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Correlations of HMGB1 and CRP with Diabetic Peripheral Neuropathy

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Abstract

Objective: To explore the correlations between high mobility group box 1 (HMGB1) and C-reactive protein (CRP) in serum of patients with diabetic peripheral neuropathy (DPN).

Methods: A total of 103 patients with diabetes mellitus (DM) hospitalized in Department of Endocrinology, The Affiliated Jiangning Hospital of Nanjing Medical University from January to December 2017 were selected, including 55 males and 48 females. They were aged 38-81 years old, with an average of (59.70 ± 9.36). According to whether peripheral neuropathy was complicated, the patients were divided into DPN group (n=43), DM group (n=34) and incipient DM group (ID group, n=26). Thirty healthy people with complete clinical data were selected as normal control group (Control group) through outpatient physical examinations. Gender, age, history of DM and smoking of all subjects were accurately recorded by detailed inquiry. Then their course of disease, smoking history, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were normatively measured and recorded in detail. Clinical indices such as biochemical indices and blood glucose and lipid levels were accurately determined and recorded. The concentrations of serum HMGB1 and CRP were detected by double antibody sandwich method and turbidimetry, respectively. Spearman's correlation analysis was conducted to assess the correlations of concentrations of serum HMGB1 and CRP with clinical parameters. High-concentration HMGB1 and CRP were taken as exposure factors to explore the correlations with DPN.

Results: The levels of serum HMGB1 and CRP in DPN, DM and ID groups were significantly higher than those in Control group ($P<0.05$), and they were significantly higher in DPN group than those in DM and ID groups ($P<0.05$). The serum HMGB1 level was positively correlated with fasting blood sugar (FBS), 2-hour postprandial blood glucose (2hPG), hemoglobin A1c (HbA1c) and low-density lipoprotein levels ($P<0.01$), whereas negatively correlated with high-density lipoprotein (HDL) level. The serum CRP level exhibited positive correlations with HMGB1, FBS, 2hPG, HbA1c and triglyceride levels ($P<0.05$), but a negative correlation with HDL level ($P<0.01$).

Conclusion: The serum levels of HMGB1 and CRP are significantly positively correlated in DM patients. The levels of serum HMGB1 and CRP in DPN patients significantly exceeded those in DM and ID patients, suggesting that abnormal increases in such levels may predict the occurrence of DPN.

Keywords: high mobility group box 1; CRP; diabetic peripheral neuropathy.

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1. Introduction

Diabetic peripheral neuropathy (DPN) causes high morbidity and mortality rates and damages the quality of life, which is mainly typified by pain, abnormal sensation and loss of sensation, affecting up to 50% of diabetes mellitus (DM) patients [1].

It is estimated that by 2030, there will be about 353 million DM patients [2] and as many as 236 million DPN patients worldwide. A series of complicated and multifactorial pathological changes have taken place in the progression from DM to DPN [3].

However, little is known about the etiology of this disease. The disease is also featured with insidious onset, i.e. some patients have suffered from peripheral nerve injury with no physical discomfort during clinical treatment, which leads to missed diagnosis and loss of the opportunities of early diagnosis and treatment, and directly affects the prognosis of patients, thus posing a serious threat to the health care in the medical industry and society. Until now, many researchers have endeavored to find effective molecular markers for the early diagnosis and monitoring of DPN from proteomics [4].

High mobility group box 1 (HMGB1), as a DNA-binding protein located in the nucleus of most mammalian cells, exerts crucial effects on structure and transcription by binding chromatins. It is a chromosome-binding protein involved in cell growth, proliferation, differentiation, migration and nerve growth, being closely related to many diseases such as tumors, autoimmune diseases and cardiovascular diseases [5-7]. HMGB1 is a pro-inflammatory mediator in the development of chronic pain, including neuropathic pain [8]. C-reactive protein (CRP) is a sensitive biomarker for subclinical systemic inflammation, which is associated with insulin resistance, hyperglycemia and dominant type 2 DM [9]. Currently, DM is considered to be a low-grade inflammatory and autoimmune disease [10]. DPN is a complication of DM, with the occurrence and development also closely related to inflammatory responses. In this study, DM patients in different states were allocated into DPN group, DM group and incipient DM group (ID group), and healthy people were included as Control group. The concentrations of serum HMGBI and CRP in patients and healthy subjects were measured by enzyme-linked immunosorbent assay (ELISA) and immunoturbidimetry, respectively, and the correlations of their expressions with clinical characteristics were analyzed.

