Glyceraldehyde-derived advanced glycation end-products are associated with left ventricular ejection fraction and brain natriuretic peptide in patients with diabetic adverse cardiac remodeling

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ABSTRACT

Objectives: Glyceraldehyde-derived advanced glycation end-products (Glycer-AGEs) have a strong binding affinity for their cognate receptor and elicit oxidative stress and inflammation. However, it remains unknown whether the levels of Glycer-AGEs correlate with the severity of cardiac function and heart failure in patients with diabetic adverse cardiac remodeling (DbCR). Fourteen heart failure patients with type 2 diabetes mellitus (DM) without other cardiac disorders (DbCR group) were enrolled. Another 14 patients with idiopathic dilated cardiomyopathy (DCM) without DM were served as a control (DCM group). All patients were assessed for serum Glycer-AGEs, nitrotyrosine (NT), and tumor necrosis factor alpha (TNF-α) and for plasma brain natriuretic peptide (BNP). The left ventricular ejection fraction (LVEF) was evaluated by echocardiography. Results: The mean serum levels of Glycer-AGEs, NT, and TNF-α in the DbCR group were significantly higher than those in the DCM group (for Glycer-AGEs, p = .0073; for NT, p = .005; for TNF-α, p < .0001, respectively). In the patients with DbCR, the levels of serum Glycer-AGEs and TNF-α were closely associated with LVEF and BNP values. Conclusions: Both Glycer-AGEs and TNF-α showed close associations with LVEF and the levels of BNP in patients with DbCR. Glycer-AGEs and TNF-α may play a pathological role in the development of DbCR.

Introduction

Diabetes mellitus (DM) is a major issue health worldwide. Hyperglycemia in DM causes various cardiovascular complications leading to loss of quality of life and death [1,2]. Recent studies have shown that patients with DM have a significantly increased risk of heart failure. The main causes of heart failure with reduced ejection fraction (HFrEF) or heart failure with preserved ejection fraction (HFpEF) in patients with DM are coronary artery disease (CAD) and hypertension [3,4]. Although recent guidelines [5] reported that the existence of diabetic cardiomyopathy remained unconfirmed, other reports have shown the existence of a condition showing diabetic adverse cardiac remodeling (DbCR) without CAD and hypertension; this DbCR condition is associated with HFrEF, HFpEF, and death is clinically important [3–7]. In recent decades, extensive studies have shown that advanced glycation end-products (AGEs), formed by continuous hyperglycemia in patients with DM, cause myocardial damage leading to diabetic cardiomyopathy [8–10]. AGEs are end-products of a nonenzymatic reaction of sugar and lipid adducts with proteins (the Maillard reaction); AGEs form cross-links among the proteins of long-living tissues causing an age-related accumulation of AGEs in the body, a process that is accelerated in patients with DM [11–13]. AGEs have chemically heterogeneous structures. Among seven immunochemically distinct classes of AGEs (glucose-derived AGEs, fructose-derived AGEs, glyceraldehyde-derived AGEs, glycolaldehyde-derived AGEs, methylglyoxal-derived AGEs, glyoxal-derived AGEs, and 3-deoxyglucosone-derived AGEs) detected in the sera of patients with type 2 DM, especially those who are on hemodialysis, glyceraldehyde-derived AGEs (Glycer-AGEs) have been reported to have the strongest binding affinity for the receptor for AGEs (RAGE), subsequently eliciting oxidative generation and vascular inflammation, leading in turn to progressive atherosclerosis in patients with DM [13]. Clinically, it remains difficult to distinguish DbCR from idiopathic dilated cardiomyopathy (DCM). AGEs, along with oxidative stress and inflammatory markers, have been reported to be related to the severity of heart failure in patients with DCM [14,15]. Therefore, we hypothesized that the levels of Glycer-AGEs, in combination with elevation of oxidative markers and inflammatory cytokines, correlate with the severity of cardiac function and heart failure in
patients with DbCR compared with those with DCM and we tested this hypothesis.

