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Research on the Correlation between Plasma BNP and the Condition and Prognosis of Chronic Heart Failure

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ABSTRACT

This study aimed to analyze the correlation between the level of brain natriuretic peptide (BNP) and the condition and prognosis of chronic heart failure (CHF). For this purpose, between January 2017 and July 2020, we recruited a total of 120 CHF patients who were treated in Cangzhou Central Hospital into this study, consisting of 40 patients in NYHA II, 40 in NYHA III and 40 in NYHA IV, and simultaneously, 40 subjects with normal heart function were enrolled into the control group. General data were collected from the patients and subjects, and laboratory tests regarding the BNP level, LVEDD and LVEF were carried out. Correlation between plasma level of BNP and the evaluation of condition and prognosis of CHF was also analyzed. For further evaluation, the expression of BNP gene expression was considered in the femoral blood of participants by the Real-time PCR technique. The results showed that comparison over the clinical data of subjects among these groups showed no significant difference ($P > 0.05$). In the research group, the averages of LVEDD and BNP in plasma were (58.53±3.75) mm and (5089.86±22.39) pg/mL, significantly higher than those in the control group, while the LVEF was (45.66±3.42) %, which was lower than that in the control group (all $P < 0.05$). For the subgroup comparison, as the NYHA class augmented, patients also had a significant increase in the mortality rate and the difference among subgroups had statistical significance ($P < 0.05$). Furthermore, with 442 pg/mL as a critical point for BNP, patients in the research group were further divided into the BNP>442 pg/mL group and BNP ≤442pg/mL group, and the statistical analysis revealed that in the BNP>442 pg/mL group, the incidence rate of cardiovascular events, re-hospitalization rate and mortality rate were 53.53%, 14.14% and 7.07%, which were all significantly higher than those in the BNP ≤ 442 pg/mL group (all $P < 0.05$). Also, BMP gene expression was increased in NYHA II, NYHA III, and NYHA IV groups compared to the control group. However, this increase was statistically significant in the NYHA III group ($P <0.05$) and in the NYHA IV group ($P <0.01$) compared to the control group. In conclusion, the level of BNP in plasma can reflect the condition of CHF and is critical to the clinical diagnosis, treatment and prognosis evaluation and evaluation of the BNP gene confirmed the result. Thus, it is worth being promoted in clinical practice.

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Introduction

Heart failure (HF) or chronic heart failure (CHF) occurs when the heart is unable to pump enough blood to the body's organs. Signs and symptoms of the disease include shortness of breath, extreme tiredness, and leg swelling (1). Shortness of breath usually worsens with exercise, lying down, and sleeping at night. Common causes of CHF include coronary artery diseases such as heart attack, high blood pressure, atrial fibrillation, alcohol abuse, and cardiomyopathy (2). The disease is often accompanied by symptoms such as increased enzymes (troponin, T creatine kinase, etc.) and heart hormones (BNP and ANP). Therefore, measuring the values of these factors can have diagnostic value in heart failure (3).

Brain natriuretic peptide (BNP), the heart hormone, is more critical with its many physiological effects, including its impact on the kidneys (increase in urine volume and electrolytes, increase in sodium excretion and consequently their natriuretic properties and increase in glomerular filtration), heart (decrease in output Ten and coronary artery compliance), lung (dilation of bronchi and abdomen), central nervous system (prevention of sympathetic activity, increased lipolysis, regulation of body temperature, increased heart rate, and decreased volume and blood pressure), other hormones (prevention of arginine secretion and Vasopressin, reduced plasma renin activity, inhibited

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catecholamine activity, decreased aldosterone and cortisol, increased testosterone, inhibited pancreatic secretion, disrupted insulin secretion and metabolism), and vascular smooth muscle (hypotension) (4-7).

