Research Article

Assessment of serum εN-carboxymethyllysine and soluble receptor of advanced glycation end product levels among type 2 diabetes mellitus patients with and without acute coronary syndrome

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ABSTRACT

Background: One of the most predominant macrovascular complications of type 2 diabetes mellitus (T2DM) is acute coronary syndrome (ACS). Advanced glycation end products (AGEs) play an important role in the development and progression of ACS in T2DM patients. The soluble receptor of AGEs (sRAGE) decoys the effect of AGEs by binding with them. **Objectives:** The objectives of the study were to measure serum εN-carboxymethyllysine (CML) and sRAGE levels among T2DM with and without ACS and to find out whether serum CML and sRAGE could be used to predict the risk of ACS among T2DM patients. **Materials and Methods:** A total of 37 T2DM patients with ACS were selected as cases and 37 T2DM as controls. Routine biochemical parameters were carried out on autoanalyzer and serum CML and sRAGE were estimated by ELISA. The data were recorded and analyzed on SPSS system and were compared by Student's *t*-test or Mann–Whitney test. **Results:** Serum CML level and CML/sRAGE ratio were significantly increased in the cases as compared to control. Correlation analyses showed that serum CML concentration and serum CML/sRAGE ratio were positively correlated with weight, waist circumference, body mass index and serum urea, and very low-density lipoprotein-cholesterol. Multivariate regression (Binary logistics) analysis after adjusted for waist circumference, weight, BMI, and systolic blood pressure showed that serum CML was significantly associated with ACS risk among T2DM patients. Receiver operating characteristic curve depicted that high CML level (>31.83 ng/ml) and CML/sRAGE ratio (>15.48) were associated with greatest risk of ACS among type 2 DM patients. **Conclusion:** T2DM patients with high serum CML concentration and CML/sRAGE ratio were at risk of ACS independent of other cardiovascular risks.

KEY WORDS: Type 2 Diabetes Mellitus; Acute Coronary Syndrome; εN-carboxymethyllysine; Soluble Receptor of Advanced Glycation End Product

INTRODUCTION

In India, type 2 diabetes mellitus (T2DM) is on the way to become a pandemic. Several risk factors have being shared between T2DM and coronary artery disease (CAD) such as hyperglycemia, obesity, dyslipidemia, physical inactivity, and stress. Hence, an increased prevalence of diabetes points toward increasing risk of a CAD.[1] Diabetic patients develop CAD 2–4 times faster than the healthy population. Diabetic subjects had 21.4% prevalence of CAD, which was higher than patients with impaired glucose tolerance (14.9%) and subjects with normal glucose tolerance (9.1%).[2]

Advanced glycation end products (AGEs) are formed by non-enzymatic glycation of proteins or lipids by high serum glucose, methylglyoxal, and 3-deoxy glucosone. AGEs,

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mainly εN-carboxymethyllysine (CML) has role in the development and progression of cardiovascular disease (CVD) in diabetes.[3] Hence, the serum level of AGEs was found to be high in T2DM patients with coronary heart disease (CHD) as compared to patients without CHD, and it was correlated with the severity of CHD.[4] The plaque concentrations of the specific AGEs, i.e., CML and MG-H1 were associated with inflammatory plaque markers and were higher in rupture-prone plaques (i.e., inflammatory atheromatous lesions).[5,6]

AGEs exert their effect by binding to AGE-specific cell surface receptors (RAGE) present on endothelial and smooth muscle cells. It promotes CVD in diabetes through endothelial dysfunction, inflammation, and inducing lipid abnormalities.[7,8] AGEs-RAGE binding activates endothelial cells, resulting in higher levels of endothelial adhesion molecules such as VCAM-1 and activation of transcription factor nuclear factor-kappa B, leading monocyte adhesivity and vascular permeability accelerating atherosclerosis.[4,5]