Methods

Patients

In the first part of this study, 53 consecutive patients with heart failure and type 2 DM were subjected to noninvasive assessment by physical examination, chest X-ray, electrocardiography, and echocardiography; these patients were assessed for duration, comorbidities (retinopathy, neuropathy, and nephropathy) and treatments of DM by clinical records, and functional capacity using the New York Heart Association (NYHA) classification guidelines. These patients were also evaluated by echocardiography for the degree of left ventricular (LV) ejection fraction (EF), with classifications including HFrEF, heart failure with a mid-range ejection fraction (HFmrEF), and HfPEF, based on the ESC guidelines for acute and chronic heart failure [16]. Furthermore, we evaluated the quality of glycemic control as <7.0% (tight control) or ≥7.0% of hemoglobin A1C (HbA1C) levels according to the recommendations for glycemic control in patients with DM as previously reported [5]. In this study, DbCR was defined as a condition showing mainly LV systolic dysfunction (EF < 55%) with DM and without hypertension, severe valvular disease, or obstructive CAD. Selection of patients for the study is shown schematically in Figure 1. Exclusion criteria in this study included hypertension, severe valvular disease, and LV function of EF < 55%, with or without obstructive CAD. In practice, a total of 17 patients was excluded from this study, including 9 patients with hypertension, 7 patients with severe valvular disease (2 with aortic valve stenosis and 5 with mitral valve regurgitation) and 8 patients with LV function of EF ≥55%, (7 patients with hypertension and LVEF ≥55%). The remaining 36 patients were subjected to coronary angiography (CAG) to exclude obstructive CAD. Twenty-two patients whose CAG results showed >50% coronary artery stenosis were excluded from the study. The treatments used in these patients are indicated in Figure 1. The remaining 14 patients (the DbCR group) were enrolled. Fourteen patients who had been previously diagnosed (according to the WHO criteria [17]) as having idiopathic DCM but lacking DM, and whose LVEF matched that of the DbCR group, were included in this study. All of the subjects with DCM were symptomatic heart failure patients without hypertension who showed enlargement of ventricles, normal LV wall thickness, and systolic dysfunction by echocardiography; all received CAG and endomyocardial biopsy. Evaluations for echocardiographic, CAG and histopathological findings were made to confirm that the DCM patients lacked CAD, secondary causes of cardiomyopathy, and valvular diseases. These DCM patients were designated the DCM group and used as the comparator to the DbCR group. Finally, the clinical records of each of the enrolled patients were inspected for the use of guideline-directed medications for heart failure.

The study protocol conformed to the Declaration of Helsinki and was approved by the Human Ethics Committee of Kanazawa Medical University. All patients provided written informed consent before enrollment in the study.

Echocardiographic analysis

All patients underwent echocardiography. Interventricular thickness (IVST), posterior LV wall thickness (PWT), LV
end-diastolic diameter (LVDd), and LV end-systolic diameter (LVDs) were measured by two-dimensional echocardiography. LV end-diastolic volume (LVEDV) and end-systolic volume (LVESV) were measured from the apical four- and two-chamber views (respectively) by the modified Simpson’s method. To assess LV systolic function, LVEF was calculated using the followed formula: 

\[
LVEF = \frac{\text{LVEDV} - \text{LVESV}}{\text{LVEDV}} \times 100\%
\]

To evaluate LV diastolic function, the E-wave (a measurement of the early ventricular filling) and the A-wave (a measurement of the atrial-driven ventricular filling) were determined using trans-mitral Doppler signals, and an E/A ratio was calculated. As tissue Doppler signals of mitral inflow, according to the algorithm for estimating the grade of LV diastolic function, as described in the previously reported guideline [18].

**Catheterization procedure**

CAG was performed through a femoral or a radial approach by using the standard Judkins technique after the administration of 2000 U of heparin. During CAG, 0.25 mg of nitroglycerin was injected routinely into each coronary artery in all patients. Angiographic analysis was carried out by two experienced cardiologists who were blinded to the identity of the patients.

**Biochemical, Glycer-AGE, NT, TNF-α, and brain natriuretic peptide measurements**

Blood samples were collected from an antecubital vein in the morning following an overnight fast. Serum and plasma samples were prepared from each blood sample by centrifugation and were stored at −80°C until assayed. Serum creatinine, plasma fasting glucose, total cholesterol, low-density lipoprotein cholesterol, triglyceride, and C-reactive protein (CRP) levels were measured by an automatic biochemical analyzer (Hitachi, Tokyo, Japan). The serum concentration of HbA1C was determined by a high-performance liquid chromatographic method using an automatic HbA1C analyzer (Tosoh, Tokyo, Japan). Brain natriuretic peptide (BNP) levels were measured automatically by a chemiluminescent immunoassay method using an ARCHITECT i 1000SR analyzer (Abbott Japan, Tokyo, Japan).

Among several oxidative stress markers and inflammatory cytokines related to cardiac dysfunction, this clinical study focused on nitrotyrosine (NT) as an oxidative stress marker and TNF-α as an inflammatory cytokine. Glycer-AGE levels were measured using competitive ELISA with immunopurified anti-Glycer-AGE antibodies: this ELISA has been shown to exhibit reliable intra-assay and inter-assay coefficients of variation (CVs) [19]. NT levels were measured as described previously [20], using a competitive time-resolved fluoroimmunoassay using dissociation-enhanced lanthanide fluorescence immunoassay reagents (PerkinElmer Life Sciences, Boston, MA, USA). The fluorescence intensity was measured at excitation/emission wavelengths of 610/340 nm, respectively, using an Arvo SX multilabel counter (Perkin-Elmer Life Sciences). For NT measurements using the time-resolved fluoroimmunoassay, the intra-assay CVs were 1.2–6.7% and the inter-assay CV across seven concentrations was 9.2% [20]. TNF-α levels were measured using a Human TNF-α Quantikine HS ELISA kit (R&D Systems, Inc., Minneapolis, MN, USA) according to the manufacturer’s instructions.

