Plasma Brain Natriuretic Peptide, Endothelin-1, and Matrix Metalloproteinase 9 Expression and Significance in Type 2 Diabetes Mellitus Patients with Ischemic Heart Disease

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
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Background: Type 2 diabetes (DMT2) combined with ischemic heart disease (IHD) promotes the occurrence and development of coronary atherosclerosis. We aimed to provide a theoretical basis for improving patient prognosis through analyzing expression of plasma brain natriuretic peptide (BNP), endothelin-1 (ET 1), and matrix metalloproteinase 9 (MMP-9).

Material/Methods: Enzyme-linked immunosorbent assay (ELISA) was used to detect BNP, ET-1, and MMP-9 levels in 50 patients with DMT2 only (group A), 47 patients with IHD only (group B), 43 patients with comorbid (both) IHD and DMT2 (group C), and 50 health controls (group D). Group C was further divided into single-branch lesion group, double-branch lesions group, and triple-branch lesion group according to coronary angiography, or cardiac function grade II, III, and IV group according to cardiac function, and their BNP, ET-1, and MMP-9 levels were compared.

Results: Compared with group D, TG, diastolic, and systolic blood pressure were all significantly elevated in groups A, B, and C. Group C exhibited obviously higher glycosylated hemoglobin than group A. Gensini score in group C was markedly higher than in group B. Compared with group D, BNP, ET-1, and MMP-9 levels were all increased in groups A, B, and C. Group C showed higher levels of BNP, ET-1, and MMP-9 than group A and B. BNP, ET-1, and MMP-9 levels in the triple-branch lesions group were higher than in the single-branch lesions group and double-branch lesions group. The cardiac function grade IV group presented higher levels of BNP, ET-1, and MMP-9 than did the grade II and III groups. BNP, ET-1, and MMP-9 showed a positive correlation to each other.

Conclusions: BNP, ET-1, and MMP-9 may participate in the occurrence and development of comorbid DMT2 and IHD. They are important objective indicators for evaluating severity and prognosis of patients with comorbid DMA2 and IHD.

MeSH Keywords: Endothelin-1 • Diabetes Mellitus, Type 2 • Matrix Metalloproteinase 9 • Myocardial Ischemia • Natriuretic Peptide, Brain

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Background

Plasma brain natriuretic peptide (BNP) is a type of diuresis sodium peptide separated from pig brain, mainly composed of 32 amino acids and 17 amino acid rings. Clinical studies [1] showed that BNP can inhibit endothelial cell proliferation, cause sympathetic excitement, dilate blood vessels, and cause diuresis and natriuresis. BNP is an important clinical indicator in evaluating stability of cardiac function. Increased ventricular wall tension and ventricular overload may accelerate BNP synthesis and secretion, causing elevation of BNP levels [2].

Endothelin (ET) is a circular peptide composed of multiple amino acid residues. It was first found in pig aortic endothelial cells in 1988 by Yanagisawa et al. It has significant effects in activation of the renin angiotensin aldosterone system, strengthening sympathetic nerve excitability, regulating sodium and water metabolism, promoting cell mitosis and vascular smooth muscle proliferation, contracting blood vessels, and enhancing myocardial contraction [3]. ET-1 is one of the strongest vasoconstrictor substances in the human body. It is mainly synthesized and secreted by vascular endocrine cells, and can directly affect blood vessels and the heart. It is an important predictor for the occurrence and development of heart failure [4].

Metalloproteinases 9 (MMP-9) is an important part of the matrix metalloproteinases (MMps) family. MMP-9 related factors are activated when the body undergoes pathological changes, resulting in a large degree of extracellular matrix degradation. It greatly weakens the plaque fibrous cap and promotes the occurrence and development of coronary atherosclerosis [5]. Type 2 diabetes (DMT2) is one of the most common chronic diseases. It is also a main factor inducing cardiovascular and cerebrovascular diseases. Its high incidence and morbidity are a serious threat human health. Ischemic heart disease (IHD) accounts for about two-thirds of the patients with heart failure. Patient prognosis is especially poor when IHD is comorbid with DMT2 [6]. This study analyzed BNP, ET-1, and MMP-9 levels in patients with DMT2 and IHD, and aimed to provide a data useful in improving prognosis of these patients.

