High serum levels of CML are associated with poor coronary collateralization in type 2 diabetic patients with chronic total occlusion of coronary artery

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Research Article

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Abstract

Background: The formation of advanced glycation end-products (AGEs) is a crucial risk factor for the pathogenesis of cardiovascular diseases. We investigated whether N-e-carboxy-methyl-lysine (CML), a major form of AGEs in vivo, was associated with poor coronary collateral vessel (CCV) formation in patients with type 2 diabetes mellitus (T2DM) and chronic total occlusion (CTO) of coronary artery.

Methods: This study consisted of 242 T2DM patients with angiographically documented CTO. Blood samples were obtained and demographic/clinical characteristics were documented. The collateralization of these patients was defined according to Rentrop score. Receiver operating characteristic (ROC) curve and multivariable regression analysis were performed.

Results: 242 patients were categorized into poor CCV group (Rentrop score 0 and 1)(n = 107) and good CCV group (Rentrop score 2 and 3)(n = 135). Serum CML levels were significantly higher in poor CCV group (110.0 ± 83.35 ng/ml) than in good CCV group (62.95 ± 58.83 ng/ml, $P < 0.001$). Moreover, these CML levels were also significantly different across the Rentrop score 0, 1, 2 and 3 groups ($P < 0.001$). In ROC curve for ascertaining poor CCV, AUCs were 0.70 (95% CI 0.64-0.77) for CML. In multivariable logistic regression, CML levels ($P < 0.001$) remained independent determinants of poor CCV after adjustment of traditional risk factors.

Conclusions: This study suggests that higher CML levels are associated to poor CCV in T2DM patients with CTO. Inhibition of AGEs including CML is a strategy in antagonizing poor CCV in diabetic patients.

Background

Diabetes causes impairment of coronary collateral vessel (CCV) formation in response to occlusion of a patent artery in patients with coronary artery disease [1]. Previous studies have evidenced that dysregulation of pro-angiogenic and anti-angiogenic elements contributes to poor CCV in ischemic tissues in diabetes [2-4]. Pathophysiologically, this pathologic feature is caused by increased formation and accumulation of advanced glycation end products (AGEs) and augmentation of oxidative stress and inflammatory reactions [2-4].

In diabetic milieu, AGEs play a central role in the pathophysiology of vascular complications including angiogenesis and arteriogenesis impairment [5,6]. Engagement of the receptor for AGEs (RAGE) with AGEs activates pathways in endothelial cells or macrophages, leading to augmented oxidative stress and inflammation in ischemic myocardial tissues, ending up with poor collateralization [5,6].

N-e-carboxy-methyl-lysine (CML) is the most abundant AGEs in vivo [7]. In diabetic condition, CML-modified proteins may exhibit structural alterations, thereby resulting in impairment of protein functions. Moreover, CML-modified protein also activates RAGE pathway, jointly accelerating the development of various vasculopathies (i.e., macrovascular and microvascular diseases) in diabetes [5,8-12]. However, relation of CML to poor collateralization in diabetic patients with CTO remains unclear.

In the present study, we performed coronary angiography and used Rentrop score system to assess the condition of CCV formation in T2DM patients with CTO. The serum levels of CML were evaluated via ELISA in the participants. Our study was designed to explore the relationship between CML levels and coronary collateralization in T2DM patients with CTO.

Methods

Study population and grouping

The study protocol was approved by the Ruijin Hospital and Shanghai Jiao Tong University School of Medicine Ethics Committee, and written informed consent was obtained from all participants.

A total of 615 T2DM patients with stable angina and at least one lesion with coronary angiographic total occlusion were enrolled between January 2012 and December 2019. This inclusion criterion was based on long-standing knowledge that a severe coronary artery obstruction was a prerequisite for spontaneous collateral recruitment [13]. Stable angina was diagnosed according to the criteria recommended by the American College of Cardiology/American Heart Association [14]. For the purpose of this research, we excluded patients with chronic heart failure (n=69), pulmonary heart disease (n=25), malignant tumors or immune system disorders (n=71), renal failure requiring hemodialysis (n=34) as well as patients who had a history of coronary artery bypass grafting (n=79) or received percutaneous coronary intervention within the prior 3 months (n=95). The remaining 242 diabetic patients with stable angina and CTO (>3 months) were eligible and categorized in this study (**Figure 1**). The diagnosis of T2DM and hyperlipidemia were made according to the 2016 guideline of ESC [15] and 2017 update of ESC/EAS on PCSK 9 inhibition [16]. Type 1 diabetes was excluded by measurement of C-peptide levels. Detailed information regarding demographics, clinical manifestation and medications used was obtained.