BNP levels increase in patients with heart disease and myocardial necrosis, a sign of left ventricular dysfunction, and higher in patients with ventricular dysfunction (7). Elevated BNP levels indicate severe coronary artery disease and may have diagnostic value in heart failure. The lower the production and secretion of this hormone and showing minor damage to the heart tissue due to myocardial infarction, the lower the risk of kidney disease and its complications (8).

In general, the CHF has already been seen as the endpoint event of most patients with cardiovascular diseases, with poor prognosis, and one of the major causes responsible for the death of patients, which emphasizes the significance in precise diagnosis and evaluation of prognosis (9, 10). Thus, early diagnosis and prognostic evaluation are of great clinical significance (11). However, insufficient quantitative indicators are evaluating the condition of CHF so far. BNP in plasma, as a bioactive, natural hormone synthesized and secreted by ventricular cells, is regulated by the tension of the ventricular wall and extension of left ventricle. Thus, heart failure may activate the natriuretic peptide system to enlarge the release of BNP, especially for the ventricular dysfunction, thereby regulating the heart function (12-14). In this study, we focused on the correlation between the level of BNP in plasma and the condition and prognosis of CHF, aiming to provide reference for clinical diagnosis and treatment.

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Materials and methods

General data

Between January 2017 and July 2020, we recruited a total of 120 CHF patients aged at an average of (61.96±4.54) years who were treated in Cangzhou Central Hospital into this study, consisting of 40 patients in NYHA II, 40 in NYHA III and 40 in NYHA IV, with the baseline diseases including dilated cardiomyopathy, valvular heart disease, hypertensive heart disease and coronary heart disease. From the patients, those with severe liver/kidney dysfunction, malignant tumors or severe infection were excluded from this study. Simultaneously, 40 subjects with normal heart function were enrolled into the control group, aged at an average of (61.86±3.43) years, consisting of 25 males and 15 females. Comparison over the general data showed that the difference had no statistical significance, suggesting that the data were compared between two groups ($P > 0.05$). This study was approved by the Ethical Board of Cangzhou Central Hospital and carried out at the prerequisite of acquiring the signature of patients on the written informed consents.

Observation indicators

Color Doppler Ultrasonic Diagnostic Machine was used to perform the color ultrasound examination for the heart by measuring the left ventricular end-diastolic diameter (LVEDD) and left ventricular ejection fraction (LVEF) by the same senior clinical physician.

Besides, 3 mL of elbow fasting blood was collected from the patients in the morning and centrifuged at 3000 r/min for 10 min to isolate the plasma that was later preserved at -80°C for measurement of BNP level by using the Enzyme-linked immunosorbent assay (ELISA) with the kit provided by the Radiometer Medical (Denmark). As per the level of BNP (442 pg/mL), patients were divided into two

subgroups (BNP ≤ 442 pg/mL group) and (BNP > 442 pg/mL group) and underwent regular anti-heart failure therapy. During the hospitalization and within 6 months after discharge, the incidence of cardiovascular events, re-hospitalization and death of patients were all recorded, followed by intergroup comparison.

BNP gene expression

4 ml of femoral blood was obtained from all participants in this experiment. After RNA extraction by the RiboPure™ RNA Purification Kit, blood (Thermo Fisher Scientific, USA) the optical absorption of the samples was examined with a spectrophotometer to evaluate the quality and quantity of the extracted RNA. Then, the cDNA was made from RNA by cDNA Synthesis Kit (Sigma-Aldrich, USA). The primer design of the GAPDH4 reference gene and BNP gene was performed with Primer Premier ver.5 software based on the information available in the NCBI gene bank. The primers were then synthesized by Macrogen Inc. (South Korea). The characteristics of the primers are shown in Table 1.