2. Methods

1 Collection of patients

A total of 103 DM patients diagnosed in Department of Endocrinology, The Affiliated Jiangning Hospital of Nanjing Medical University from January 2017 to December 2017 were selected, including 43 DPN patients, 34 DM patients and 26 ID patients. They were aged 38-81 years old, with an average of (59.70 ± 9.36). Meanwhile, 30 healthy people with normal physical examination results were enrolled into Control group. Informed consent of all subjects was obtained, and their clinical data were collected.

Inclusion criteria for DM and ID patients: 1) Patients meeting the diagnostic criteria for DM proposed by the WHO in 1999, i.e. fasting blood sugar (FBS) concentration ?7 mmol/L; 2) those with clinical features of DM, such as polyuria, thirst, polydipsia, fatigue and weight loss due to unknown reasons; 3) those without symptoms of peripheral neuropathy or abnormality in electromyography. Exclusion criteria for DM and ID patients: 1) Patients with other types of DM such as gestational DM; 2) those suffering from severe heart, liver or kidney dysfunction.

Inclusion criteria for DPN patients: Patients meeting the diagnostic criteria for DPN proposed in the Guidelines for the Prevention and Control of Type 2 DM in China (2010 Edition), and those with neurological symptoms: a. Sensory disturbance, such as acupuncture pain, numbness in hands and feet and loss of temperature sensation. b. Dyskinesia, such as myasthenia and skeletal muscle atrophy. c. In electromyography, conduction disorder occurred in at least two nerves and the conduction velocity slowed down. Exclusion criteria for DPN patients: 1) Patients suffering from severe heart, liver or kidney dysfunction; 2) those suffering from cerebrovascular diseases.

2 Baseline clinical data

(1) The age, gender, smoking history, course of disease and other data of patients were recorded by inquiry.

(2) The weight and height of the subjects were measured to calculate body mass index (BMI) based on the following formula: BMI= weight (kg)/height $(m)^2$.

(3) The diastolic blood pressure and systolic blood pressure (mmHg) were recorded using a mercury sphygmomanometer at an interval of 5 min, and the average was taken.

(4) FBS, 2-hour postprandial blood glucose (2hPG), triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) levels were determined using a Hitachi-912 automatic analyzer (Hitachi, Mannheim, Germany). High-pressure liquid chromatography

(Bio-Rad, Hercules, CA) was utilized to detect HbA1c level.

3 Measurement of serum HMGB1 and CRP concentrations

Sample collection: The subjects were fasted for at least 8 h in advance, and 8 mL of venous blood was extracted in the morning of the next day, which was centrifuged in a centrifuge at 1000 rpm for 5 min. Then the upper serum was transferred to an EP tube with a pipette. Afterwards, the concentration of HMGB1 was detected using an ELISA kit (Shanghai Hengyuan Biochemical Reagent Co., Ltd., China). Subsequently, immunoturbidimetry was conducted to determine serum CRP level.

4 Statistical analysis

All data were statistically analyzed and plotted by using GraphPad Prism 6.0 and SPSS 21.0 software. The quantitative data conforming to normal distribution were expressed as mean ± standard deviation. Comparisons between two groups were performed by the t test, and those among multiple groups were carried out with one-way analysis of variance. The numerical data were represented as n, and intergroup comparisons were conducted with the χ^2 test. Correlations between clinical indices were subjected to Spearman's analysis. P<0.05 was considered statistically significant.

3. Results

1 Baseline clinical data

The baseline clinical data of all subjects are listed in Table 1. There were no statistically significant differences in gender, age, smoking history, BMI and presence or absence of hypertension among DPN group, DM group, ID group and Control group (P>0.05), indicating that the baseline clinical data of the four groups were comparable.

Table 1. *Baseline clinical data*

	DPN group (n=26)	DM group (n=34)	ID group (n=30)	Control group (n=30)	F/χ^2	P
Gender (male/female)	14/12	19/15	22/21	15/15	0.280	0.964
Age	57.27±10.06	59.03±9.85	62.02±11.04	62.97±8.40	2.091	0.105
Smoking history (yes/no)	22/4	28/6	42/1	28/2	6.351	0.096
BMI (kg/m^2)	24.90±2.82	24.29±2.97	24.57±2.96	24.11±3.05	0.382	0.766
Hypertension (yes/no)	5/21	1/33	3/40	1/29	6.928	0.074

2 Levels of biochemical indices

FBS, 2hPG, HbA1c, TG, LDL and HDL significantly differed among DPN group, DM group, ID group and Control group (P<0.05). Specifically, they were significantly higher in DPN group, DM group and ID group than those in Control group. No significant difference was detected in the TG level among the four groups (P>0.05) (Table 2).