Coronary angiography

Coronary angiography was performed through the femoral or radial approach. All angiograms were reviewed by two experienced interventional cardiologists, according to lesion classification scheme of the American College of Cardiology/American Heart Association [17]. Both of them were blinded to the study protocol and clinical data. Any differences in interpretation were resolved by a third reviewer.

The condition of CCV was determined using Rentrop score as in previous studies [18-20], as follows: grade 0=no collaterals, grade 1=side branch filling of the recipient artery without visualization of the epicardial artery, grade 2=partial filling of the main epicardial coronary artery, grade 3=complete filling of the main epicardial coronary artery [21]. Patient with Rentrop 0 or 1 were categorized as poor CCV group and those with Rentrop 2 or 3 were belong to good CCV group.

Thus, the present study contained 242 patients altogether: Rentrop 0 (n=46), Rentrop 1 (n=61), Rentrop 2 (n=66), Rentrop 3 (n=69). Poor CCV (Rentrop 0 or 1) group had 107 patients and good CCV (Rentrop 2 or 3) group had 135 patients.

Sample acquisition and biochemical measurement

Blood samples were obtained from patients undergoing angiography after 12h fasting. Samples were collected by centrifugation at the speed of 3000 rpm for 10 min. All serum samples were stored at -80°C until analysis. Serum glucose, glycosylated hemoglobin A1c (HbA1c), blood urea nitrogen, creatinine, uric acid, and lipid profiles were measured with standard laboratory techniques on a Hitachi 912 Analyzer (Roche Diagnostics, Germany). Modified estimated glomerular filtration rate (eGFR) was calculated.

CML Quantification

Serum CML levels were measured with Cell BioLabs CML Competitive ELISA kit (STA-816) according to the manufacturer's instructions. The CML ELISA kit used a colorimetric immunoassay method and CML levels of samples were determined by comparing samples OD values with a standard curve of gradient dilution of CML-modified BSA, in which higher CML modification correlates with lower OD signal. The final CML levels were shown with ng/ml unit by calculation of CML-modified BSA/CML. The inter-assay variation was controlled in acceptable range.

Statistical analysis

Continuous variables are presented as mean $\pm$ standard deviation (SD), and categorical data are summarized as frequency (percentage). For categorical clinical variables, differences between groups were evaluated by the chi-square test followed by Bonferroni's correction. For continuous variables, normal distribution was evaluated with the Kolmogorov–Smirnov test. Differences among groups were analyzed by one-way analysis of variance (ANOVA) followed by *post-hoc* analysis (Bonferroni's correction). Receiver operating characteristic (ROC) curves were plotted to assess the power of CML for detecting poor collateralization and to compare its power with HbA1c and with combined risk factors. Area under the curve (AUC) was compared using the DeLong method. Risk factors for CAD including gender, age, body mass index (BMI), hypertension, smoke, HbA1c, eGFR and high-sensitivity C reactive protein (hsCRP) were recruited into multivariable logistic regression analyses with or without CML measurements to assess determinants for poor CCV. All analyses used 2-sided tests with alpha value set at 0.05. All statistical analyses were performed with IBM SPSS Version 26 for Mac (IBM SPSS Inc, Chicago, IL, USA) and Prism 9 for macOS (1994 - 2021 GraphPad Software, LLC).

Results

Baseline characteristics

The baseline characteristics and biochemical measurements of all the patients with poor CCV and good CCV group are presented in **Table 1**. Patients of poor CCV group were older, more smokers, poor glycemic control, had lower ratio of male, hypertension history, higher serum levels of creatinine and hsCRP, and lower eGFR values in comparison with those of good CCV (for all comparison, $P < 0.05$).

Serum CML levels are significantly increased in patients with poor CCV

Serum CML levels were significantly increased in poor CCV group (110.0 ± 83.35 ng/ml) than in good CCV group (62.95 ± 58.83 ng/ml, $P < 0.001$) (**Figure 2**). CML levels were also significantly different across the groups categorized according to Rentrop score (Rentrop score 0, 120.8 ± 75.12 ng/ml; Rentrop score 1, 101.8 ± 88.78 ng/ml; Rentrop score 2, 67.01 ± 64.78 ng/ml; Rentrop score 3, 59.07 ± 52.70 ng/ml, respectively) (P for trend < 0.001) (**Figure 3**).