**Statistical analyses**

Continuous variables were tested for normal distribution of data using the Kolmogorov–Smirnov test. Data for normally distributed continuous variables are expressed as mean ± SD, and a two-tailed nonpaired Student’s t test was used to compare the variables between the two groups. On the other hand, continuous variables with the skewed distribution (total cholesterol, low-density lipoprotein cholesterol, triglyceride, fasting glucose, and BNP) are expressed as median and interquartile range and were logarithmically transformed for further analysis, and Mann-Whitney’s U test was used to compare the variables between the two groups. Categorical variables of the patient’s clinical background and echocardiographic findings were compared using the χ² test. The Spearman’s correlation coefficient was used to assess the relationships between continuous variables. All statistical analyses were performed using JMP software, version 9 (SAS Institute Japan Co., Ltd., Tokyo, Japan). Statistical significance was defined when p < .05.

**Results**

**Clinical characteristics and echocardiographic findings of the studied patients**

Table 1 shows the clinical characteristics and echocardiographic findings of the two groups. In the DbCR group, the duration of DM (mean ± SD) was 14.9 ± 16.6 years, with 11 patients (78.6%) showing HbA1C levels ≥7.0%, 11 (78.6%) patients using insulin and almost all patients exhibiting complications (retinopathy, neuropathy and/or nephropathy) of DM. Fasting glucose and HbA1C levels in the DbCR group were significantly higher than those in the DCM group. There were no significant differences in mean age, sex, body mass index, lipid profiles, creatinine, or CRP levels. Additionally, the two groups did not differ significantly in their BNP levels, a parameter that represents the severity of heart failure. Consistent with the BNP observation, there were no significant differences between the two groups in the frequencies of three phenotypes of heart failure. However, the use of β-blockers and mineralocorticoid receptor antagonists in the DCM group was significantly higher than that in the DbCR group.

Although there were no significant differences between the two groups in LVEF, a parameter that represents the severity of cardiac function, LVDd and LVDs in the DCM
The mean E/A ratio in the DbCR group was nominally lower than that in the DCM group, but this difference did not achieve statistical significance. There were no significant differences between the two groups in the grade of LV diastolic function.

The levels of serum Glycer-AGEs, NT, and TNFα

The mean levels of serum Glycer-AGEs, NT, and TNFα in the two groups are shown in Figure 2(A–C) (respectively): values are presented in Table 2. The mean levels of serum Glycer-AGEs, NT, and TNFα in the DbCR group were significantly higher than those in the DCM group, with values of 10.0 ± 3.10 vs. 7.11 ± 2.04 U/mL (DbCR vs. DCM, respectively), p = .0073 for Glycer-AGEs (Figure 2(A)); 12.1 ± 6.60

Table 1. Clinical characteristics and echocardiographic findings of the study patients.

|                      | DbCR group | DCM group | p-Value |
|----------------------|------------|-----------|---------|
| n                    | 14         | 14        |         |
| Age (years)          | 62.0 ± 13.8| 53.6 ± 12.8| .11    |
| Male/female patients (n) | 10/4       | 13/1      | .14    |
| BMI (kg/m²)          | 24.8 ± 5.9 | 23.0 ± 3.1 | .34    |
| T-Chol (mg/dL)       | 184 (153-223) | 185 (147-221) | .98    |
| LDL-C (mg/dL)        | 111 (82-127) | 119 (99-126) | .61    |
| TG (mg/dL)           | 152 (89-249) | 102 (74-195) | .18    |
| Fasting glucose (mg/dL) | 185 (141-270) | 104 (99-126) | .0035  |
| HbA1c (%)            | 8.6 ± 1.7  | 5.7 ± 0.4  | <.0001 |
| Cr (mg/dL)           | 1.0 ± 0.28 | 1.30 ± 1.01 | .31    |
| BNP (pg/mL)          | 261 (75-729) | 242 (152-586) | .78    |
| CRP (mg/dL)          | 0.5 ± 0.6  | 0.3 ± 0.4  | .29    |
| Duration of diabetes (years) | 14.9 ± 16.6 |         |        |
| Quality of glycemic control as HbA1c levels, number (%) |                      |
| <7.0%                | 3 (21.4)   |           |        |
| ≥7.0%                | 11 (78.6)  |           |        |
| Phenotype of heart failure, number (%) |                      |
| HFrEF                | 9 (64.2)   | 2 (14.3)  | .62    |
| HFmrEF               | 4 (28.6)   | 3 (21.4)  | .26    |
| HFpEF                | 1 (7.1)    | 1 (7.1)   | .31    |
| NYHA classification, number (%) |                      |
| I                    | 2 (14.3)   |           |        |
| II                   | 9 (64.2)   | 5 (35.7)  | .26    |
| III                  | 3 (21.4)   | 4 (28.6)  | .66    |
| IV                   | 0 (0)      | 1 (7.1)   | .31    |
| Diabetes treatment, number (%) |                      |
| Diet only            | 2 (14.3)   |           |        |
| Metformin            | 5 (35.7)   |           |        |
| Sulfonylureas        | 0 (0)      |           |        |
| α-Glucosidase inhibitors | 1 (7.1)   |           |        |
| DPP-4 inhibitors     | 6 (42.9)   |           |        |
| SGLT2 inhibitors     | 5 (35.7)   |           |        |
| Insulin              | 11 (78.5)  |           |        |
| Guideline-directed medications for heart failure, number (%) |                      |
| β-Blockers           | 8 (57.1)   | 14 (100)  | .0057  |
| ACEI/ARB             | 10 (71.4)  | 12 (85.7) | .36    |
| MRA                  | 0 (0)      | 6 (42.9)  | .0057  |
| Complications of diabetes, number (%) |                      |
| Retinopathy          | 10 (71.4)  |           |        |
| Neuropathy           | 7 (50.0)   |           |        |
| Nephropathy          | 12 (85.7)  |           |        |
| Echocardiographic findings |                      |
| IVST (mm)            | 10.3 ± 2.3 | 10.6 ± 2.2 | .74    |
| PWT (mm)             | 10.7 ± 1.5 | 10.7 ± 1.5 | 1.0    |
| LVDd (mm)            | 51.5 ± 8.4 | 64.6 ± 8.7 | .0004  |
| LVDs (mm)            | 40.5 ± 9.2 | 57.0 ± 8.5 | <.0001 |
| LVEF (%)             | 37.3 ± 8.8 | 33.0 ± 6.7 | .17    |
| Peak E wave velocity (cm/sec) | 79.3 ± 32.0 | 74.9 ± 32.5 | .75    |
| E/A ratio            | 1.1 ± 0.8  | 2.4 ± 2.5  | .18    |
| Grade of LV diastolic dysfunction, number (%) |                      |
| Grade I              | 4 (28.6)   | 3 (21.4)  | .66    |
| Grade III            | 2 (14.2)   | 4 (28.6)  | .36    |
| Not determined       | 8 (57.1)   | 7 (50.0)  | .70    |