Material and Methods

General Information

We enrolled 50 patients with DMT2 alone, 47 patients with IHD alone, and 43 patients with comorbid (both) IHD and DMT2 between Aug. 2011 and Dec. 2013. Our definition of DMT2 conformed to the diabetes diagnosis standards promulgated by the WHO Diabetes Experts Committee in 1999 [7] and our definition of IHD conformed to the diagnosis standard prescribed by the ACC/AHA adult chronic heart failure diagnosis and treatment

guideline issued in 2009 [8]. Study participants were divided into 4 groups. Group A included 50 DMT2 patients (28 males and 22 females, ages 37–77 years, 59.4±8.2). Group B included 49 IHD patients (27 males and 22 females, ages 34–76years, 58.1±8.3). Group C included 47 DMT2 patients with comorbid IHD (26 males and 21 females, ages 36–79 years, 59.4±9.2). Group D included 50 healthy controls (29 males and 21 females, ages 35–78 years, 58.7±8.5). Inclusion criteria were: (a) 30–80 years old; (2) had disease within the last year; (3) body mass index 19–35 kg/m^2; (4) without diabetes complications such as diabetic nephropathy and diabetic foot; and (5) heart failure caused by myocardial ischemia or coronary heart disease. Exclusion criteria were: (1) hypohepatia and renal insufficiency; (2) acute myocardial infarction; (3) cardiac function class I; (4) heart failure caused by congenital heart disease, cardiomyopathy, valvular heart disease, or thyroid disease; (5) cardiac shock; (6) acute decompensated heart failure, (7) patients with acute or chronic infectious diseases, rheumatic disease, malignant tumor, or in pregnancy or lactation; and (8) diabetes other than type 2. We excluded 2 patients in group B due to acute myocardial infarction. In group C we excluded 4 patients: 3 with acute myocardial infarction and 1 with acute decompensated heart failure. Therefore, there were 50 participants in group A, 47 in group B, and 43 in group C.

Methods

Reagents and instruments

Instruments used included an automatic biochemical analyzer (type 722, Shimadzu, Japan), a microplate reader (type ELX-800, BIO-TCK, USA), a –80°C low temperature refrigerator (Haier, Qingdao, China), a spectrophotometer (type 722, Shimadzu, Japan), MMP-9 ELISA kit (Lengton Biotech, Shanghai, China), and the ET-1 ELISA kit (Jinma Biotech, Shanghai, China).

Glycolipids detection

We extracted 5 ml of venous blood from fasting participants. Fasting plasma glucose (FPG), glycosylated hemoglobin (HbAlc), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and 2-h postprandial blood glucose (2hPG) were detected by automatic biochemical analyzer.

BNP, ET-1, and MMP-9 detection

We centrifuged 5 ml of venous blood at 3000 rpm for 15 min and the plasma was separated. Electrochemiluminescence immunoassay was used for BNP detection, and ELISA was used for ET-1 and MMP-9 detection according to the manufacturer's instructions.

Blood pressure measurement

We measured participants' systolic and diastolic blood pressure at 7 am and 9 am. Before testing, participants were asked to empty their bladders, to abstain from drinking coffee or smoking, and to rest for 5 min. Blood pressure was measured 3 times and averaged.

Coronary artery severity score

The Gensini score system was used to evaluate the severity of coronary arteries in group B and C participants [9]. Gensini score was calculated as vascular stenosis degree × lesions section coefficient, with 100% vascular stenosis=32 points, 91–99%=16 points, 76–90%=8 points, 51–75%=4 points, 26–50%=2 points, and P≤25%=1 point. The various coefficients correspond to the lesions. Lesions in the 1st and 2nd diagonal branch, distal end of circumflex artery, distal end of anterior descending branch, posterior branch of left ventricle, distal end, and middle of right coronary artery, ×1.0; in the middle of the anterior descending branch, ×1.5; in the near end of the anterior descending branch, or near the end of the circumflex artery, ×2.5; in the left main coronary artery, ×5.0; in the rest, ×0.5.

