Correlation between advanced glycation end-products and the expression of fatty inflammatory factors in type II diabetic cardiomyopathy

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Abstract

Diabetic cardiomyopathy (DCM) is one of the most severe complications of diabetes without a clear pathogenesis. This study investigated the adiponectin (APN) and leptin levels in type II DCM, as well as their correlation with advanced glycation end-products (AGEs). From 2011–2013, 78 type II diabetes mellitus (T2DM) cases (40–65 years old) in the Taian region were randomly selected. Based on the results of colour Doppler ultrasonography and coronary angiography, the cases were divided into a simple T2DM group (40 cases) and a DCM group (38 cases). Forty healthy subjects were used as normal control (NC). An enzyme-linked immunosorbent assay was performed to determine the levels of fatty inflammatory factors such as APN, leptin and AGEs, and a correlation analysis was conducted. In the T2DM group, the APN levels were decreased but the leptin and AGE levels were significantly increased compared to the NC group. In the DCM group, the APN levels were decreased but the leptin and AGE levels were significantly increased (P<0.01) compared to the T2DM group. The AGE levels were positively correlated with disease progression and with fasting plasma glucose levels, glycated haemoglobin, insulin resistance and leptin, but were negatively correlated with APN levels. Additionally, the APN and leptin levels were independently related to the AGE levels. Fatty inflammatory factors play a significant role in the progression of both simple T2DM and DCM. The results of this study revealed the pathogenesis of DCM and indicated the potential significance of AGEs in DCM prevention and treatment.

KEY WORDS: Cardiomyopathy; adiponectin; leptin; advanced glycation end-products
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INTRODUCTION

Diabetic cardiomyopathy (DCM) is a special group of secondary cardiomyopathies related to diabetes mellitus (DM) that excludes hypertensive heart disease, coronary artery disease, valvulopathy and other myocardial lesions caused by known diseases [1]. DM-related cardiovascular diseases have become major causes of death in DM patients [2], and mortality in these diseases is 2–3 times higher than in non-DM-associated cardiovascular diseases.

A publication of the American Diabetes Association (2004) revealed the significant role of oxidative stress in the progression of chronic DM complications, including DCM. In particular, excessive reactive oxygen species (ROS) are produced in hyperglycaemia-stimulated cells, which then induce excessive advanced glycation end-products (AGEs) [3]. AGEs can prompt collagen crosslinking, resulting in collagen elasticity loss and decreases pliancy of the arteries and myocardium [4]. Recent studies have shown that type II DM (T2DM) is a chronic inflammatory disease mediated by inflammatory cytokines whose levels have been shown to increase in T2DM. These factors play a significant role in the development of DM and DM-related complications, and they are closely associated with insulin resistance (IR), endothelial dysfunction and apoptosis, and atherosclerosis [5,6]. Studies have specifically revealed that tumour necrosis factor-α (TNF-α) and transforming growth factor-β1 (TGF-β1) are causative factors in myocardial apoptosis and myocardial fibrosis [7,8], while insulin-like growth factor down-regulates myocardial apoptosis and fibrosis [9]. However, few studies have focused on the roles of adiponectin (APN) and leptin in T2DM, although a primary study has indicated that resistance to leptin in T2DM promotes myocardial lesions [10], and a decrease in APN has been shown to promote myocardial lesions [11]. Nevertheless, no study investigating the correlation between inflammatory cytokines and AGEs has been published.

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Based on previous studies, herein we explored the functions of APN and leptin in the development of myocardial lesions and the correlations between these molecules and AGEs.

MATERIALS AND METHODS

Study subjects

A total of 78 T2DM patients (41 men and 37 women, 40–65 years old) that received treatment at the Affiliated Hospital of Taishan Medical University from 2011–2013 were included in this study. All patients were confirmed to have T2DM based on the oral glucose tolerance test (OGTT) according to the 1999 diagnostic standards of the WHO. All included patients underwent routine blood examinations, blood biochemistry analysis, 24-h microalbumin testing, fundus examinations, electrocardiography, cardiac colour ultrasounds, and coronary angiography.