Data are presented as mean ± standard deviation or median (interquartile range). Mann-Whitney’s U test was used to compare the variables with skewed distribution (T-Chol, LDL-C, TG, fasting glucose, and BNP) between the DbCR and DCM groups.

ACEI: angiotensin-converting enzyme inhibitors; ARB: angiotensin II receptor blockers; BMI: body mass index; BNP: brain natriuretic peptide; Cr: creatinine; CRP: C-reactive protein; DbCR: diabetic adverse cardiac remodeling; DCM: dilated cardiomyopathy; DPP-4: dipeptidyl peptidase-4; E/A: ratio of early to late mitral valve flow velocity; HbA1c: hemoglobin A1c; HFmrEF: heart failure with a mid-range ejection fraction; HFpEF: heart failure with preserved ejection fraction; HFrEF: heart failure with reduced ejection fraction; IVST: interventricular thickness; LDL-C: low-density lipoprotein cholesterol; LV: left ventricular; LVDd: left ventricular end-diastolic diameter; LVDs: left ventricular end-systolic diameter; LVEF: left ventricular ejection fraction; MRA: mineralocorticoid receptor antagonists; NYHA: New York Heart Association; PWT: posterior LV wall thickness; SGLT2: sodium-glucose cotransporter 2; T-Chol: total cholesterol; TG: triglycerides.

group were significantly larger than those in the DbCR group. There were no significant differences between the two groups in IVST and PWT, indices of LV hypertrophy. The mean E/A ratio in the DbCR group was nominally lower than that in the DCM group, but this difference did not achieve statistical significance. There were no significant differences between the two groups in the grade of LV diastolic function.

The levels of serum Glycer-AGEs, NT, and TNFα

The mean levels of serum Glycer-AGEs, NT, and TNFα in the two groups are shown in Figure 2(A–C) (respectively): values are presented in Table 2. The mean levels of serum Glycer-AGEs, NT, and TNFα in the DbCR group were significantly higher than those in the DCM group, with values of 10.0 ± 3.10 vs. 7.11 ± 2.04 U/mL (DbCR vs. DCM, respectively), p = .0073 for Glycer-AGEs (Figure 2(A)); 12.1 ± 6.60
The existence of diabetic cardiomyopathy is a matter of debate and was not confirmed in the recent guidelines [5]. Nonetheless, patients with DM are known to exhibit a condition showing adverse cardiac remodeling without CAD, hypertension, or valvular disease, but with HFrEF or HFpEF [3–7]. We defined DbCR as the condition of LV systolic dysfunction (mainly HFrEF) in patients with DM and heart failure. Clinically, patients with DbCR had a long duration of DM, and high rates of poor glycemic control, insulin failure. Clinically, patients with DbCR had a long duration of DM, and high rates of poor glycemic control, insulin failure.

**Table 2.** Comparison of Glycer-AGEs, NT and TNFα between the two groups.

|                      | DbCR group | DCM group | p-Value |
|----------------------|------------|-----------|---------|
| Glycer-AGEs (U/mL)   | 10.0 ± 3.10| 7.11 ± 2.04| .0073   |
| NT (µg/dL)           | 12.1 ± 6.60| 6.17 ± 3.10| .005    |
| TNFα (pg/mL)         | 2.20 ± 0.92| 0.81 ± 0.20| <.0001  |

Data are presented as mean ± standard deviation.