Soluble isoforms of RAGE include endogenous secretory RAGE (esRAGE) and soluble receptor of AGEs (sRAGE). Both of them lack a transmembrane domain and intracellular domain as that of RAGE, so they are devoid of intracellular signaling. Synthesis of esRAGE is by the splicing of truncated RAGE mRNA and that of sRAGE by the action of sheddase and metalloproteinase (MMP) 10 on RAGE. sRAGE has a high affinity for the ligand (AGEs) than RAGE and thus inhibits the deleterious effect of AGE-RAGE signaling.[9-11] Recombinant sRAGE blocks the development and progression of several pathological diseases such as CVS complication and neural dysfunction in animal models.[3,9] Increase in serum sRAGE levels in T2DM patients acts as a compensatory mechanism to inhibit the deleterious effect of AGE-RAGE signaling. Therefore, sRAGE was considered as antiatherogenic.[3,5,12,13]

Most studies in literature have analyzed the serum AGE and sRAGE separately so that the association between them in T2DM with acute coronary syndrome (ACS) is lacking. The exact relationship between serum AGEs and sRAGE levels among T2DM patients was not explored till date to understand the deleterious effects of AGEs and beneficial effect of sRAGE. Hence, the novelty of the study would be to collect data on serum levels of CML and sRAGE measured simultaneously among T2DM with ACS. Therefore, the objectives of the study were to measure serum CML and sRAGE levels among T2DM with and without ACS and to find out whether serum CML and sRAGE can be used to predict the risk of ACS among T2DM patients.

MATERIALS AND METHODS

Study Participants

The study was conducted in Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER) Hospital, Puducherry-06. The study was approved from Institute Research Council, and permission from Institute Human Ethics Committee was obtained (JIP/IEC/SC/2015/23/840 Dated: 21/12/2015). A written informed consent was taken from study subjects, before recruitment. 37 T2DM patients as controls were recruited based on the American Diabetes Association, 2010 criteria.[14] 37 T2DM (>18 years old) with ACS including unstable angina (UA), non-ST-elevation myocardial infarction (NSTEMI), and ST-segment elevation myocardial infarction (STEMI) were recruited based on clinical presentation, electrocardiographic changes and elevation of cardiac enzymes (Troponin-I, creatine kinase-total, and creatine kinase-MB).[14] Patients with type 1 diabetes mellitus, chronic renal disease, liver cirrhosis, congestive heart failure, chronic lung diseases, chronic infections, and smokers were excluded from the study.

Sample size Calculation

The sample size was estimated with an expected difference in the serum sRAGE level between cases and controls (mean difference of 0.20 ng/ml, with an SD of 0.17 ng/ml) at 5% level of significance and 80% power, based on the previous study.[6] It was calculated as 37 in each group.

Clinical and Biochemical Measurements

Medical history (i.e., date of birth, smoking, alcohol consumption, and medical treatments) for all subjects were recorded. Height, weight, waist circumference, and seated blood pressure were measured by the same observer. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (meter). Under the strict aseptic condition, fasting 5 mL venous blood was collected from all subjects enrolled in the study and was used for routine biochemistry investigations and CML and sRAGE analysis. Routine biochemistry investigations were assessed on fully automated analyzer by appropriate method. Serum CML and sRAGE levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Ray Biotech) according to the manufacturer's protocol.

Compliance with Ethical Standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Statistical Analysis

Categorical data were described as percentages, and continuous data were described as means ± SD. A Student's *t*-test (for data that were normally distributed) or a Mann–Whitney test (for data that were not normally distributed) was used to compare between cases and controls. Correlations between serum CML,

sRAGE levels, and CML/sRAGE ratio and continuous variables were determined using Pearson's correlation (for normally distributed data) or Spearman's correlation (for non-normally distributed data). Receiver operating characteristic (ROC) curve was performed to investigate the value of serum CML, sRAGE levels, and CML/sRAGE ratio in differentiating T2DM patients with or without ACS. Multiple logistic regression analysis was done to look for the independent association between serum CML, sRAGE levels, and CML/sRAGE ratio and ACS adjusted for weight, waist circumference, systolic blood pressure (SBP), and BMI. A value of $P < 0.05$ was considered statistically significant. All analyses were performed using software package SPSS 12.0 for Windows 10.

RESULTS

Baseline Clinical Characteristics and Biochemical Measurements

Details for the (T2DM with ACS) cases and (T2DM) controls were shown in Table 1. The cases had a male predominance over the control group. Of 37 ACS patients, 7 had UA, 11 had NSTEMI, and 19 had STEMI. The cases had significantly high weight, height, waist circumference, and BMI and more alcoholics as compared to controls. There was no significant difference in blood pressure, duration of diabetes mellitus, and serum lipid profile among cases and controls.