Moreover, CML levels were inversely correlated with Rentrop score before (Spearman's $r = -0.319$, $P < 0.001$) and after (Spearman's $r = -0.311$, $P < 0.001$) adjustment of gender, age, BMI, smoke, hypertension, HbA1c, eGFR and hsCRP serum levels. The percentage of poor CCV increased stepwise from the lowest tertile (< 38.76 ng/mL) to the highest tertile of CML (> 95.75 ng/mL) (P for trend < 0.001) (**Table 2**). Odds ratio for poor CCV increased to 6.802 (95% CI 2.980-15.526) in the highest tertile in comparison with those in the lowest tertile ($P < 0.001$), after adjustment of multiple variables including gender, age, BMI, smoke, hypertension, HbA1c, eGFR and hsCRP levels (**Table 2**).

ROC curve for detecting poor CCV exhibited that AUC was 0.67 for CML (95% CI 0.60-0.74, $P < 0.001$) and the cutoff value was 45.20 ng/ml according to Youden's index with a diagnostic sensitivity of 79.44% and specificity of 52.59%.

Multivariable analysis

Multivariate logistic regression analysis was performed to ascertain independent determinants of poor CCV. In Model 1, we included major parameters in **Table 1**, including gender, age, BMI, hypertension, smoking, HbA1c, eGFR and hsCRP. The results showed that hypertension, poor glycemic control, low eGFR and high hsCRP levels were independent determinants for poor collateralization. After adjustment for these variables, serum CML levels remained independently associated with poor CCV (OR=1.966, 95% CI 1.510-2.559, $P < 0.001$) (model 2) (**Table 3**). The calibrations of both models were good ($P = 0.731$ for Model 1 and $P = 0.967$ for Model 2 in Hosmer-Lemeshow test). The addition of CML in Model 2 significantly improved predictive performance with an increase of Nagelkerke R^2 by 12.3% ($P < 0.001$). In addition, ROC curve for both models showed that addition of CML in Model 2 effectively elevated the AUC value (AUC=0.79, 95% CI 0.73-0.85 $P < 0.001$) comparing with Model 1 (AUC=0.67, 95% CI 0.60-0.74 $P < 0.001$) ($P = 0.011$) (**Figure 4**).

Discussion

Patients with diabetes usually exhibit poor coronary collateralization after ischemia [1]. Our study has demonstrated that serum CML levels are significantly increased in T2DM CTO patients with poor CCV as compared with those with good CCV. Serum CML levels are inversely correlated with Rentrop score in these patients. ROC curve for detecting poor CCV has demonstrated that AUC is 0.67 for CML. In logistic regression analysis, serum CML level is an independent determinant of poor CCV in patients with T2DM and CTO. Our study supported a notion that increased CML levels contributes to poor coronary collateralization in T2DM patients with CTO.

Hyperglycemia-associated formation of AGEs and subsequent engagement of AGEs with RAGE causes augmented oxidative stress and robust inflammation, leading to diabetic cardiovascular complications (macrovascular and microvascular vasculopathies), and robust production of AGEs, which in return results in a vicious cycle [2-6].

Among various AGEs, CML modifications predominate in vivo in diabetes[7]. Previous studies have evidenced the impact of CML in the pathogenesis of cardiovascular diseases associated with diabetes. Specific AGEs including CML are associated with incident cardiovascular events with T2DM [22,23]. Glycation and CML levels in skin collagen predict future 10-year progression of diabetic retinopathy and nephropathy in controls and in intervention and complication patients of type 1 diabetes [24]. Serum levels of AGEs (mainly CML) are associated with impaired renal function and pathogenic mechanisms of chronic kidney disease [25,26]. Circulating Levels of CML are closely related to central obesity and inflammation [27], carotid diameter [28], and differentiate early to moderate Alzheimer's disease [29]. Moreover, plasma AGEs, in particular CML levels, are found to be related to the severity and prognosis of CHF [30]. Consistent with above-mentioned studies, our study has showed that high CML levels are associated with poor collateralization in type 2 diabetic patients with CTO. Our findings have further added novel information regarding CML, indicating that CML impairs the repairing mechanisms of CCV formation in ischemic tissues.

