Analysis of Endogenous Antioxidant Peptides and Oxidative Stress Levels in Diabetic Cardiomyopathy

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Abstract. To investigate endogenous carnosine, glutathione system and oxidative stress levels in serum of patients with diabetic cardiomyopathy, and analyze relationships among endogenous carnosine, glutathione system and oxidative stress of patients with diabetic cardiomyopathy. The serum of 68 healthy people (NC), 75 patients with type 2 diabetes mellitus (DM) and 57 patients with diabetic cardiomyopathy (DCM) of the third affiliated hospital of QiQihar Medical University were collected. Carnosine content was measured by ELISA. T-GSH, GSH and GSSG were measured by micro-enzyme labeling. Clinical datas were compared: In comparison to NC group, BMI, FPG, 2hPG, C peptide and HbA1c in the DCM group and DM group increased significantly (P<0.05). The average value of human serum carnosine content, GSH content, GSH/GSSG ratio in the DM and DCM group were significantly lower than that in the control one (P<0.05). Serum endogenous antioxidant peptides and reduced glutathione (GSH) level drop and the imbalance of redox state in diabetic cardiomyopathy patients, which promote the occurrence and development of diabetic cardiomyopathy.

Introduction

With the changes in economy situation and life styles, the morbidity of diabetics is mounting up each year, which severely threatens people's health. Diabetic patients for a long lasting exposure to high glucose could produce more ROS and enhance oxidative stress, which could induce cardiomyocyte apoptosis, finally resulting in diabetic cardiomyopathy (DCM) [1]. DCM has been defined as ventricular dysfunction that occurs independently of coronary artery disease and hypertension. It is the major cause of morbidity and mortality in subjects suffering from diabetes mellitus, and gets extensive attention of academe increasingly [2, 3]. Mechanisms of diabetic cardiomyopathy involve glucose and lipid metabolism disorder, insulin resistance, oxidative stress, renin-angiotensin-aldosterone system activation, cardiac autonomic nerve...
dysfunction, cell death. Among them, the enhancement of oxidative stress of cardiac cells might be the early molecular phenomenon of diabetic cardiomyopathy.[4-6].

The imbalance of redox state under diabetics resulted in a series of endogenous antioxidants changes, carnosine and glutathione system of them are significant components of cardiomyocytes redox system[7]. This study investigates endogenous carnosine, glutathione system and oxidative stress levels in patients with diabetic cardiomyopathy in serum, and analyze relationships among endogenous carnosine, glutathione system and oxidative stress of patients with diabetic cardiomyopathy. To explore the molecular biology mechanism of the protective effect of carnosine for diabetic cardiomyopathy. In order to provide a new way and theory for prevention and treatment of diabetic cardiomyopathy.

Material and Methods

General Information

From Jun 2014 to Mar 2015 clinical datas of the third hospital affiliated to QiQihar Medical University were collected, including healthy people 68 cases, of which 34 men and 34 women between ages 45 and 65; type 2 diabetes patients 75 cases, of which 38 men and 37 women with an average age of 55.9; diabetic cardiomyopathy 57 cases, of which 30 men and 27 women with an average age of 57.2. Accepting criteria: (1) In accordance with diagnosis criteria on DCM and T2DM made by the Chinese Diabetes Society (CDS) in 2004[8]; (2) Patients gave and signed informed consent. Fasting plasma glucose (FPG), 2 hours plasma glucose (2hPG), total cholesterol (TC), total triglyceride(TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), C peptide and glycosylated hemoglobin A1C(HbA1C) of all patients were reported.

Specimen Collection

After a 10-hour fast the morning fasting 5ml venous blood of all subjects were obtained to glass, adding of heparin as anticoagulant, after 4℃ high-speed centrifugal (3500 r/min) 10 minutes extract supernatant into test tube in an ice-box under -80℃, sample can be tested for at one time.

Methods

Carnosine content was measured by ELISA in serum levels. T-GSH, GSH and GSSG were measured by micro-enzyme labeling. Assay kits were purchased from Nanjing Jiancheng Bioengineering institute. The absorbance was measured by BioTek Elx800 multifunctional microplate reader.