Serum CML Concentration in Groups

Fasting serum CML concentration was higher in the cases (45.80 ± 22.25 ng/ml) than in the controls (26.04 ± 13.80 ng/ml), and the difference was statistically highly significant ($P < 0.001$). Patients with UA (37.24 ± 15.57 ng/ml) had low serum CML level as compared to NSTEMI (51.04 ± 25.76 ng/ml) and STEMI (45.51 ± 22.27 ng/ml) patients [Figure 1], and the difference was statistically significant. Utilizing the ROC curve for values of CML and risk of ACS among T2DM patients, it was seen that the increase in the risk of ACS was seen at CML level more than 31.83 ng/ml (Area under curve = 0.804, sensitivity of 76%, and specificity of 78%, LR = 0.31, $P < 0.0001$).

Table 1: Clinical and biochemical properties of the study population

Characteristic	Patients with T2DM	
	Without ACS (Controls)	With ACS (Cases)
Number of patients	37	37
Age (years)	56.30±13.98	54.62±9.84
Male/Female	20/17	30/7
Weight (Kg)	67.51±12.58	80.73±8.96*
Height (cm)	162.00±7.35	167.11±8.15*
Waist circumference (cm)	86.41±5.90	93.49±3.70*
BMI	25.72±4.50	29.02±3.85*
Systolic blood pressure (mm of Hg)	122.86±8.39	126.48±9.50
Diastolic blood pressure (mm of Hg)	81.62±4.52	83.24±4.75
Duration of Diabetes (years)	4.89±4.77	4.42±3.43
History of smoking, *n* (%)	0 (0%)	0 (0%)
History of alcohol, *n* (%)	11 (30%)	18 (49%)*
Fasting plasma glucose (mg/dl)	193.62±80.77	207.60±64.30
Total Cholesterol (mg/dl)	178.38±45.27	175.81±39.34
Triglyceride (mg/dl)	156.81±79.04	131.08±34.48
HDL-C (mg/dl)	42.16±11.25	41.40±12.27
LDL-C (mg/dl)	105.05±40.26	104.75±36.24
VLDL-C (mg/dl)	31.32±15.82	26.22±7.03
CK-Total (U/l)	116.62±77.35	258.64±327.60*
CK-MB (U/L)	6.47±5.19	29.63±30.38*
Urea (mg/dl)	19.97±5.70	26.81±11.13*
Creatinine (mg/dl)	0.89±0.17	1.06±0.32*
CML (ng/ml)	26.04±13.80	45.80±22.25*
sRAGE (ng/ml)	2.16±0.80	2.46±1.43
CML/sRAGE ratio	13.17±7.77	20.31±10.35*

**P*-value: < 0.05, Continuous variables were described as mean±SD, categorical variables were presented as frequencies. HDL-C, high-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, VLDL-C: Very low-density lipoprotein cholesterol, CK-total: Creatine Kinase-Total, T2DM: Type 2 diabetes mellitus, ACS: Acute coronary syndrome, BMI: Body mass index, CK-MB: Creatine kinase-MB; CML: Carboxymethyl lysine, sRAGE: Soluble Receptor of Advance Glycation End product.

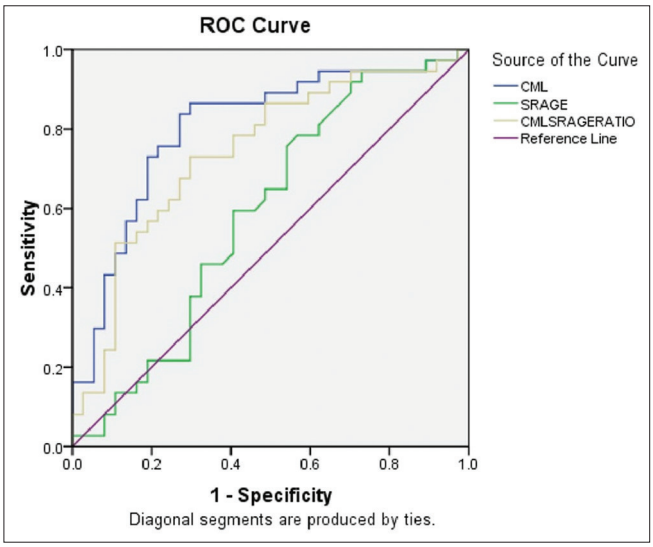

Figure 1: Construction of receiver operating characteristic curve confirmed that serum carboxymethyllysine (CML) concentration (ng/ml) and CML/soluble receptor of advanced glycation end product ratio significantly differentiated type 2 diabetes mellitus patients with or without acute coronary syndrome