Sufficient evidence has revealed that reduction of AGEs levels may be effective to alleviate diabetic vasculopathies [31]. Alagebrium, capable of breaking cross-link structure in AGEs, targets the miR-27b/TSP-1 signaling pathway to attenuate CML-induced endothelial dysfunction [32]. Soluble RAGE (sRAGE) is a RAGE isoform generated through alternative splicing or shedding from cell membrane. sRAGE combines with AGEs to prevent the engagement of AGEs with RAGE and subsequent activation of RAGE pathway [33]. Animal studies have shown that administration of sRAGE remarkably stabilizes atherosclerotic plaque, and inhibits inflammatory factors such as cyclooxygenase-2 (COX-2), VCAM-1, and monocyte chemoattractant protein-1 (MCP-1), thereby attenuating atherosclerosis progression [33]. Moreover, antioxidants (e.g., vitamin C, vitamin E), ACEIs, ARBs and statins are capable of inhibiting AGEs formation [31]. These data jointly suggest that inhibition of renin-angiotensin system, modulation of dyslipidemia, AGE inhibition, RAGE pathway inhibition and oxidative stress reduction are therapeutic strategies for preventing cardiovascular complications in diabetes, partially through antagonizing AGEs formation.

Limitations

We recognize limitations in our study. First, this study is a cross-sectional study, and all the enrolled patients were from a single center. Second, the Rentrop scoring system is not a most precise way for evaluation of coronary collateralization. It is more accurate by calculating collateral flow index, which requires measurement of pressure within aorta and the distal culprit segment at the same time. Last, CMLs have been traditionally quantified by gas chromatography/mass spectrometry (GC/MS). Thereby, the correlation between CML and poor collateralization needs further investigated by prospectively study.

Conclusion

In conclusion, our results suggest that higher CML is associated with poor collateralization in T2DM patients with CTO.

Abbreviations

CCV coronary collateral vessel; AGEs advanced glycation end-products; CML N-e-carboxy-methyl-lysine; RAGE receptor for AGEs; T2DM type 2 diabetes mellitus; CTO chronic total occlusion; BMI body mass index; HbA1c glycosylated hemoglobin A1c; eGFR estimated glomerular filtration rate; hsCRP high-sensitivity C reactive protein; sRAGE soluble RAGE; COX-2 cyclooxygenase-2; MCP-1 monocyte chemoattractant protein-1; ROC receiver operating characteristic; AUC area under the curve; SD standard deviation.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ruijin Hospital and Shanghai Jiao Tong University School of Medicine Ethics Committee, and written informed consent was obtained from all participants.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

None by any of the authors.

Funding

Not applicable.

Authors' contributions

LYL, YD, XQW and FHD participated in study design, data analysis and interpretation. LYL, YD and LL drafted the main manuscript and prepared all figures and tables. YS, LYL, FFL, ZMW and QJC performed data collection. YD, LL and WFS revised the manuscript before final approval. All authors read and approved the final manuscript.

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Tables

Table 1. Baseline characteristics and biochemical measurements in patients with poor and good collateralization