These patients constituted the DCM and simple T2DM groups. The DCM group included 38 patients (21 males and 17 females) and the simple T2DM group included 40 patients (21 males and 19 females). The DCM group was obtained based on the following inclusion criteria described in the literature [12]: 1) DM history ≥ 5 years; 2) age ≤ 65 years; 3) with or without clinical manifestations of cardiac insufficiency measuring below Killip level II; 4) a cardiac colour ultrasound showing local or extensive decreases in myocardial contraction or increased thicknesses of the left ventricular posterior wall and the interventricular septum, left atrial enlargement, and decreased left ventricular function; 5) at least one microangiopathy (diabetic retinopathy or diabetic nephropathy stage I-III); and 6) coronary angiography showing no severe stenosis (stenosis rate <50%). Among these criteria, 4) and 5) were the critical criteria for determining the existence of DCM. The simple T2DM group was obtained based on the following exclusion criteria: 1) age > 65 years old; 2) severe DM complications such as diabetic retinopathy, stage IV-V diabetic nephropathy, or diabetic foot ulcers; 3) heart disease such as hypertensive heart disease, coronary artery disease, or rheumatic valvulopathy; 4) severe liver and kidney function deficiency, cerebrovascular disease, or autoimmune disease; and 5) a history of using an anti-oxidative-stress drug, such as α-lipoic acid or vitamin C or E, within the past month.

After the exclusion of DM patients by glucose tolerance testing (75 g), 40 healthy subjects (20 men and 20 women) were randomly chosen as the normal control (NC) group based on physical examinations.

This study was approved by the Ethics Committee of the Affiliated Hospital of Taishan Medical University. All procedures were performed in accordance with the provisions of the Declaration of Helsinki. All participants signed written informed consent forms.

General physical examination

The items included in the general physical examination included age, disease progression, height, waist circumference, hip circumference, blood pressure and the waist-to-hip ratio (WHR).

Sample collection

After the patients fasted for 10 h, blood samples were obtained to measure the fasting blood glucose (FBG), triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and glycated haemoglobin (HbA1c) levels using an automatic biochemical analyser (7600-020; Hitachi, Tokyo, Japan).

Enzyme-linked immunosorbent assay (ELISA)

The ELISA kit used to determine the leptin and APN levels was manufactured by the Beijing North Biotechnology Research Institute, and the ELISA kit used to determine the AGE levels was purchased from Tianjin Reagent Biotech Company Ltd. A 5-mL sample of vascular blood was first collected in the early morning following fasting and was centrifuged at 3000 rpm for 15 min after standing for 30 min. The supernatant was then harvested and stored at -70°C. ELISAs were performed to determine the levels of leptin, APN and AGEs, and a radioimmunoassay was performed to determine the fasting insulin (FINS) level. Additionally, the IR index was calculated using the Haffner formula: IR=FBG×FINS/22.5.

Statistical analysis

SPSS software version 17.0 was used for the statistical analysis. The measured data are expressed as x±s. *t*-tests were used for comparisons between 2 groups, and variance analysis was performed for comparisons of 3 groups. Moreover, q tests were used for comparisons between any two means, and Pearson's test was applied for correlational analyses between indicators. $P<0.05$ was considered statistically significant.

RESULTS

Comparison of clinical data between groups

The results demonstrated no significant differences among the 3 groups with respect to age, the number of cases, disease progression, composition, the WHR, systolic pressure (SP), or diastolic pressure (DP) or with respect to the TG, TC, HDL or LDL levels. Compared with the NC group, indicators such as FBG, FINS, IR and HbA1c were significantly increased in

the T2DM and DCM groups (P<0.05), whereas a comparison between the T2DM group and the DCM group showed no significant differences (P>0.05) (Table 1).

Changes in the serum levels of APN, leptin and AGE among the 3 groups

Normal APN levels were detected in the NC group, whereas the APN levels were significantly decreased in the T2DM group (P=0.00015). However, the APN levels were significantly lower in the DCM group compared to the T2DM group (P=0.0002). For leptin and AGE, normal levels were detected in the NC group, whereas significantly increased levels were detected in the T2DM group (leptin, P=0.005; AGEs, P=0.0035). However, the leptin and AGE levels in the DCM group were significantly higher than in the T2DM group (leptin, P=0.0012; AGEs, P=0.0027) (Table 2).