DbCR: diabetic adverse cardiac remodeling; DCM: idiopathic dilated cardiomyopathy; Glycer-AGEs: glyceraldehyde-derived advanced glycation end-products; NT: nitrotyrosine; TNFα: tumor necrosis factor alpha.

vs. 6.17 ± 3.10 µg/dL (respectively), p = .0050 for NT (Figure 2(B)); and 2.20 ± 0.92 vs. 0.81 ± 0.20 pg/mL (respectively), p < .0001 for TNFα (Figure 2(C)).

**Association of Glycer-AGE, TNFα and LVEF**

The associations of Glycer-AGE, TNFα with LVEF are shown in Figure 3(A–C) (respectively) for the DbCR group and Figure 3(D–F) (respectively) for the DCM group. In the DbCR group, Glycer-AGE, NT, and TNFα levels showed significant negative associations with LVEF (r = –0.60, p = .022 for Glycer-AGEs; r = –0.54, p = .046 for NT; and r = –0.64, p = .014 for TNFα) (Figure 3(A–C), respectively). In contrast, in the DCM group, there were no associations with LVEF (r = –0.06, p = .82 for Glycer-AGE; r = –0.17, p = .57 for NT; r = –0.02, p = .95 for TNFα) (Figure 3(D–F), respectively).

**Association of Glycer-AGE, NT and TNFα with BNP**

The association of Glycer-AGEs, NT and TNFα with BNP are shown in Figure 4(A–C) (respectively) for the DbCR group and in Figure 4(D–F) (respectively) for the DCM group. The Glycer-AGEs and TNFα levels in the DbCR group showed significant positive associations with BNP (r = 0.77, p = .013 for Glycer-AGEs; and r = 0.82, p = .0003 for TNFα) (Figure 4(A,C), respectively). TNFα in the DCM group also showed significant positive association with BNP (r = 0.84, p = .0002) (Figure 4(F)). However, no associations were detected between NT and BNP levels in the DbCR group (r = 0.08, p = .79) (Figure 4(B)), or Glycer-AGE and NT with BNP in the DCM group (r = –0.14, p = .64 for Glycer-AGE; and r = 0.15, p = .61 for NT) (Figure 4(D,E), respectively).

**Association of NT and TNFα with Glycer-AGE**

The associations of NT and TNFα with Glycer-AGE in the DbCR group are shown in Figures 4 and 5(A,B), respectively; the associations in the DCM group are shown in Figures 4 and 5(C,D), respectively. There was no association between Glycer-AGEs and NT in the DbCR group (r = 0.28, p = .33) (Figures 4 and 5(A)), nor of NT and TNFα with Glycer-AGE in the DCM group (r = 0.34, p = .23 for NT; and r = –0.02, p = .94 for TNFα) (Figures 4 and 5(C,D), respectively). However, the Glycer-AGE levels showed significant positive association with TNFα levels in the DbCR group (r = 0.73, p = .003) (Figures 4 and 5(B)).

**Discussion**

In this clinical study, we showed that serum levels of Glycer-AGE, NT and TNFα in the DbCR group were significantly higher than those in the DCM group. We also showed that the levels of Glycer-AGE and TNFα were strongly associated with LVEF and BNP values, respectively. While the levels of TNFα were significantly associated with BNP, Glycer-AGE and NT were not associated with either LVEF or BNP in patients with DCM. These results suggested that Glycer-AGEs, along with inflammation, play an important role in the development of DbCR; the pathophysiological roles of Glycer-AGEs in DbCR may be distinct from those in DCM.
As a longer duration of DM and microvascular complications of these findings are consistent with previous reports [5,21], these items may be important indications in the patients with DbCR.

The precise mechanisms of the development of DbCR have not been established. However, it has been proposed that both glucotoxicity and lipotoxicity induce vasculomycocardial damage, including microvasculopathy, myocardial hypertrophy, fibrosis, and cell death [3]. This damage is thought to be the result of oxidative stress and chronic inflammation, and to be mediated via the AGE-RAGE axis in patients with DM [3]. Among these causes, the AGE-RAGE axis itself is known to elicit oxidative stress and inflammation [22]. The circulating levels of AGEs have

**Figure 3.** Association of systemic levels of Glycer-AGE (r = -0.60, p = .022) (A), NT (r = -0.54, p = .046) (B), and TNFα (r = -0.64, p = .014) (C) with LVEF in patients of the DbCR group. Association of systemic levels of Glycer-AGEs (r = -0.06, p = .82) (D), NT (r = -0.17, p = .57) (E), and the TNFα (r = -0.02, p = .95) (F) with LVEF in patients of the DCM group. DbCR: diabetic adverse cardiac remodeling; DCM: idiopathic dilated cardiomyopathy; Glycer-AGEs: glyceraldehyde-derived advanced glycation end-products; LVEF: left ventricular ejection fraction; NT: nitrotyrosine; TNFα: tumor necrosis factor alpha.