Classification	Poor CCV (n=107)	Good CCV (n=135)	P value
Male, n (%)	74 (69.16)	114 (84.44)	0.005
Age, year	67.31±11.22	64.19±10.10	0.024
BMI, kg/m ²	25.25±3.74	24.98±3.34	0.546
Smoke, n (%)	41 (38.32)	34 (25.19)	0.036
Hypertension, n (%)	71 (66.36)	106 (78.52)	0.041
SBP, mmHg	134.93±21.58	136.06±19.96	0.672
DBP, mmHg	73.45±10.55	75.11±11.43	0.246
FBG, mmol/L	8.44±3.41	7.69±2.77	0.060
HbA1c, %	6.95±1.43	6.37±1.58	0.003
Dyslipidemia, n (%)	29 (27.10)	22 (16.30)	0.056
Triglyceride, mmol/L	1.77±0.93	1.70±1.22	0.650
Total cholesterol, mmol/L	3.98±1.29	3.87±1.08	0.496
LDL-C, mmol/L	2.33±1.05	2.25±0.89	0.516
HDL-C, mmol/L	1.01±0.20	1.06±0.28	0.133
ApoA, g/L	1.12±0.22	1.15±0.23	0.312
ApoB, g/L	0.80±0.27	0.77±0.23	0.367
Lp(a), g/L	0.36±0.86	0.30±0.29	0.411
BUN, mmol/L	7.18±4.84	6.93±3.77	0.648
Serum creatinine, μ mol/L	101.74±53.74	85.10±66.51	0.037
eGFR, ml/min/1.73 m ²	68.51±20.80	85.17±20.49	<0.001
UA, μ mol/L	348.69±104.15	338.62±97.02	0.438
hsCRP, mg/L	14.58±33.21	7.14±20.63	0.034
Data are mean $\pm$ SD or number (%).			
Abbreviations: CCV coronary collateral vessel, BMI body mass index, SBP systolic blood pressure, DBP diastolic blood pressure, FBG fasting blood glucose, HbA1c glycosylated hemoglobin A1c, LDL-C low-density lipoprotein cholesterol, HDL-C high-density lipoprotein cholesterol, BUN blood urea nitrogen, UA uric acid, eGFR estimated glomerular filtration rate, hsCRP high-sensitivity C reactive protein.			

Table 2. odds ratio of poor collateralization according to CML in diabetic patients

Tertiles of CML (n, range ng/ml)	Poor CCV, n (%)	Crude OR (95% CI)	^a Adjusted OR (95% CI)
Tertile 1 (n=80, <38.76)	19 (23.75)	1	1
Tertile 2 (n=80, 38.76-95.75)	37 (46.25)	2.763 (1.404-5.437) *	2.556 (1.161-5.624) *
Tertile 3 (n=82, >95.75)	51 (62.20)	5.282 (2.672-10.441) **	6.802 (2.980-15.526) **
Per tertile	/	2.278 (1.626-3.192) **	2.610 (1.729-3.941) **
P value for tertile trend	<0.001	<0.001	<0.001
Abbreviations: CCV coronary collateral vessel; CI confidence interval; OR odds ratio.			
*P<0.05; **P<0.001.			
^a Multiple-adjustment for gender, age, body mass index, hypertension, smoke, HbA1c, estimated glomerular filtration rate, total-to-HDL cholesterol ratio and serum level of high sensitive C reactive protein			

Table 3. Logistic regression analyses for poor collateralization in diabetic patients

	Variables	OR (95% CI)	P value
Model 1	Male	0.800 (0.374-1.712)	0.566
Nagelkerke R ² = 0.307	Age per 10 years	1.123 (0.837-1.508)	0.439
Hosmer-Lemeshow test: P = 0.731	BMI	1.057 (0.966-1.157)	0.230
	Hypertension	0.415 (0.213-0.810)	0.010
	Smoke	2.278 (1.205-4.307)	0.011
	HbA1c	1.297 (1.064-1.580)	0.010
	Total-to-HDL cholesterol ratio	1.176 (0.955-1.449)	0.127
	eGFR	0.967 (0.950-0.983)	<0.001
	Log hsCRP	1.134 (1.004-1.282)	0.043
Model 2	Male	0.736 (0.315-1.721)	0.480
Nagelkerke R ² = 0.430	Age per 10 years	1.143 (0.837-1.560)	0.401
Hosmer-Lemeshow test: P = 0.967	BMI	1.041 (0.947-1.145)	0.401
	Hypertension	0.325 (0.155-0.682)	0.003
	Smoke	2.035 (1.040-3.980)	0.038
	HbA1c	1.275 (1.034-1.572)	0.023
	Total-to-HDL cholesterol ratio	1.134 (0.908-1.416)	0.267
	eGFR	0.964 (0.947-0.982)	<0.001
	Log hsCRP	1.179 (1.030-1.349)	0.017
	Log2 CML	1.966 (1.510-2.559)	<0.001
BMI body mass index; HbA1c, glycosylated hemoglobin A1c; HDL, high-density lipoprotein; eGFR, estimated glomerular filtration rate; hsCRP, high sensitive C reactive protein			
Model 1, adjusted for conventional cardiovascular factors; Model 2, adjusted for the factors included in Model 1 with the addition of CML.			

Figures

Figure 1

Legend not included with this version

Figure 2

Legend not included with this version

Figure 3

Legend not included with this version

Figure 4

Legend not included with this version

Supplementary Files

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