Table 1. The characteristics of the primers for BNP and GADPH genes

Gene	Primer Sequence (5'-3')	Gen Bank	Amplicon	Ta, °C	Efficiency	R^2
BNP	F: ATCTGTCGCCGCTGGGAGGT R: TGGATCCGGAAGGCGCTGTCT	NM_031545	187 bp	60	101.1%	0.999
GAPDH	F: GGCAAGTTCAACGGCACAG R: GACGCCAGTAGACTCCACGAC	NM_017008.4	144 bp	58	104.7%	0.998

After performing normal PCR reactions and obtaining the desired binding conditions and temperature for the genes, the Syber Green method performed real-time PCR using the Corbett 6000 Rotor gene. Real-time PCR reactions were performed at a final volume of 20µl, and each reaction was duplicated. The reaction mixture consisted of 3µl (50 ng/µl cDNA), 8µl RealQ Plus 2x Master Mix Green (Amplioqon, Denmark), 0.4µl of each reciprocating primer (10pmol) and 8.2µl of ribonuclease-free water.

The temperature program used in Real-Time PCR includes a temperature cycle of 95°C for 13 minutes, 40 temperature cycles (95°C for 30 seconds, 60°C for binding of BNP gene primers, 58°C for binding of GAPDH primers, and 72°C for 40 seconds, and extension for 30 seconds). The expression of the genes was measured by $2^{-\Delta\Delta CT}$ method.

Statistical analysis

In this study, data were analyzed in the SPSS 17.0 software. Measurement data were presented by mean±standard deviation, where the difference was validated by the t-test. Enumeration data were presented by the number of cases (or percentage), where the difference was validated by the chi-square test. P<0.05 suggested that the difference had statistical significance.

Results and discussion

Comparison of the clinical data

In comparison over the clinical data between the control group and NYHA II, NYHA III, NYHA IV groups, we found no significant differences (P > 0.05) (Figure 1).

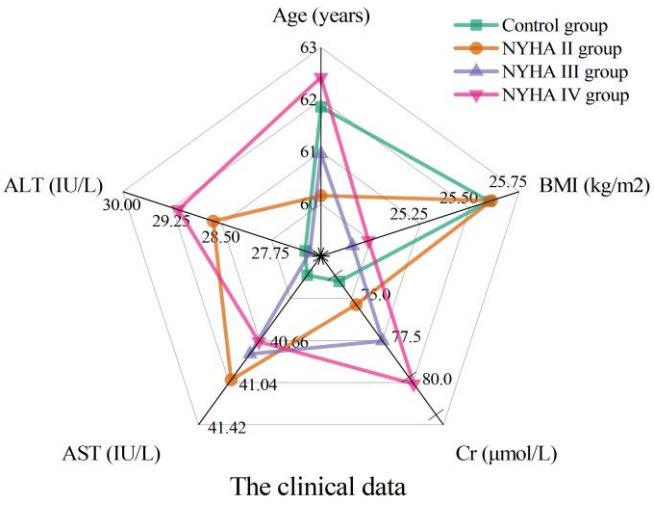

Figure 1. Comparison of the clinical data

Comparison of the levels of LVEDD, LVEF and BNP between two groups

As shown in Figure 2, patients in the research group had significantly elevated LVEDD and BNP, obviously higher than those in the control group (P < 0.05), while the LVEF in the research group was lower (P < 0.05).

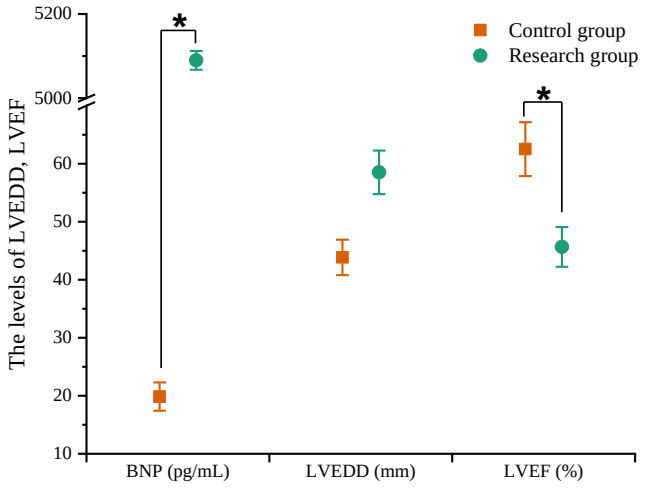

Figure 2. Comparison of the levels of LVEDD, LVEF and BNP between two groups

Comparison of the levels of LVEDD, LVEF and BNP between patients with different NYHA classes

As the NYHA class of patients increased, LVEDD and BNP were increasing, while LVEF was decreasing (all P < 0.05) (Table 2).