Statistical Analysis

GraphPad Prism software, version 5.0 was used for all statistical analysis. Laboratory data is represented by mean ± standard deviation, student t-test was used to compare two group means.

Results

Comparison Analysis of Clinical Data among the Groups

Comparison analysis of datas among the groups, cases, sex, TC,TG, LDL, HDL of the groups were not significantly different from those of NC group (P>0.05); BMI, FPG, 2hPG, C
peptide, HbA1c were significantly increased and different from those of NC group (P<0.05), (Table 1).

Table 1. Comparison analysis of clinical data among the groups.

<table>
<thead>
<tr>
<th></th>
<th>NC</th>
<th>DM</th>
<th>DCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>68</td>
<td>75</td>
<td>57</td>
</tr>
<tr>
<td>Man/Woman</td>
<td>34/34</td>
<td>38/37</td>
<td>30/27</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.2±0.5</td>
<td>25.2±0.5*</td>
<td>25.5±0.4*</td>
</tr>
<tr>
<td>FPG (mM)</td>
<td>4.7±0.5</td>
<td>9.3±0.7*</td>
<td>9.8±0.9*</td>
</tr>
<tr>
<td>2hPG (mM)</td>
<td>6.1±0.5</td>
<td>11.9±1.5*</td>
<td>15.8±1.6*</td>
</tr>
<tr>
<td>TC (mM)</td>
<td>4.4±0.4</td>
<td>4.8±0.5</td>
<td>4.9±0.5</td>
</tr>
<tr>
<td>TG (mM)</td>
<td>1.5±0.5</td>
<td>1.9±0.5</td>
<td>1.8±0.6</td>
</tr>
<tr>
<td>LDL (mM)</td>
<td>2.9±0.3</td>
<td>3.1±0.5</td>
<td>3.1±0.6</td>
</tr>
<tr>
<td>HDL (mM)</td>
<td>1.1±0.2</td>
<td>1.0±0.3</td>
<td>1.0±0.3</td>
</tr>
<tr>
<td>C peptide</td>
<td>0.3±0.1</td>
<td>0.6±0.2*</td>
<td>0.7±0.2*</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>4.8±0.5</td>
<td>7.5±0.9*</td>
<td>8.2±2.1*</td>
</tr>
</tbody>
</table>

NC: healthy people; DM: diabetes; DCM: Diabetic cardiomyopathy. *P<0.05 vs NC.

The Change of Human Serum Carnosine Content among the Groups

The average scores of human serum carnosine content were all in a normal range in the NC group, and were under the normal average level in the DM and DCM group. Carnosine content was reduced and was statistically significant difference in DCM group (P<0.05) but not in DM group (P>0.05) compared with NC group. No significant difference and statistical significance were seen between DCM group and DM group (P>0.05). These results showed that disorders are caused in diabetes mellitus and diabetic cardiomyopathy including enhanced oxidative stress, higher antioxidant peptides consumption, lower levels of human serum carnosine, (Figure 1).

![Carnosine Content](image1.png)

Figure 1. To detect human serum carnosine by ELISA method. NC: healthy people; DM: diabetes; DCM: diabetic cardiomyopathy. *P<0.05 vs NC.

Glutathione System Level Analysis in Human Serum among each Group

There was no significant variety among different groups in T-GSH content (P>0.05). GSH content were under the normal average level in the DM and DCM group, and it was statistically significant difference in DCM group (P<0.05). GSSG content in the DM group and DCM group were higher than that of NC group, compared with DCM group and NC group, it was obviously increased and statistically significant (P<0.05). GSH/GSSG ratio in the DM group and DCM
group were lower than that of NC group (P<0.01). GSH-Px activity in the DM group were slightly higher than NC group, DCM group were lower than that of NC group, but these differences showed no significance (P>0.05). GRAC in the DM group and DCM group were lower than that of NC group and it was significantly reduced and statistically significant in DCM group (P<0.05). GST activity in the DM group and DCM group were lower than that of NC group (P<0.01). T-SH content in the DM group and DCM group were lower than that of NC group, and it was reduced in both DM group (P<0.05) and DCM group (P<0.01), (Figure 2). These results showed that disorders of glutathione system are caused in diabetes mellitus and diabetic cardiomyopathy including the descent of reduced GSH, the increasing of oxidized GSSG, reduction ratio of GSH/GSSG, lower activities of GRAC and GST, which indicated that patients appeared abnormal including enhanced oxidation, reduced antioxidant capacity, the imbalance of redox state.