Serum sRAGE Concentration in Groups

Fasting serum sRAGE concentration was higher in the cases (2.46 ± 1.43 ng/ml) than in the controls (2.16 ± 0.80 ng/ml), but the difference was not statistically significant. No statistically difference was found in serum sRAGE among UA (1.91 ± 0.63 ng/ml), NSTEMI (2.90 ± 2.44 ng/ml), and STEMI (2.40 ± 0.64 ng/ml) patients.

Serum CML/sRAGE Ratio in Groups

Fasting serum CML/sRAGE ratio was higher in the cases (45.80 ± 22.25) than in the controls (13.17 ± 7.77), and the difference was statistically highly significant ($P < 0.0001$). Patients with NSTEMI (19.88 ± 6.85) had lower serum CML/sRAGE ratio as compared to UA (20.11 ± 10.29) and STEMI (20.63 ± 12.35) patients, and the difference was statistically significant. Utilizing the ROC curve for values of CML/sRAGE ratio and risk of ACS among T2DM patients, it was seen that the increase in the risk of ACS was seen at CML/sRAGE ratio of more than 15.48 (Area under curve = 0.744, sensitivity of 70%, and specificity of 70%, LR = 0.42, $P < 0.001$).

Correlation between Serum CML, sRAGE Concentration, CML/sRAGE Ratio, and Clinical Parameters

Correlation analyses undertaken on all T2DM patients showed that serum CML concentration and serum CML/sRAGE ratio were positively correlated with weight, waist circumference, BMI and serum urea, and very low-density lipoprotein cholesterol (VLDL-C). Serum sRAGE concentration did not show significant correlation with any of the physical and biochemical parameters [Table 2]. Multivariate regression (Binary logistics) analysis after adjusted for waist circumference, weight, BMI, and SBP showed that serum CML was significantly associated with ACS risk among T2DM patients [Table 3].

DISCUSSION

As the prevalence of ACS is rising, it has become necessary to find the biochemical markers that are involved in the pathogenesis of ACS. Therefore, the aim of the study was to evaluate the association between serum CML, sRAGE, and CML/sRAGE ratio with the risk of ACS in T2DM patients. In the study, there was a significant difference between cases and controls with respect to their anthropometric characters and percentage of alcoholics. There was no significant difference in the blood pressure, duration of diabetes mellitus, and serum lipid profile among cases and controls. We established that there was a high fasting serum CML concentration in cases as compared to controls which were statistically significant. Our study had demonstrated that fasting serums sRAGE concentration was low in cases compared to controls. Serum sRAGE concentration was not correlated to any physical and biochemical parameters. Correlation analyses had shown the positive association between serum CML level, CML/sRAGE ratio and weight, waist circumference, BMI and serum urea, and VLDL-C. However, serum CML level was not correlated with age, height, blood pressure, and diabetes of duration and serum total cholesterol, and triglyceride levels. Using the ROC curve, the increased risk of ACS among T2DM patients was seen at serum CML level more than 31.83 ng/ml with 76% sensitivity and 78% specificity. Although serum CML level has low sensitivity and specificity, multivariate regression (Binary logistics) analysis after adjusted for waist circumference, weight, BMI, and SBP showed that serum CML level was significantly associated with ACS risk among T2DM patients.

Falcone *et al*.[15] also have shown that plasma levels of sRAGE were not associated with age, duration of diabetes, smoking, duration of hypertension, BMI, creatinine, glomerular filtration rate, and C-reactive protein, but they demonstrated the inverse correlation with total cholesterol and triglycerides values. There has been an inconsistent association between serum sRAGE level and physical and biochemical parameters, raising the question of sRAGE level as a biomarker in ACS. Serum sRAGE level may not be considered as a biomarker for ACS as it is produced by proteolytic cleavage of RAGE by MMP; hence, its level depends on MMP activities and the time of blood collection.[11] Kiuchi *et al*.[16] also had shown no correlation between serum AGEs and age, gender, and percentage of hypertensive subjects and smoking.