Table 2. Comparison of the levels of LVEDD, LVEF and BNP between patients with different NYHA classes (mean ± standard deviation)

Group	Cases (n)	BNP (pg/mL)	LVEDD (mm)	LVEF (%)
NYHA II	40	567.85±14.66	51.65±3.01	54.21±3.64
NYHA III	40	3173.65±20.66	58.65±3.64	45.87±3.80
NYHA IV	40	7658.85±54.22	65.31±4.20	39.05±3.01
P		<0.05	<0.05	<0.05

Comparison of the prognosis between patients with different levels of BNP

In the BNP > 442pg/mL group, the incidence rate of cardiovascular events, re-hospitalization rate and death rate of patients were 53.53%, 14.14 and 7.07%, all significantly higher than those in the BNP ≤ 442 pg/mL group (all P < 0.05) (Figure 3).

Comparison of BNP gene expression between the control, NYHA II, NYHA III, and NYHA IV groups

The results of this part showed that BMP gene expression was increased in NYHA II, NYHA III, and NYHA IV groups compared to the control group. However, this increase was statistically significant in the NYHA III group (P <0.05) and the NYHA IV group (P <0.01) compared to the control group ((Figure 4).

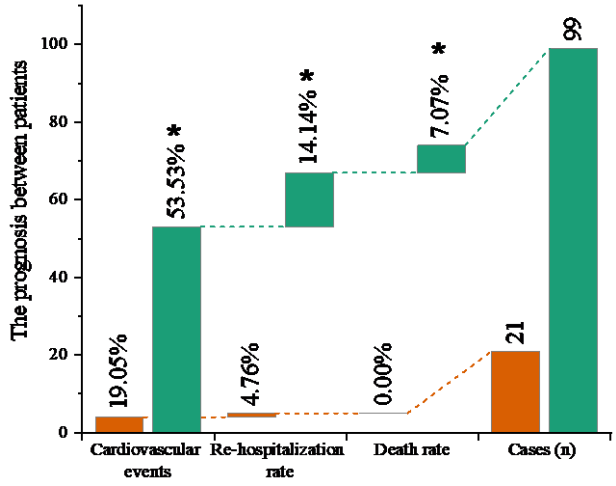

Figure 3. Comparison of the prognosis between patients with different levels of BNP

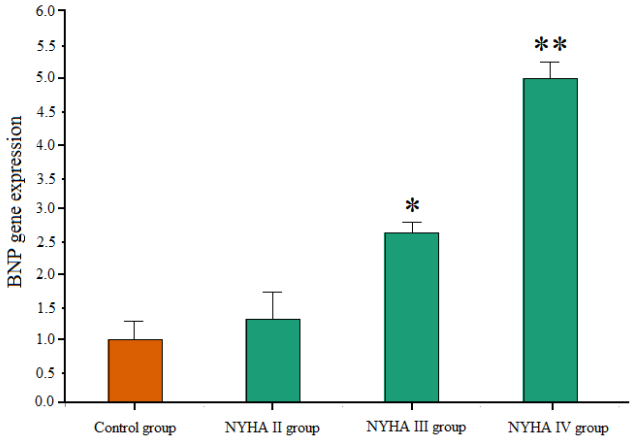

Figure 4. Comparison of BNP gene expression between the control, NYHA II, NYHA III, and NYHA IV groups; *: P<0.05 and **: P<0.01