![Figure 2. Glutathione system level analysis in human serum among each group. NC: healthy people; DM: diabetes; DCM: diabetic cardiomyopathy. *P<0.05, †P<0.01 vs NC.](image)

**Discussion**

Cardiovascular complications in diabetics is one of the main death causes in diabetic at present. The earlier performance of diabetic cardiomyopathy is asymptomatic diastolic dysfunction, eventually lead to a decrease in ventricle compliance, impaired contractile function, which eventually lead to congestive heart failure. The mechanism of diabetic cardiomyopathy is unknown, it mainly involves several factors, including disorder of calcium balance, elevation of renin-angiotensin system activity, mitochondria disorder, abnormal metabolic substrate utility [9-11], enhanced oxidative stress is the central nodes of these factors [12,13]. Oxidative stress induced myocardial cells injury in diabetic, the treatment of exogenous antioxidant should be provided, but the curative effect is not so good by its toxic and side effect.

The imbalance of redox state in diabetic, it caused the changes a series of endogenous antioxidants, antioxidant peptides were important components of cardiac myocytes oxidative stress system. Natural antioxidative peptide in organism, mainly included carnosine and glutathione system. Carnosine is a kind of natural water-soluble dipeptide which has high effective bioactivity, can effectively eliminate oxygen derived free radicals, aldehydes,
lipoidase, ROS and so on, with strong antioxidant activity\cite{14}. Carnosine could inhibit intracellular peroxidation but to inhibit membranal peroxidation that it was able to inhibit every oxidation reaction of the whole peroxidation\cite{15}. Liu Y, etc. using HPLC-UV a novel method for quantitation of cardiac muscle carnosine levels, the concentration of carnosine was significantly lower in the diabetes rats group, compared to that in the healthy control rats\cite{16}. Nevertheless, what about human serum carnosine levels in diabetes mellitus and diabetic cardiomyopathy? It is currently not reported. Our results show that serum carnosine levels decrease in diabetes mellitus and diabetic cardiomyopathy patients, DCM group reduced with NC group, a statistically significant difference (P<0.05). Our results coincide with the reports, and further to be confirmed in diabetic cardiomyopathy patients.

Glutathione system is important for anti-oxidant recovery, and plays an important role in maintaining redox state, preventing free radical damage. Research shows the activity of serum GSH, GSH-Px, GR reduce in diabetic\cite{17}, but related indexes of serum glutathione system in diabetic cardiomyopathy has not been published. Our results show that abnormal glutathione system, lower GSH, higher GSSG, decrease in GSH/GSSG ratio, which indicate the enhancement of oxidation, the decrease of antioxidation capacity, the imbalance of redox state in diabetes mellitus and diabetic cardiomyopathy.

Carnosine and glutathione are primary natural antioxidant in organisms, then there are a large amount of peroxiredoxins, such as glutaredoxin and thioredoxin. Glutathione is cofactor of glutaredoxin and can maintain its reducing state. When oxidative stress or energy-deprivation, the lack of glutathione and accumulation of GSSG can cause the increase of oxidized glutaredoxin 1. In our preliminary experiment, the activation of eNOS/NO system is regulated by Grx 1 and coupled with inhibition of JNK and NF-κB signaling pathway which could alleviate the oxidative stress and apoptosis damage in coronary arteries endothelial cells induced by high glucose\cite{18}. Grx 1 resisting cardiocyte apoptosis induced by high glucose is associated with suppressing the apoptotic signal pathway of caspase-8/3 and JNK /c-Jun\cite{19,20}. Our past research also found that carnosine against cardiomyocyte apoptosis induced by high glucose is attributed to the suppression of expression levels of caspase- 9/3 and p-JNK /p-c-Jun\cite{20,21}. So carnosine could be important in preventing or treating diabetic cardiomyopathy in the future, but if it has interact effect with Grx1/GSH system, other endogenous antioxidant such as CAT, so further research is necessary.

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