This was the first kind study which showed the association between serum CML and sRAGE levels in T2DM with ACS. Increase in serum CML was one of the reasons for the occurrence of ACS in T2DM patients. Limitations of the study were as follows: (1) Diabetic patients were controls which cannot exclude confounding effects such as the effects

Table 2: Correlation between serum CML, sRAGE, CML/sRAGE and other variables

Parameter	Patients with T2 DM					
	Total (*n*=74)		Without ACS (Control, *n*=37)		With ACS (Cases, n=37)	
	r	*P*-value	*r*	*P*-value	*r*	*P*-value
Serum CML level						
Weight (Kg)	0.344	0.003*	-0.014	0.389	0.385	0.190
Waist circumference (cm)	0.439	0.0001*	0.212	0.207	0.285	0.087
(BMI)	0.338	0.003*	-0.020	0.907	0.372	0.024*
Total Cholesterol (mg/dl)	0.052	0.658	0.397	0.015*	-0.147	0.386
LDL-C (mg/dl)	0.046	0.695	0.430	0.008*	-0.200	0.236
Urea (mg/dl)	0.262	0.024*	-0.040	0.812	0.162	0.339
sRAGE (ng/ml)	0.449	0.0001*	0.186	0.270	0.533	0.001*
CML/sRAGE ratio	0.725	0.0001*	0.785	0.0001*	0.624	0.0001*
Serum sRAGE level						
Systolic blood pressure (mm of Hg)	0.052	0.661	0.343	0.037*	-0.127	0.453
Duration of Diabetes (years)	0.098	0.404	0.407	0.013*	-0.089	0.600
CML (ng/ml)	0.449	0.0001*	0.186	0.270	0.533	0.001*
CML/sRAGE Ratio	0.725	0.0001*	-0.394	0.016*	-0.283	0.090
Serum CML/sRAGE ratio						
Weight (Kg)	0.304	0.008*	-0.038	0.822	0.345	0.037*
Waist circumference (cm)	0.342	0.003*	0.152	0.370	0.209	0.214
(BMI)	0.314	0.007*	-0.016	0.927	0.408	0.012*
VLDL-C (mg/dl)	-0.245	0.035*	-0.158	0.349	-0.304	0.067
CK-MB (U/L)	0.287	0.013*	-0.035	0.835	0.180	0.288
CML (ng/ml)	0.725	0.0001*	0.785	0.0001*	0.624	0.0001*
sRAGE (ng/ml)	-0.240	0.039*	-0.394	0.016*	-0.283	0.090

**P*-value: < 0.05 . T2DM: Type 2 diabetes mellitus, ACS: Acute coronary syndrome, CML: εN-carboxymethyllysine, sRAGE: Soluble receptor of advanced glycation end products, VLDL-C: Very low-density lipoprotein cholesterol, CK-MB: Creatine kinase-MB, LDL-C: Low-density lipoprotein cholesterol, BMI: Body mass index

Table 3: Multivariate regression (Binary Logistics) analyses in all subjects

Independent variables	Beta coefficient	Adjusted Odd ratio (95%Cl)	*P*-value
Weight	-0.220	0.802 (0.683-0.943)	0.007*
Waist circumference	-0.399	0.671 (0.526-0.856)	0.001*
BMI	0.296	1.344 (0.850-2.125)	0.206
SBP	-0.040	0.961 (0.879-1.051)	0.385

**P*-value: < 0.05 . BMI: Body mass index, CI: Confidence interval, SBP: Systolic blood pressure

of diseases itself and drugs treatment, (2) the different duration and different treatment of each T2DM patient might incorporate a possible source of selection bias, (3) the study population was relatively small, and (4) cause-effect relationship between serum CML levels and ACS was not possible from a cross-sectional study.

CONCLUSION

In T2DM patients with ACS, high level of serum CML and CML/sRAGE ratio was observed. The study had demonstrated that T2DM patients with high serum CML concentration and CML/sRAGE ratio were at the threat of ACS independent of other CVD risk factors and utility of measuring serum CML concentration and CML/sRAGE ratio in the prevention of future ACS in T2DM patients.

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