatic β -cell function

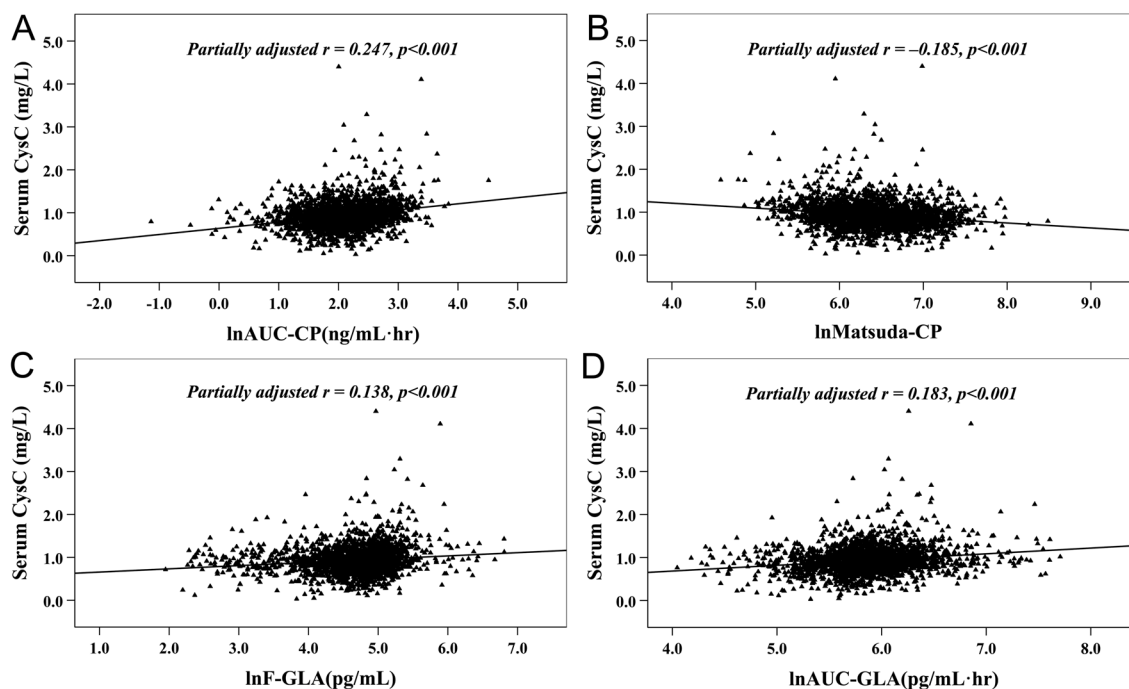


Figure 2

Scatter plots for relationships between serum CysC levels and pancreatic α - and β -cell function indices partially adjusted for HbA1c, eGFR_M and glucose-lowering therapies (A) lnAUC-CP; (B) lnMatsuda-CP; (C) lnF-GLA; (D) lnAUC-GLA.

(lnAUC-CP and lnMatsuda-CP) and α -cell function (lnF-GLA and lnAUC-GLA) by multivariable linear regression analysis. In the crude Model 0, serum CysC levels were significantly associated with lnAUC-CP ($\beta = 0.241$, $t = 12.747$, $P < 0.001$ and $R^2 = 0.058$), lnMatsuda-CP ($\beta = -0.195$, $t = -10.206$, $P < 0.001$ and $R^2 = 0.038$), lnF-GLA ($\beta = 0.131$, $t = 6.782$, $P < 0.001$ and $R^2 = 0.017$), lnAUC-GLA ($\beta = 0.208$, $t = 10.921$, $P < 0.001$ and $R^2 = 0.043$). After controlling for other clinical relevant variables in model 1, model 2 and model 3, the adjusted R^2 was exhibited to be progressively increased from model 0 to model 3. In the completely adjusted Model 3, serum CysC levels were exhibited to remain independently account for lnAUC-CP ($\beta = 0.178$, $t = 10.518$, $P < 0.001$, partial $R^2 = 2.89\%$ and total $R^2 = 54.7\%$), lnMatsuda-CP ($\beta = -0.137$, $t = -7.118$, $P < 0.001$, partial $R^2 = 2.16\%$ and total $R^2 = 41.3\%$), lnF-GLA ($\beta = 0.049$, $t = 2.263$, $P = 0.024$, partial $R^2 = 0.24\%$, and total $R^2 = 30.1\%$), lnAUC-GLA ($\beta = 0.121$, $t = 5.730$, $P < 0.001$, partial $R^2 = 1.46\%$ and total $R^2 = 32.8\%$).