Table 2. *Biochemical index levels of DPN, DM, ID and Control groups*

	DPN group (n=26)	DM group (n=34)	ID group (n=30)	Control group (n=30)	F/χ^2	P
FBS (mmol/L)	9.28±3.25[a]	9.18±2.86[a]	9.05±2.30[a]	5.27±0.28	19.963	**0.000**
2hPG (mmol/L)	17.03±6.52[a]	13.74±5.04[a]	14.63±5.62[a]	6.70±0.80	23.136	**0.000**
HbA1c (%)	9.93±1.70[a]	9.22±2.28[a]	10.60±7.15[a]	5.64±0.44	8.430	**0.000**
TC (mmol/L)	4.58±1.28	4.03±1.16	4.63±1.32	4.47±0.54	1.998	0.118
TG (mmol/L)	2.55±1.62[a]	2.17±1.97[a]	1.80±1.06[a]	1.35±0.47	3.957	**0.010**
LDL (mmol/L)	2.51±1.06[a]	2.11±0.75[a]	2.45±0.98[a]	1.74±0.34	5.626	**0.001**
HDL (mmol/L)	1.24±0.34[a]	1.31±0.48[a]	1.33±0.54[a]	2.38±0.43	41.952	**0.000**

[a]Compared with Control group, P<0.05.

3 Serum HMGB1 and CRP concentrations

The concentrations of serum HMGB1 and CRP were compared among the four groups (Table 3 and Figure 1). The serum HMGB1 levels of DPN, DM and ID groups were significantly higher than that of Control group (P<0.05), while it was significantly higher in DPN group than that in DM and ID groups (P<0.05). Similarly, DPN group, DM group and ID group showed a higher CRP level in serum than Control group (P<0.05), and it was significantly

higher in DPN group than that in DM group and ID group (P<0.05).

Table 3. *Serum HMGB1 and CRP concentrations of DPN, DM, ID and Control groups*

	DPN group	DM group	ID group	Control group	F	P
HMGB1 (unit)	10.11±1.61[a]	8.64±1.97[ab]	8.39±2.93[ab]	5.38±1.65	23.164	**0.000**
CRP (mg/L)	5.19±3.26[a]	3.12±1.81[ab]	2.27±1.31[ab]	1.94±1.03	16.359	**0.000**

[a]Compared with Control group, P<0.05; [b]compared with DPN group, P<0.05.

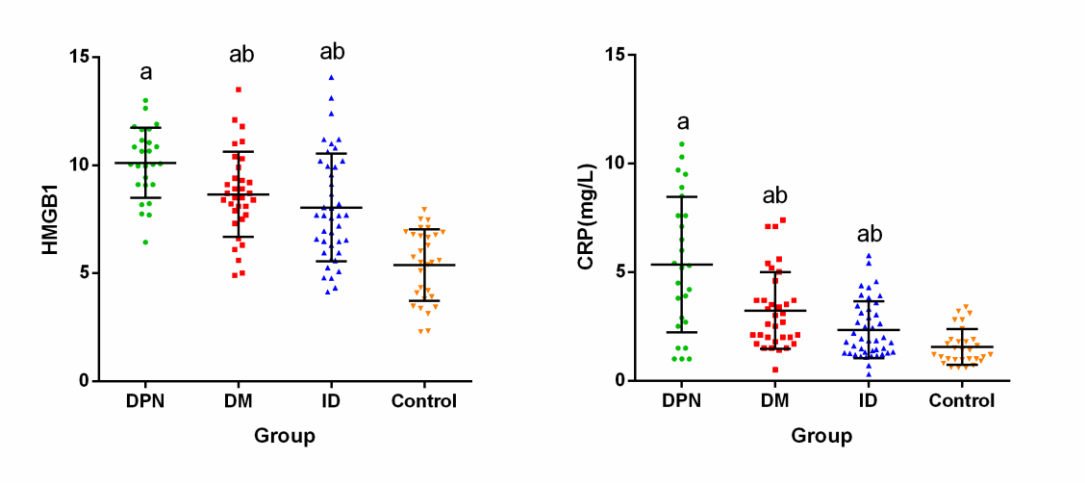

Figure 1. *Serum HMGB1 and CRP concentrations of DPN, DM, ID and Control groups.*

4 Correlations of serum HMGB1 and CRP concentrations with clinical parameters

The correlations of serum HMGB1 and CRP concentrations with clinical parameters were assessed for the four groups (Table 4). The concentration of serum HMGB1 had significant positive correlations with FBS, 2hPG, HbA1c and LDL levels (P<0.01), a significant negative correlation with HDL level (P<0.01), and no correlation with TC or TG level (P>0.05). The concentration of serum CRP displayed significant positive correlations with FBS, 2hPG, HbA1c, TG and HMGB1 levels (P<0.05), a significant negative correlation with HDL level (P<0.01), and no correlation with TC or LDL level (P>0.05).