**Figure 4.** Association of systemic levels of Glycer-AGEs (r = 0.77, p = .013) (A), NT (r = 0.08, p = .79) (B), and TNFα (r = 0.82, p = .0003) (C) with systemic levels of BNP in patients of the DbCR group. Association between of systemic levels of Glycer-AGEs (r = -0.14, p = .64) (D), NT (r = 0.15, p = .61) (E), and TNFα (r = 0.84, p = .0002) (F) with systemic levels of BNP in patients of the DCM group. BNP: brain natriuretic peptide; DbCR: diabetic adverse cardiac remodeling; DCM: idiopathic dilated cardiomyopathy; Glycer-AGEs: glyceraldehyde-derived advanced glycation end-products; NT: nitrotyrosine; TNFα: tumor necrosis factor alpha.
been shown to correlate strongly with both diabetes and congestive heart failure in children with type I DM [9]. Previous studies have demonstrated that, among several AGEs, Glycer-AGEs have the strongest binding affinity for RAGE and that activation of RAGE leads to progressive atherosclerosis in patients with DM \[22–24\]. On the other hand, the clinical features of DCM, such as cardiac enlargement and impaired LV systolic function, resemble those of DbCR. AGEs, as well as oxidative stress and inflammatory markers, have been reported to contribute to the severity of heart failure in patients with DCM \[14,15\]. Therefore, we explored the effect of Glycer-AGEs, which are known to have the strongest binding affinity for RAGE, on the severity of LV systolic dysfunction and heart failure in patients with DbCR compared with effects in patients with DCM who have the same extent of LVEF. In the present study, we demonstrated significant elevation in serum Glycer-AGEs levels in patients with DbCR compared with individuals with DCM. These results suggested that serum Glycer-AGEs may be an important and simple diagnostic marker for distinguishing DbCR and DCM.

The levels of AGEs are increased in patients with chronic heart failure, and the concentrations of such products have been shown to correlate inversely with LVEF and to be related to the severity and prognosis of the disease \[15,25\]. However, there have not been (to our knowledge) any studies showing definite association between AGEs and LVEF or BNP in patients with DbCR. To the best of our knowledge, the present work represents the first to show an association of Glycer-AGEs to LVEF and BNP in patients with DbCR.

TNFα is produced primarily in macrophages, and elevated levels of TNFα in patients with DM have been shown to be associated with micro- and macrovascular complications \[26\]. TNFα is known to reduce myocardial contractility and to increase tissue fibrosis, contributing to cardiac failure \[27\]. A previous study reported that accumulation of RAGE and of RAGE ligands can propagate further inflammation and result in long-term complications in patients of DM \[28\]. The cardiac accumulation of AGEs in diabetes also represents an important inflammation trigger. Sustained activation of TNF signaling is known to induce cardiomyocytes apoptosis and remodeling through the activation of cell death pathways \[29\]. In the present study, serum TNFα also was elevated, and levels of this cytokine were highly associated with LVEF and BNP in patients with DbCR. Furthermore, there was a significant positive association between Glycer-AGE and TNFα levels in patients with DbCR, suggesting the importance of inflammation induced by the AGE-RAGE axis on the progression of DbCR.

In the present study, there were no significant associations between the levels of CRP (a nonspecific inflammatory marker) and LVEF or BNP levels in either the DbCR or DCM groups (data not shown). Although we did not measure high-sensitivity CRP (hs-CRP), it has been reported that higher levels of hs-CRP are associated with a higher risk of incidence or progression of heart failure in patients with
DM [30]. Further research will be needed to evaluate whether hs-CRP contributes to the progression of DbCR.

Hyperglycemia induces the production of reactive oxygen species (ROS) and increases nitric oxide (NO) synthase activity, resulting in NO production [31]. Subsequently, peroxynitrite (generated in the vasculature from the reaction of NO with ROS) can oxidize many molecules, leading to endothelial dysfunction and the formation of atherosclerotic lesions [32]. Direct measurement of short-lived ROS is extremely difficult. However, the production of peroxynitrite can be inferred indirectly by the presence of NT [33]. It has been reported that ROS-mediated cell death may promote abnormal cardiac remodeling, which ultimately may contribute to the characteristic morphological and functional abnormalities that are associated with diabetic cardiomyopathy [34]. In our study, serum NT levels showed significant elevation and association with LVEF, but NT levels did not show a significant association with BNP or Glycer-AGEs in patients with DbCR. A previous study reported that myocardial levels of NT (detected by immunohistochemical analysis) in patients with diabetic cardiomyopathy were fivefold higher than those in patients with DCM [35]. Therefore, circulating levels of NT may not reflect myocardial ROS leading to LV systolic dysfunction and heart failure.