Therefore, after adjusting for other clinical relevant variables, serum CysC levels may independently explain 2.89% variation of AUC-CP, explain 2.16% variation of Matsuda-CP, explain 0.24% variation of F-GLA, and 1.46% variation of AUC-GLA. Insulin secretion index, AUC-CP, may be more involved in the serum concentration of CysC when compare to other α - and β -cell function indices.

Discussion

In the present study, we explore the relationship between serum CysC levels and pancreatic α - and β -cell functions in 2634 patients with T2D. The main findings of our study were as follows: first, with quartiles of serum CysC levels ascending, AUC-CP, F-GLA and AUC-GLA were increased, while Matsuda-CP was decreased; second, after controlling for other various clinical variables, serum CysC levels were positively and independently responsible for AUC-CP, F-GLA and AUC-GLA, and negatively and independently responsible for Matsuda-CP; third, serum CysC levels may independently explain 2.89% variation of AUC-CP, explain 2.16% variation of Matsuda-CP, explain 0.24% variation of F-GLA, and 1.46% variation of AUC-GLA; fourth, insulin secretion index (AUC-CP) may be more involved in the serum concentration of CysC when compare to other α - and β -cell function indices. In brief, serum CysC levels are closely connected to pancreatic α -cell and β -cell dysfunctions.

CysC serves as a functional protein that directly plays pleiotropic roles in many pathophysiological processes in the human body, let alone a marker for early kidney dysfunction. There are always two-sided natures of everything, and this is also true for CysC levels. On one hand, increased serum CysC levels have

Table 2 Multivariable linear regression models exhibiting the effects of serum CysC levels on outcomes of pancreatic α - and β -cell function.

Models	B (95% CI)	β	t	P	Partial R ² for CysC (%)	Total R ² for model (%)
In AUC-CP						
Model 0: crude	0.472 (0.399 to 0.544)	0.241	12.747	<0.001	5.81	5.81
Model 1: age, sex, BMI, SBP, DBP, diabetes duration, hypertension and statins	0.494 (0.422 to 0.565)	0.253	13.579	<0.001	6.60	22.0
Model 2: model 1 + ALT, AST, lipid profiles, UA, eGFR _M , F-GLA and AUC-GLA	0.322 (0.242 to 0.403)	0.160	7.856	<0.001	2.46	28.3
Model 3: model 2 + HbA1c and glucose-lowering therapies	0.357 (0.290 to 0.423)	0.178	10.518	<0.001	2.89	54.7
In Matsuda-CP						
Model 0: crude	-0.317 (-0.378 to -0.256)	-0.195	-10.206	<0.001	3.81	3.81
Model 1: age, sex, BMI, SBP, DBP, diabetes duration, hypertension and statins	-0.318 (-0.378 to -0.258)	-0.196	-10.328	<0.001	3.96	19.5
Model 2: model 1 + ALT, AST, lipid profiles, UA, eGFR _M , F-GLA and AUC-GLA	-0.191 (-0.259 to -0.123)	-0.114	-5.504	<0.001	1.21	26.4
Model 3: model 2 + HbA1c and glucose-lowering therapies	-0.231 (-0.294 to -0.167)	-0.137	-7.118	<0.001	2.16	41.3
In F-GLA						
Model 0: crude	0.211 (0.150 to 0.272)	0.131	6.782	<0.001	1.72	1.72
Model 1: age, sex, BMI, SBP, DBP, diabetes duration, hypertension and statins	0.213 (0.148 to 0.279)	0.133	6.371	<0.001	1.54	2.12
Model 2: model 1 + ALT, AST, lipid profiles, UA, eGFR _M , AUC-CP, Matsuda-CP and AUC-GLA	0.065 (-0.001 to 0.132)	0.040	1.932	0.053	0.16	28.5
Model 3: model 2 + HbA1c and glucose-lowering therapies	0.081 (0.011 to 0.151)	0.049	2.263	0.024	0.24	30.1
In AUC-GLA						
Model 0: crude	0.252 (0.207 to 0.297)	0.208	10.921	<0.001	4.33	4.33
Model 1: age, sex, BMI, SBP, DBP, diabetes duration, hypertension and statins	0.260 (0.211 to 0.309)	0.215	10.450	<0.001	4.04	5.18
Model 2: model 1 + ALT, AST, lipid profiles, UA, eGFR _M , AUC-CP, Matsuda-CP and F-GLA	0.152 (0.103 to 0.201)	0.122	6.064	<0.001	1.49	31.6
Model 3: model 2 + HbA1c and glucose-lowering therapies	0.151 (0.100 to 0.203)	0.121	5.730	<0.001	1.46	32.8