Grouping

Group C was divided into a single-branch lesion group, a double-branch lesions group, and a triple-branch lesion group according to coronary angiography, or cardiac function grade II, III, and IV group according to the cardiac function. The cardiac function classification conformed to New York Heart Association (NYHA) standards [10].

Statistical analysis

All statistical analyses were performed using SPSS20.0 software (Chicago, IL). Numerical data are presented as means and standard deviation (±SD). Differences between groups and times were analyzed by ANOVA. Two-group comparison was performed by Bonferroni test. Linear regression analysis was used for correlation analysis. P<0.05 was considered as statistically significant.

Results

Baseline comparison

The body mass index, sex ratio, age, and LDL-C showed no significant differences between groups (P>0.05). Compared with group D (healthy controls), TG and diastolic and systolic blood pressure were all significantly elevated in groups A, B, and C (P<0.05). TG in group C was the highest (P<0.05). Group

C exhibited obviously higher glycosylated hemoglobin than group A (P < 0.05). The Gensini score in group C was markedly higher than in group B (P<0.05) (Table 1).

BNP, ET-1, MMP-9 comparison

Compared with group D, BNP, ET-1, and MMP-9 levels were all increased in groups A, B, and C. Group C showed higher levels of BNP, ET-1, and MMP-9 than group A and B (P<0.05). There were no significant differences in BNP, ET-1, and MMP-9 expressions between groups A and B (P>0.05) (Table 2).

BNP, ET-1, MMP-9 comparison among groups with different numbers of lesion branches in patients with comorbid DMT2 and IHD

BNP, ET-1, and MMP-9 levels in the triple-branch lesion group were higher than in the single-branch lesion group and double-branch lesion group (P<0.05), and they had higher levels in the double-branch lesion group than in the single-branch lesion group (P < 0.05) (Table 3).

BNP, ET-1, MMP-9 comparison among different cardiac functions in patients with comorbid DMT2 and IHD

The cardiac function grade IV group had higher levels of BNP, ET-1, and MMP-9 than that of the grade II and III groups (P<0.05); and their expressions were significantly higher in the grade III group than in the grade II group (P<0.05) (Table 4).

BNP, ET-1, and MMP-9 correlation analysis in patients with comorbid DMT2 and IHD

Linear regression analysis showed that BNP, ET-1, and MMP-9 were positively correlated to each other (r>0, P<0.05) (Table 5).

Discussion

Previous studies [11] showed that 15% of DMT2 patients present heart failure symptoms, and about 4.2% of the DMT2 cases are complicated with heart failure every year. Heart failure is the end stage of various cardiovascular diseases, including myocardial infarction, ventricular remodeling, and ventricular enlargement. Clinical studies [12] suggested that changes of humoral factors and neurohormonal mechanism are an important mechanism for the occurrence and development of heart failure. Body fluid variation includes the changes in arginine vasopressin (AVP), ET, and BNP. Variations in neurohormonal mechanisms include renin-angiotensin-aldosterone system activation and enhanced vagus nerve excitability. Epidemiological data [13] indicate that the cardiovascular disease morbidity and mortality in diabetics were significantly

Table 1. Baseline comparison.