Diabetic cardiomyopathy is characterized by LV diastolic dysfunction and/or systolic dysfunction, pathological LV hypertrophy, and interstitial fibrosis, independent of hypertension and CAD [36]. Some of the studied patients had various degree of LV diastolic dysfunction combined with LV systolic dysfunction. On the other hand, very few of the patients in the present study showed remarkable LV hypertrophy on echocardiography. Therefore, further studies will be needed to determine any association between Glycer-AGEs and the extent of diastolic dysfunction, and histopathological analysis will be needed to evaluate myocardial hypertension and/or interstitial fibrosis in patients with DbCR.

Limitations

The present study has some limitations. Firstly, in this clinical study, we could not evaluate Glycer-AGE levels in normal subjects as controls, given ethical concerns regarding the use of various tests in otherwise healthy subjects. A previous paper has reported serum levels of serum Glycer-AGEs can oxidize many molecules, leading to endothelial dysfunction and the formation of atherosclerotic lesions [32]. Direct measurement of short-lived ROS is extremely difficult. However, the production of peroxynitrite can be inferred indirectly by the presence of NT [33]. It has been reported that ROS-mediated cell death may promote abnormal cardiac remodeling, which ultimately may contribute to the characteristic morphological and functional abnormalities that are associated with diabetic cardiomyopathy [34]. In our study, serum NT levels showed significant elevation and association with LVEF, but NT levels did not show a significant association with BNP or Glycer-AGEs in patients with DbCR. A previous study reported that myocardial levels of NT (detected by immunohistochemical analysis) in patients with diabetic cardiomyopathy were fivefold higher than those in patients with DCM [35]. Therefore, circulating levels of NT may not reflect myocardial ROS leading to LV systolic dysfunction and heart failure.

Diabetic cardiomyopathy is characterized by LV diastolic dysfunction and/or systolic dysfunction, pathological LV hypertrophy, and interstitial fibrosis, independent of hypertension and CAD [36]. Some of the studied patients had various degree of LV diastolic dysfunction combined with LV systolic dysfunction. On the other hand, very few of the patients in the present study showed remarkable LV hypertrophy on echocardiography. Therefore, further studies will be needed to determine any association between Glycer-AGEs and the extent of diastolic dysfunction, and histopathological analysis will be needed to evaluate myocardial hypertension and/or interstitial fibrosis in patients with DbCR.

This study used a small sample size. Further studies with a larger sample population will be needed to confirm whether Glycer-AGEs play a critical role in the development of DbCR.

Conclusions

Both Glycer-AGEs and TNFα showed close negative association with LVEF and positive with the levels of BNP in patients with DbCR. Glycer-AGEs and TNFα may play a pathological role in the development of DbCR, and may be of use as diagnostic markers of DbCR.

Disclosure statement

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References

[1] Di Angelantonio E, Kaptoge S, Wormser D, Emerging Risk Factors Collaboration, et al. Association of cardiometabolic multimorbidity with mortality. JAMA. 2015;314(1):52–60.
[2] Taccredi M, Rosengren A, Svensson AM, et al. Excess mortality among persons with type 2 diabetes. N Engl J Med. 2015;373(18):1720–1732.
[3] Bando YK, Murohara T. Diabetes-related heart failure. Circ J. 2014;78(3):576–583.
[4] Lehrke M, Marx N. Diabetes mellitus and heart failure. Am J Cardiol. 2017;120(15):S37–S47.
[5] Cosentino F, Grant PJ, Aboyans V, ESC Scientific Document Group, et al. 2019 ESC guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. Eur Heart J. 2020;41(2):255–323.
[6] Lorenzo-Almorós A, Tuñón J, Orejas M, et al. Diagnostic approaches for diabetic cardiomyopathy. Cardiovas Diabetol. 2017;16(1):28.
[7] Boudina S, Abel ED. Diabetic cardiomyopathy revisited. Circulation. 2007;115(25):3213–3223.
[8] Bodiga VL, Eda SR, Bodiga S. Advanced glycation end products: role in pathology of diabetic cardiomyopathy. Heart Fail Rev. 2014;19(1):49–63.
[9] Brunvand L, Heier M, Brunborg C, et al. Advanced glycation end products in children with type 1 diabetes and early reduced diastolic heart function. BMC Cardiovasc Disord. 2017;17(1):133.
[10] Zhao J, Randive R, Stewart JA. Molecular mechanisms of AGE/RAGE-mediated fibrosis in the diabetic heart. World J Diabetes. 2014;5(6):860–867.
[11] Miyata T, Sugiyama S, Saito A, et al. Reactive carbonyl compounds related uremic toxicity (“carbonyl stress”). Kidney Int Suppl. 2001;78:525–531.
[12] Schleicher ED, Wagner E, Nerlich AG. Increase accumulation of the glycoxidation product N"-(carboxymethyl) lysine in human tissues in diabetes and aging. J Clin Invest. 1997;99(3):457–468.

[13] Takeuchi M. Serum levels of toxic AGEs (TAGE) may be a promising novel biomarker for the onset/progression of lifestyle-related disease. Diagnostics. 2016;6(2):23.

[14] Wojciechowska C, Romuk E, Tomasik J, et al. Oxidative stress markers and c-reactive protein are related to severity of heart failure in patients with dilated cardiomyopathy. Mediators Inflamm. 2014;2014:1–10.