Table 4. *Correlations of serum HMGB1 and CRP concentrations with clinical parameters*

Clinical parameter	HMGB1 (unit)		CRP (mg/L)	
	r	P	r	P
FBS (mmol/L)	0.521	**0.000**	0.256	**0.003**
2hPG (mmol/L)	0.453	**0.000**	0.325	**0.000**
HbA1c (%)	0.592	**0.000**	0.247	**0.004**
TC (mmol/L)	0.045	0.605	- 0.031	0.724
TG (mmol/L)	0.061	0.490	0.209	**0.016**
LDL (mmol/L)	0.240	**0.005**	0.105	0.228
HDL (mmol/L)	- 0.320	**0.000**	- 0.309	**0.000**
HMGB1 (unit)	1	**0.000**	0.289	**0.001**

4. DISCUSSION

Up to 50% of DM patients will suffer from peripheral neuropathy, which is a result of the change of microenvironment of peripheral sensory nerves caused by microangiopathy and the direct effect of high glucose on peripheral sensory neurons. Owing to the lack of glucose intake regulated by insulins, sensory neurons are

particularly vulnerable to hyperglycemia. Upon DM, the injury of peripheral sensory fibers of the nerve trunk induces the development of local inflammatory responses and neuropathic pain, including hyperalgesia symptoms. The initiation of long-term inflammation and immune-mediated neuropathophysiology has a close correlation with the occurrence and maintenance of neuropathic pain.

HMGB1, a ubiquitous nuclear DNA-binding protein, is able to modulate gene expression. It can be transferred from the nucleus to the cytoplasm and extracellular environment via discrete secretory pathways or passive pathways of apoptotic and necrotic cells under pathogenic or stress conditions. HMGB1 released out of cells interacts with advanced glycation end product receptor or Toll-like receptor 4, thereby activating inflammatory pathways and stimulating inflammatory responses. The binding of HMGB1 to the receptor triggers the activation of NF-?B, thereby leading to the secretion of various pro-inflammatory cytokines, including interleukin-1 (IL-1), IL-6 and tumor necrosis factor (TNF-α). Besides, HMGB1-mediated receptor binding initiates the phosphorylated downstream signaling pathways of extracellular signal-regulated kinase, p38 mitogen-activated protein kinase and c-Jun N-terminal kinase, and such a positive feedback loop further facilitates the release of pro-inflammatory cytokines. Shibasaki et al. found through anti-HMGB1 antibody treatment in the neuropathic pain model that the HMGB1 expression was increased in peripheral nerves after nerve injury, suggesting that this protein participates in the occurrence of pain hypersensitivity. Herein, the concentration of serum HMGB1 in DM patients was significantly higher than that in normal people, and it was significantly higher in DPN patients than that in DM patients, revealing the correlations of the serum HMGB1 level with DPN and DM. The body of DM patients is in a state of low-grade inflammation, and the abnormal high expression of HMGB1 may influence the evolution process from DM to DPN in patients. Additionally, the results of correlation analysis demonstrated that the concentration of serum HMGB1 displayed positive correlations with FBS, 2hPG, HbA1c and LDL of DM patients, and a negative correlation with HDL, which further proved the involvement of HMGB1 in the pathogenetic processes of DPN and DM.

CRP is a sensitive biomarker for subclinical systemic inflammation, which is associated with insulin resistance, hyperglycemia and obvious type 2 DM. Wang et al. found that the CRP level was significantly correlated with the incidence rate of DPN. Studies in China have proven that serum CRP was related to endocrine disorders, insulin resistance and obesity. In this study, the serum CRP concentration in DPN, DM and ID patients was significantly higher than that in normal people, and it was significantly higher in DPN patients than that in DM and ID patients. The above results indicate that CRP is associated with the occurrence and development of DPN. CRP is modulated by IL-6 and TNF-α, which stimulates the release of vascular endothelial growth factors, thus increasing the basement membrane thickness of capillary vessels, reducing the blood flow in nerve tissues, causing ischemic necrosis and finally inducing the evolution into nerve injury. However, HMGB1 can facilitate the secretion of IL-6 and TNF-α, thereby indirectly contributing to the influence of CRP on the pathogenesis of DPN. We also verified that there was a significant positive correlation between HMGB1 and CRP. Moreover, CRP had significant positive correlations with FBS, 2hPG, HbA1c and TG levels, and a significant negative correlation with HDL level, revealing the involvement of CRP in both DPN and blood lipid metabolism.

In summary, serum HMGB1 and CRP are abnormally highly expressed in DM patients, and their levels in DPN patients are significantly higher than those in DM and ID patients. Furthermore, the two indices are positively associated with each other and may play a synergistic role in the occurrence and development of DPN, so they are expected to work as effective predictors for monitoring DPN.

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