Correlation analysis for AGEs

AGEs, which are irreversible products, are important markers of chronic complications of DM [13]. Pearson's correlation analysis showed positive correlations between AGE levels and both IR (r=0.278, P=0.025) and leptin (r=0.598, P=0.006) but a negative correlation between AGE and APN (r=-0.579, P=0.008). Subsequently, a multiple stepwise regression analysis was performed with AGEs as the dependent variable and the correlated factors as independent variables. The results showed that a decrease in APN and an increase in leptin were independently correlated with AGE levels (Table 3).

DISCUSSION

It has been proven that T2DM is a chronic inflammatory disease related to a variety of inflammatory factors [6, 14]. Complications associated with diabetes are closely related to the control of blood glucose, and DCM is no exception. At present, the pathogenesis of DCM is not fully understood, though it is known that DCN results from damage induced by multiple factors. In addition to oxidative stress, changes in APN [15, 16], leptin [17, 18], and AGEs in patients with diabetes are primarily related to blood glucose control, and changes in the levels of these factors promote the occurrence and development of DCM. However, the correlations between these factors are not yet clear. Thus, this is a hot topic in current DCM research.

There are currently no efficient, direct methods for diagnosing DCM in the clinic. Thus, most DCM cases are confirmed by the presentation of clinical symptoms and auxiliary examinations. AGEs, which are the end-products of the non-enzymatic glycosylation of amino acids, lipids and lipoproteins, are closely related to the development of vascular diseases and DM complications. AGEs are widely distributed in the blood, cells and tissues, and the binding of AGEs to their corresponding receptors can activate multiple intracellular signalling pathways that function in oxidative stress, inflammatory reactions and thrombogenesis [19]. A high rate of blood glucose-induced glycosylation of mitochondrial proteins increases free radical levels and prompts the production of AGEs, constituting a vicious cycle [20]. This irreversible production of AGEs results in a failure to maintain blood glucose at normal levels, which not even AGEs can restore. Moreover, accumulation usually increases in tissues. Therefore, AGEs play essential roles in the development of DCM and are considered to be important markers of DCM.

APN is a specific protein secreted by adipocytes. An epidemiological investigation showed that serum APN, a protein closely associated with energetic metabolism, is an independent risk factor associated with decreased T2DM morbidity [21]. The activities of fatty acid oxidation, glycometabolism improvement, anti-inflammation and

TABLE 1. Comparison of clinical data among the three groups

Clinical index	NC	Type 2 diabetes mellitus	DCM
Number of cases (M/F)	40 (20/20)	40 (21/19)	38 (21/17)
Age (years)	54.6±7.9	55.7±7.6	56.5±7.2
Course (years)	-	12.1±3.6	11.6±3.2
TG (mmol/L)	1.57±0.25	1.68±0.30	1.71±0.31
TC (mmol/L)	5.18±1.07	5.15±1.20	5.20±1.21
HDL (mmol/L)	1.13±0.23	1.08±0.21	1.05±0.20
LDL (mmol/L)	3.32±0.37	3.38±0.46	3.45±0.53
WHR	0.89±0.06	0.92±0.08	0.94±0.09
SP (mmHg)	131±15	133±20	135±23
DP (mmHg)	82±10	84±10	86±13
FBG (mmol/L)	5.0±0.8	11.3±3.5*	11.8±4.1*
FINS (mU/L)	8.4±1.8	21.4±5.3*	22.5±4.5*
IR	1.87±0.41	10.75±2.51*	10.50±2.79*
HbA1c (%)	4.83±0.82	8.68±2.53*	8.81±2.60*

*p<0.01 vs. the control group

TABLE 2. Comparison of serum APN, leptin, and AGE levels among the three groups

Group	Case number (M/F)	APN (mg/L)	Leptin (μg/L)	AGEs (kU/L)
NC	40 (20/20)	12.31±3.23	5.51±2.13	34.90±8.25
Type 2 diabetes mellitus	40 (21/19)	10.78±2.76*	7.41±2.54*	75.67±11.56*
DCM	38 (21/17)	5.34±2.12*#	11.65±4.77*#	101.33±15.79*#