Index	Group A (n=50)	Group B (n=47)	Group C (n=43)	Group A (n=50)
Age (year)	59.4±8.2	57.9±8.1	59.1±8.9	58.7±8.5
Gender (male/female)	28/22	26/21	24/19	29/21
Systolic blood pressure (mmHg)	134±15*	132±17*	139±18*	117±10
Diastolic blood pressure (mmHg)	80±10	82±11*	81±12*	71±7
BMI (kg/m^2)	23.61±1.32	24.02±2.56	24.31±1.8	24.88±1.89
FPG (mmol/L)	7.17±0.88*	4.97±0.49	7.54±1.07*	4.88±0.42
2h PG (mmol/L)	10.7±4.4	–	10.9±4.8	–
HbAlc (%)	7.2±0.9	–	8.1±1.2##	–
TG	1.74±0.99*	1.79±0.92*	2.23±0.74*,**	1.23±0.43
TC	4.38±0.51	4.23±0.69	4.68±0.67*	4.24±0.71
HDL-C	1.07±0.20	1.06±0.14	0.85±0.11*	1.11±0.12
LDL-C	2.42±0.51	2.47±0.56	2.63±0.47	2.36±0.42
Gensini score	–	16.1±5.8#	53.4±17.1	–
LVEF (%)	58.97±7.64	39.26±4.43*	40.37±5.17*	65.31±9.76
DMT2 duration (year)	1.24±0.59	–	1.29±0.63	–

* Compared with group D, $P<0.05$; ** compared with group A and B, $P<0.05$; # compared with group B, $P<0.05$; ## compared with group A, $P<0.05$.

Table 2. BNP, ET-1, MMP-9 comparison.

Index	Group A (n=50)	Group B (n=47)	Group C (n=43)	Group D (n=50)	P value
BNP (ng/L)	6.05±1.61*,#	6.22±1.79*	24.45±6.03*,**	4.64±1.38	<0.05
ET-1 (ng/ml)	65.26±19.17*,#	66.45±18.35*	96.27±24.29*,**	49.34±13.18	<0.05
MMP-9 (pg/ml)	2257.49±467.13*,#	2318.26±487.51*	3586.54±768.29*,**	1443.79±312.25	<0.05

* Compared with group D, $P<0.05$; ** Compared with group A and B, $P<0.05$; # Compared with group B, $P<0.05$.

Table 3. BNP, ET-1, MMP-9 comparison among different number of lesion branches in patients with DMT2 and IHD.

Index	Single branch lesion group (n=14)	Double branch lesions group (n=15)	Triple branch lesion group (n=14)	P value
BNP (ng/L)	12.21±2.96*,**	20.54±4.03*	27.67±6.91	<0.05
ET-1 (ng/ml)	79.32±20.56*,**	86.11±22.38*	100.14±26.73	<0.05
MMP-9 (pg/ml)	2956.13±410.26*,**	3247.68±589.04*	3971.23±792.52	<0.05

* Compared with triple branch lesion group, $P<0.05$; ** Compared with double branch lesions group, $P<0.05$.

Table 4. BNP, ET-1, MMP-9 comparison among different cardiac functions in patients with DMT2 and IHD.

Index	Grade II (n=9)	Grade III (n=21)	Grade IV (n=13)	P value
BNP (ng/L)	14.19±3.85*,**	22.47±4.76*	30.06±7.22	<0.05
ET-1 (ng/ml)	81.52±21.63*,**	89.41±23.56*	106.17±27.42	<0.05
MMP-9 (pg/ml)	3012.25±437.42*,**	3429.81±624.57*	4329.16±871.74	<0.05

* Compared with grade IV, $P<0.05$; ** Compared with grade III, $P<0.05$.

Table 5. BNP, ET-1, and MMP-9 correlation analysis in patients with DMT2 and IHD.

Index	BNP		ET-1		MMP-9	
	r	*P*	*r*	*P*	*r*	*P*
BNP	–	–	0.641	0.001	0.479	0.013
ET-1	0.436	0.024	–	–	0.583	0.004
MMP-9	0.389	0.017	0.547	0.003	–	–

higher than in patients without diabetes. Worse degree of atherosclerosis may cause heart failure, severe arrhythmia, or sudden cardiac death.