[15] Hartog JWL, Voors AA, Schalkwijk CG, et al. Clinical and prognostic value of advanced glycation end-products in chronic heart failure. Eur Heart J. 2007;28(23):2879–2885.

[16] McDonagh TA, Metra M, Adano M, ESC Scientific Document Group, et al. 2021 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J. 2021;42(36):3599–3726.

[17] Richardson P, McKenna W, Bristow M, et al. Report of the 1995 World Health Organization/International Society and Federation of Cardiology task force of the definition and classification of cardiomyopathies. Circulation. 1996;93:841–842.

[18] Naghieh SF, Smiseth OA, Appleton CP, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr. 2016;29(4):277–314.

[19] Tahara N, Yamagishi S, Takeuchi M, et al. Positive association between serum level of glyceraldehyde-derived advanced glycation end products and vascular inflammation evaluated by [18F] fluorodeoxyglucose positron emission tomography. Diabetes Care. 2012;35(12):2618–2625.

[20] Watanabe M, Kawai Y, Kitayama M, et al. Diurnal glycemic fluctuation is associated with severity of coronary artery disease in prediabetic patients: possible role of nitrotyrosine and glycer-aldehyde-derived advanced glycation end products. J Cardiol. 2017;69(4):625–631.

[21] Paulus WJ, Tschöpe C. A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. J Am Coll Cardiol. 2013;62(4):263–271.

[22] Takeuchi M, Takino J, Yamagishi S. Involvement of the toxic AGEs (TAGE)-RAGE system in the pathogenesis of diabetic vascular complications: a novel therapeutic strategy. Curr Drug Targets. 2010;11(11):1468–1482.

[23] Yonekura H, Yamamoto Y, Sakurai S, et al. Novel splice variants of the receptor for advanced glycation end-products expressed in human vascular endothelial cells and pericytes, and their putative roles in diabetes-induced vascular injury. Biochem J. 2003;370(Pt 3):1097–1109.

[24] Yamamoto Y, Yonekura H, Watanabe T, et al. Short-chain aldehyde-derived ligands for RAGE and their action on endothelial cells. Diabetes Res Clin Pract. 2007;77(3):530–540.

[25] Simm A, Wagner J, Gursinsky T, et al. Advanced glycation endproducts: a biomarker for age as an outcome predictor after cardiac surgery? Exp Gerontol. 2007;42(7):668–675.

[26] Vulesevic B, McNell B, Giacco F, et al. Methylglyoxal-induced endothelial cell loss and inflammation contribute to the development of diabetic cardiomyopathy. Diabetes. 2016;65(6):1699–1713.

[27] Westermann D, Van Linthout S, Dhayat S, et al. Tumor necrosis factor-alpha antagonism protects from myocardial inflammation and fibrosis in experimental diabetic cardiomyopathy. Basic Res Cardiol. 2007;102(6):500–507.

[28] Xue J, Ray R, Singer D, et al. The receptor for advanced glycation end products (RAGE) specifically recognizes methylglyoxal-derived AGEs. Biochemistry. 2014;53(20):3327–3335.

[29] Frati G, Schirone L, Chimenti I, et al. An overview of the inflammation signaling mechanisms in the myocardium underlying the development of diabetic cardiomyopathy. Cardiovasc Res. 2017;113(4):378–388.

[30] Ohkuma T, Jun M, Woodward M, et al. Cardiac stress and inflammatory markers as predictors of heart failure in patients with type 2 diabetes: the ADVANCE trial. Diabetes Care. 2017;40(9):1203–1209.

[31] Hua K, Wang S, Dong W, et al. High glucose increases nitric oxide generation in lipopolysaccharide-activated macrophages by enhancing activity of protein kinase C-ε and NF-κB. Inflamm Res. 2012;61(10):1107–1116.

[32] Peluffo G, Radi R. Biochemistry of protein tyrosine nitration in cardiovascular pathology. Cardiovasc Res. 2007;75(2):291–302.

[33] Ischiropoulos H. Biological tyrosine nitration: a pathophysiological function of nitric oxide and reactive oxygen species. Arch Biochem Biophys. 1998;356(1):1–11.

[34] Khullar M, Al-Shudiefat AAS, Ludke A, et al. Oxidative stress: a key contributor to diabetic cardiomyopathy. Can J Physiol Pharmacol. 2010;88(3):233–240.

[35] Frustaci A, Ciccossanti F, Chimenti C, et al. Histological and proteomic profile of diabetic versus non-diabetic dilated cardiomyopathy. Int J Cardiol. 2016;203:282–289.

[36] Jia G, Whaley-Connell A, Sowers JR. Diabetic cardiomyopathy: a hyperglycaemia- and insulin-resistance-induced heart disease. Diabetologia. 2018;61(1):21–28.

[37] Hyogo H, Yamagishi S, Iwamoto K, et al. Elevated levels of serum advanced glycation end products in patients with non-alcoholic steatohepatitis. J Gastroenterol Hepatol. 2007;22(7):1112–1119.