In recent years, people have witnessed tremendous changes in the lifestyle and diet structure, and also a significant increase in the incidence rate of cardiovascular events (15). CHF, as the endpoint event in the progression of a variety of heart diseases, is a kind of clinical syndrome caused by insufficient ventricular filling due to the functional diseases or deficiency in anatomical structure, or by the damaged ejection of the heart (16). Clinically, CHF is divided into left heart failure, right heart failure and combined heart failure. Generally, CHF is caused by the increased backflow in the ventricular dilation due to the overstressed preload, with clinical manifestation of mitral valve insufficiency or aortic insufficiency

which can lead to the excessive load for the left ventricle in dilation, thereby triggering the heart failure; besides, CHF can be caused by the overstressed afterload resulting from the hypertension or aortic valve stenosis, thus resulting in the left ventricle; furthermore, decreased myocardial contractility is able to induce the myocardial ischemia or cardiomyopathy, which can also contribute to the CHF, and such patients may also exhibit conditions like the insufficient myocardial contraction or incoordination caused by the coronal heart disease or complications of local myocardial ischemia; in addition, due to the weakened ventricular compliance, ventricular dilation is affected, resulting in the alteration of heart function, like ventricular hypertrophy or hypertrophic cardiomyopathy (17-20).

Generally, clinical physicians would evaluate the condition of CHF according to the performance of 6 min walking test, LVEF, LVEDD, functional class of left ventricle and the clinical symptoms, while for some patients with limitation in daily activity or complications of multiple baseline diseases, echocardiogram (ECG) examination is not available for the diagnosis (21). Recent advance in clinical research has uncovered the close correlation of the condition of CHF with a variety of substances or cytokines in a laboratory test. BNP, as a kind of neurohormone derived from the human ventricular muscle, is the polypeptide generated by myocardial cells consisting of 32 amino acids that are in association with the plasma, ventricle, myocardium and neuroendocrine system, manifesting the diuretic effect and vasodilating effect; meanwhile, BNP can inhibit the activity of sympathetic nervous system to compensate the effect caused by the increased myocardial dilation in the ventricular wall, which suggests that heart failure gives rise to the increase in the secretion of BNP and the increase in the BNP content in blood, so BNP level in the blood is critical to the clinical diagnosis, treatment and prognosis of heart failure patients (22, 23). Thus, to investigate the application value of BNP level in plasma in diagnosis and evaluation of prognosis for CHF patients is significant for clinical treatment and improvement of prognosis for these patients.

Our work focused on the correlation between the level of BNP in plasma and the condition and prognosis of CHF patients. As a result, CHF patients had increases in LVEDD (58.53±3.75) mm and BNP (5089.86±22.39) pg/mL, significantly higher than those in the control group, while they had a lower LEVEF of (45.66±3.42) %. Thus, an increase in ventricular load can promote the secretion of BNP and increase the level of BNP in plasma. Furthermore, we noted that LVEDD and BNP increased with the augmentation of NYHA class of patients, while LVEF manifested the opposite changes. Hence, the level of BNP in plasma can reflect the condition of CHF patients precisely. Otherwise, augmentation of NYHA class of patients also contributed to the increase in the mortality rate, while in patients with BNP levels higher than 442pg/mL, the incidence rate of cardiovascular events, re-hospitalization rate and mortality rate were 53.53%, 14.14% and 7.07%, higher than those in the patients with BNP level ≤ 442pg/mL. Hence, the increased level of BNP in plasma can affect the prognosis, with an increase in the risk of death, especially those at NYHA IV. Also, BMP gene expression was increased in NYHA II, NYHA III, and NYHA IV groups compared to the control group. However, this increase was statistically significant in the NYHA III group ($P < 0.05$) and in the NYHA IV group ($P < 0.01$) compared to the control group.

In conclusion, the level of BNP in plasma can reflect the condition of CHF and is critical to the clinical diagnosis, treatment and prognosis evaluation and evaluation of the BNP gene confirmed the result. Thus, it is worth being promoted in clinical practice.

Acknowledgements

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Interest conflict

None.

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