In this study we measured BNP, ET-1, and MMP-9 expressions in patients with DMT2 and IHD. Upon comparing the baseline of each group, patients with comorbid DMT2 and IHD exhibited higher TG than patients with DMT2 alone and patients withe IHD alone. HbAlc was significantly higher in patients with comorbid DMT2 and IHD than in patients with DMT2 alone. The Gensini score is an international general objective standard used to evaluate coronary heart disease severity. A higher Gensini score indicates worse coronary heart disease severity [14]. In this study, patients with comorbid DMT2 and IHD had obviously higher Gensini scores than did patients with IHD alone. This suggests that comorbid DMT2 and IHD increased the degree of coronary atherosclerosis.

BNP is an important indicator of cardiac function improvement. Higher BNP level represents worse heart disease patient prognosis [15]. ET-1 level slightly increases when the cardiovascular load significantly increases in healthy persons; under pathological condition of ventricular wall overload, it clearly up-regulates and leads to myocardial fibrosis, vasospasm, and smooth muscle cell apoptosis or cracking [15]. MMP - 9 is involved in inflammation, angiogenesis, and extracellular matrix destruction, and leads to plaque production and rupture. This results in a large amount of MMP-9 in the blood and raises its level in serum [16]. Table 2 shows that BNP, ET-1, and MMP-9 levels increased in all groups, but they were higher in patients with comorbid DMT2 and IHD. This indicates that BNP, ET-1, and MMP-9 expression is elevated in patients with comorbid DMT2 and IHD.

Elgebaly et al. [17] suggested that BNP and ET-1 levels in coronary heart disease patients were significantly higher than in healthy subjects, and they have a close relationship with disease severity and cardiac function classification. Table 3 shows that BNP, ET-1, and MMP-9 levels in the triple-branch lesion group were higher than in the single-branch and double-branch lesions groups, and they exhibited higher levels in the double-branch lesions group than in the single-branch lesion group. This suggests that BNP, ET-1, and MMP-9 increased more obviously following the increase of vascular lesion branches. Table 4 shows that the cardiac function grade IV group presented higher levels of BNP, ET-1, and MMP-9 than did the grade II and III groups, and their expressions were significantly higher in the grade III group than in the grade II group, indicating that BNP, ET-1, and MMP-9 levels increased markedly following the increase in cardiac function grade. When heart failure occurs, the renin-angiotensin-aldosterone system and neuroendocrine system are activated, leading to significantly increased angiotensin II secretion and synthesis. ET-1 concentration increased significantly under the influence of cytokines, ischemia, hypoxia, and endothelial dysfunction [18]. BNP interferes with functioning of the sympathetic nervous system, the renin-angiotensin-aldosterone system, and water sodium retention, and also effectively reduces myocardial fibrosis, and regulates ventricular remodeling and vasodilation [19]. BNP, ET-1, and MMP-9 levels are closely related to the disease severity of patients with comorbid DMT2 and IHD.

Our research also found that BNP, ET-1, and MMP-9 exhibited a positive correlation with each other in patients with comorbid DMT2 and IHD. Papazafiropoulou et al. [20] reported that many smooth muscle cells and macrophages exist in the atherosclerotic plaque of CHD patients, and MMP-9 expression in macrophages and smooth muscle cells up-regulates significantly. MMP-9 may participate in the formation of atherosclerotic plaque in CHD. ET-1 concentration increase directly acts on the vascular smooth muscle and the heart, progressively aggravating ventricular pressure load and stimulating BNP secretion. Increased BNP is an endogenous ET-1 antagonist that counteracts or mitigates ET-1 damage to the heart. The limitations of this study are that the sample size was relatively small and that some patients had no increase in BNP, ET-1, or MMP-9, which may influence the accuracy of our results. Further research with larger sample sizes is needed.

Conclusions

BNP, ET-1, and MMP-9 may participate in the occurrence and development of comorbid DMT2 and IHD synthetically. BNP, ET-1, and MMP-9 are important objective indicators for evaluation of comorbid DMA2 and IHD disease severity and prognosis.

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