*p<0.01 vs. the NC group; #p<0.01 vs. the T2DM group

TABLE 3. Results of the multiple stepwise regression analysis

Variable	Regression coefficient	Standard error	Standardized standard error	t	P
Constant	23.124	6.807	-	3.397	0.001
APN	0.030	0.041	0.029	0.725	<0.001
Leptin	0.878	0.063	0.511	13.908	<0.001
IR	3.147	0.203	0.498	15.483	0.470

anti-atherosclerosis also confirmed that APN is a protective factor in DCM [15]. The results of a study by Schulze, in which a 5-year follow-up was conducted in confirmed cases of T2DM, demonstrated a correlation between APN and cardiovascular events [16]. In the present study, decreased APN levels were found in the T2DM and DCM groups, and the decreases in the DCM group appeared to be more significant than those in the T2DM group. APN also demonstrated a negative correlation with AGEs that was consistent with previous studies [11, 22]. These findings verified that APN is a protective factor in DCM, indicating that the protective activity of APN is associated with AGE production.

Leptin is a peptide hormone secreted by adipocytes that suppresses appetite, decreases intake and increases energy consumption. However, leptin has a protective role that applies only under certain conditions. In particular, leptin resistance, when coupled with either excessive or deficient leptin levels, can result in many pathological changes. At normal levels, leptin inhibits myocardial fibrosis by increasing the synthesis of collagen, which plays a protective role in the heart via left ventricular remodelling [17, 18]. As a result, deficiencies in leptin can cause left ventricular hypertrophy in experimental animals. In obese populations with low leptin levels, leptin can be used in the treatment of obesity and myocardial fibrosis, preventing or relieving harmful ventricular remodelling. Generally, various levels of obesity are found in T2DM cases, with the condition stimulating the increased secretion of leptin. High levels of leptin function in two manners. The first is the prevention of lipotoxicity and excessive fat accumulation, protecting lipid-intolerant, non-fat organs under a continuous caloric surplus and concomitant high blood leptin levels [23]. Second, high blood leptin levels and leptin resistance can aggravate the inflammatory response and oxidative stress in myocardial cells, resulting in increased angiotensin-converting enzyme activity [24]. These increases can stimulate sympathetic outflow, and the autonomic outflow to the kidney can cause increased arterial pressure [25]. These composite factors then cause myocardial hypertrophy, increased fibrous protein levels and an increased cardiac load, resulting in the development of DCM. In the present study, increased leptin was found in the T2DM and DCM groups, and the levels in the DCM group appeared to be more significant. These results were consistent with those of previous studies [26, 27]. Leptin production was also positively correlated with AGE levels, indicating that increased levels of leptin play a facilitating role in the development of DCM. In addition to these factors, the pathogenesis of DCM might be related to the production of AGE, which serves as a non-protective factor.

Although this study confirmed the correlations between AGE levels and APN and leptin levels, it did not explore the molecular mechanisms underlying these correlations. Therefore, causality between AGE levels and APN and leptin levels could not be determined. In particular, the role played by high leptin levels could not be completely determined based on a correlation analysis alone. In the future, the role of leptin in cardiac muscle must be quantified from the perspective of molecular mechanisms. DCM is a complex disease, and therefore its incidence cannot be explained by a single cause. To further clarify the aetiology of DCM, more pro-inflammatory factors such as IL-6, IL-1, TNF-α, CRP, and other indicators of myocardial damage should be investigated in the future. Due to time limitations, we did not research the correlations between APN, glucose, fatty acids, leptin, glycaemia, IR, and cardiac dysfunction in DCM. These correlations or potential causal relationships will be determined in future studies.

CONCLUSION

This study revealed the pathological significance of an imbalance between protective and harmful inflammatory factors in DCM development. Correlation analyses indicated that APN and leptin levels were independently related to AGE levels. The results of this study could play a constructive role in the examination, treatment, evaluation and prognostic assessment of DCM.

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DECLARATION OF INTEREST

The authors declare no conflicts of interest.

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