AUC-CP: C-peptide area under curve; AUC-GLA: glucagon area under the curve; ; F-GLA: Fasting glucagon; Matsuda-CP: C-peptide-substituted Matsuda's index .

been demonstrated to be associated with several adverse consequences, such as overweight (28), obesity (29), metabolic syndrome (21), hypertension (30), Hashimoto's thyroiditis (31), cancer prognosis (10), progression to pre-diabetes (22), and incidence of diabetes (23). What's more, serum CysC levels were dose dependent and positively related to cardio-cerebrovascular risks of the general population (13, 32) and diabetic complications in the population with T2D (15, 16, 17, 18, 20). On the other hand, CysC-mediated neuroprotective effects had also been found in preclinical models of the disease. Reduced serum CysC levels are highly associated with

Alzheimer's disease and maybe an independent prediction marker for Alzheimer's disease (33). Upregulation of CysC expression is identified to be a potential therapeutic target for Parkinson's disease (34). Approach to increase CysC is also a potential candidate against stroke through preserving lysosomal membranes integrity (35, 36). In our present study, increased serum CysC levels were closely associated with greater BMI and the prevalence of hypertension in patients with T2D. Additionally, increased serum CysC levels were also shown to be associated with indices of pancreatic α - and β -cell dysfunctions in those patients.

Now that increased serum CysC levels are initially linked to obesity-related diseases, and can predict the incidence of pre-diabetes and diabetes, it is very likely that CysC overexpression plays a central role in the pathophysiological processes of T2D. A previous study by Uruska *et al.* (37) reported that higher levels of serum CysC were indicative of a higher degree of insulin resistance evaluated by glucose disposal rate in type 1 diabetes. Reutens and colleagues (38) demonstrated that both central adiposity and insulin resistance partially mediated the relationship between increased serum CysC levels and the progression of T2D. Lee *et al.* (39) also found that there were independent relationships between increased serum CysC levels, insulin resistance and inflammation biomarkers, which may interpret the linkage between serum CysC levels and CVD in T2D independent of kidney function. Moreover, Yokoyama *et al.* (40) conducted a study in 40 patients with T2D, and they revealed that serum CysC levels were positively correlated with postprandial insulin secretion after antidiabetic treatment (insulin-stimulated mitiglinide therapy) independent of postprandial glucose levels. In our present study, we observed that increased serum CysC levels were responsible for blunted insulin sensitivity evaluated by Matsuda-CP and increased insulin secretion of β -cells evaluated by AUC-CP in patients with T2D. Matsuda-CP is a surrogate indicator of the overall insulin sensitivity that can efficiently assess the sensitivity of the visceral and peripheral tissues to insulin (41), and AUC-CP is a reliable indicator for measurement of post-challenge β -cell secretion function (26). Therefore, serum CysC presented a bidirectional regulatory effect on pancreatic β -cell function. Increased serum CysC levels seemed to on one hand decrease systemic insulin sensitivity and on the other hand increase insulin secretion from β -cells in patients with T2D.

There are some possible mechanisms for increased CysC expression linked to aggravated insulin resistance and increased insulin secretion from β -cells. It is widely accepted that the pathogenesis of insulin resistance is involved with ectopic lipid accumulation and systemic inflammation (42). CysC mRNA expression and CysC release by subcutaneous and omental adipose tissue increased two- to three-fold in obese when compared to these in nonobese subjects (43). Meanwhile, systemic inflammation indicators, such as interleukin-6, tumor necrosis factor alpha (TNF- α) and C-reactive protein, are closely related to serum CysC levels (14, 30, 44, 45). These evidences suggested that increased CysC expression directly participated in the pathogenesis of insulin resistance. At the same time, insulin resistance may in turn induce a compensatory insulin secretory

response. Moreover, CysC exerted dual neuronal-vascular roles in promoting neuronal survival and angiogenesis by the regulation of the secreted protein vascular endothelial growth factor (VEGF) in the Parkinson's disease model (34). VEGF is abundantly expressed in islet β -cells, serving as an essential modulator of the islet microvasculature (46). CysC may promote β -cells survival and islet angiogenesis by the regulation of VEGF-mediated pathways. These evidences supported that increased CysC may facilitate insulin secretion from β -cells.

Up to now, no previous study has examined the association between serum CysC levels and pancreatic islet α -cell function. Our study found that increased serum CysC levels were associated with elevated fasting and post-challenge glucagon levels (F-GLA and AUC-GLA, respectively) in patients with T2D. Increased CysC expression may contribute directly to the pathogenesis of insulin resistance, which subsequently leads to fasting hyperglucagonemia, less early glucagon suppression and elevated postload 2-h plasma glucagon levels (3, 47, 48). In addition, increased CysC concentration may induce an inflammatory response by enhancing TNF- α expression (30, 49), and inflammation may in turn lead to β -cell dedifferentiation (50, 51, 52), characterized by loss of β -cell identity and expression of glucagon in these β -cells (53). Moreover, our previous study has demonstrated that fatty acid-binding protein 4, an inflammatory factor primarily originated from adipose tissue, was positively associated with fasting and postprandial glucagon levels in T2D (54). Collectively, our present study and the relevant literatures indicated that increased serum CysC levels may be contributed to the elevated fasting and postprandial glucagon in patients with T2D.

We do need to address several limitations of the present study. First, we used a cross-sectional observational design to conduct this study. Consequently, causality may not be inferred between increased serum CysC levels and responses of pancreatic α - and β -cells in T2D. We need a longitudinal study to improve this defect. Second, our study is confined to the cases in a single center, and the finding may have limited generalizability. Third, our present study only revealed the clinical relevance, so basic research was needed to investigate the role of CysC expression in the pathophysiological processes of T2D.

Conclusions

In summary, increased serum CysC levels may be independently responsible for increased insulin secretion

from β -cells, decreased systemic insulin sensitivity, and elevated fasting and postprandial glucagon secretion from α -cells in T2D, which indicate that increased serum CysC levels may take part in the impaired pancreatic α - and β -cell functions in patients with T2D.

Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/EC-21-0597>.

Declaration of interest

The authors declared that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Ethical statement

The study design was reviewed and approved by the Human Study Review Committee of Affiliated Hospital 2 of Nantong University. The conduction of study was adhered to the Declaration of Helsinki involving research of human subjects, and all patients signed an informed consent when admitted to the study.

Availability of data and materials

The study data could be provided to the interested researchers upon reasonable requests. The requests for data should be made to the corresponding author of the study.

Author contribution statement

J b S and D m Z contributed to the conception and design of the study. F X, X h W, C h W and C Y contributed to the data collection and assembly. H q Y, J x M, J p X, S x Z and J b S contributed to the data analysis and interpretation. H q Y and J x M contributed to the initial drafting of the manuscript. J b S and X q W revised the manuscript. X q W provided the administrative support. All authors were in agreement regarding the final manuscript.

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