Research Article

Assessment of copeptin, ghrelin and pro BNP in metabolic syndrome

A Emre, K Sibel, B Burcu, A A Gülçin, K Alpaslan, B Cumhur

Abstract

This study was aimed to assessment the relationship between MetS and three different biomarkers (Copeptin, Ghrelin and NT-proBNP) that related to metabolism and cardiovascular system. Metabolic syndrome (MetS) is a multifactorial disorder with metabolic abnormalities including central obesity, insulin resistance, atherogenic dyslipidemia, and hypertension that increase the risk of the development of diabetes and cardiovascular disease (CVD) at highly rates.

Methods: Our study was evaluated according to the metabolic syndrome diagnostic criteria of the American Heart Association/National Heart, Lung and Blood Institute (AHA/NHLBI) and the International Diabetes Federation (IDF). A total of seventy four subjects (44-MetS and 30-healthy) included in this study. Copeptin and ghrelin were determined using the ELISA. The amino-terminal pro-brain natriuretic peptide (NT-proBNP) was measured through chemiluminescence method. The biochemical parameters including blood glucose-insulin values, lipid profiles were also measured.

Results: Total cholesterol, fasting blood glucose, insulin, LDL were significantly higher in the MetS than healthy and HDL was significantly lower. There was no significant difference between thyroid tests. Copeptin was significantly higher in MetS than healthy (p=0.007). Ghrelin was lower in MetS than in the healthy (p=0.002). ProBNP was higher in Mets compared to the control (p=0.028).

Conclusion: Copeptin, Ghrelin and ProBNP are directly related not only in itself but also to various metabolic syndrome components. With the data obtained in our study, it is thought to be a guide in follow-up of high risk patients.

Keywords: Copeptin, Ghrelin, Metabolic syndrome, ProBNP gluconeogenesis; Glycogenolysis

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Introduction

Metabolic syndrome (MetS) is a multifactorial disorder with metabolic abnormalities including insulin resistance, central obesity and hypertension.[1] High blood pressure, increased fasting glucose, decreased HDL cholesterol, and rised triglycerides, all of which are associated with increased weight gain, fat accumulation and enhanced waist size and circumference, are hallmarks of MetS.[2] MetS is highly associated with the development of diabetes and cardiovascular disease (CVD).[2,3]

Natriuretic peptides are cardiac hormones that play crucial roles in the control of metabolic homeostasis by potassium transport, sodium reabsorption, fat mobilization, and regulation of body water and blood pressure. There are three natriuretic peptide (NP) hormones including atrial natriuretic peptide (ANP), brain natriuretic peptide which is also known as B-type natriuretic peptide (BNP), and C-type natriuretic peptide (CNP).[2,4,5] BNP is a circulating peptide with natriuretic and vasodilatory properties that synthesized and released as an inactive prohormone (proBNP) in response to increased mycordial wall stress due to excessive pressure or volume and other circumstances such as inflammation.[6-8] Upon secretion, inactive hormone proBNP is enzymatically cleaved to active mature BNP and N-terminal-pro-B-type natriuretic peptide (NT-proBNP).[8] Both BNP and NT-proBNP are widely endorsed in clinical guidelines as biomarkers to help diagnosis of heart failure and also to monitor progression of the disease.[6,9] The NT-proBNP and BNP are decreased in obese individuals, however, there are a limited number of studies on comparing this relationship in diabetes, overweight/obesity, and MetS.[10]

Arginine vasopressin (AVP), also known as antidiuretic hormone (ADH), is one of the crucial hormones that plays roles in cardiovascular homeostasis.[11] AVP secreted from the neurohypophysis, sustains water homeostasis, effects on osmoregulation, produces vasoconstriction, plays roles in secretion of adrenocorticotropic hormone (ACTH) and glucose and lipid metabolism.[12] Copeptin that comprises the C-terminal part of the AVP precursor (CT-proAVP), is a stable peptide, reflects circulating levels of AVP and can be used as a novel biomarker of AVP release.[12,13] Recently, copeptin has been suggested as a novel biomarker for a diversity of several diseases.[14]

The hormones involved in energy balance regulation, are of special interest as possible for MetS and Type 2 diabetes.15 Ghrelin is an orexigenic circulating peptide with pleiotropic affects stimulates appetite and regulates energy balance, modulates vascular function.[16,17] Whether ghrelin plays a role in the metabolic syndrome development and related diseases, is unknown.[15]

Our study was evaluated according to the metabolic syndrome diagnostic criteria of the American Heart Association/National

Heart, Lung and Blood Institute (AHA/NHLBI) and the International Diabetes Federation (IDF).

Our aimed to determine the relationship among MetS and three different markers including copeptin, proBNP and ghrelin, and whether they are directly related to metabolism and cardiovascular system in this study.

Materials and methods

Metabolic syndrome definition and laboratory measurements

Patients with MetS who were followed up in the Internal Medicine Department of Hitit University Faculty of Medicine and healthy volunteers were included in the study. Detailed anamnesis forms were prepared and filled for each patient. MetS was diagnosed according to the International Diabetes Federation (IDF)/American Heart Association (AHA)/National Heart, Lung and Blood Institute (NHLBI) and the National Cholesterol Education Program Adult Treatment Panel III (NCEP) criteria.[18]

Samples number was determine according to Power analysis before the study. The fourty-four patients with MetS diagnosis and 30 healthy volunteers were included in this study. Blood samples were collected appropriately, centrifuged and their sera were separated and stored at -86 degrees until the study, protected from light until the study was performed.

Routine biochemical examinations of all patients were performed and the levels of various parameters were determined in the blood samples taken after 12 hours of fasting to evaluate the MetS grade. This study was approved by the local ethics committee. Informed consent of the patient was obtained.

Measurement of copeptin and ghrelin levels

Copeptin levels: The Enzyme-Linked Immuno-sorbent Assay (ELISA) technique was used to measure copeptin values in samples according to the manufacturer's instructions. Sunred Biological Technology Co., Shanghai). Optical density (OD) below 450 nm wavelengths were used in the measurement samples.

Ghrelin levels: The kit for the determination of human ghrelin in samples according to the manufacturer's protocol used a double antibody sandwich enzyme-linked immunosorbent assay (ELISA) (200-12-0973; Sunred Biological Technology Co., Shanghai). Optical density (OD) below 450 nm wavelengths was used in the measurement samples.

NT-proBNP levels: Plasma pro-BNP measurements were performed by electrochemiluminescence (ECL) using the Roche Elecsys NT-proBNP kit on the Roche Cobas E411 Test Analyzer (Series no. 16H6-11; 2017, Roche Diagnostics, Germany).

Biochemistry and hormone levels: Routine biochemistry tests were performed spectrophotometrically on the Roche Cobas 6000 C501 autoanalyser (Serial no: 15L2-02; 2015, Roche Diagnostics, Germany). Hormone tests were measured by electrochemiluminescence method on Roche Cobas 6000 E601 (Serial no: 28D9-01; 2017, Roche Diagnostics, Germany).

Statistical analysis

The findings of the study were evaluated statistically using IBM SPSS Statistic 22.0 (IBM Co., Armonk, NY, USA). All data were given as mean ± standard deviation. Statistical significance level was defined as 0.05. Shapiro-Wilk test was used to check whether the data were suitable for normal distribution. Mann Whitney-U test, one of the non-parametric tests, was used due to the lack of normal distribution of data.

Results

A total of 74 inviduals, 44 of whom were diagnosed with MetS and 30 of whom were healthy volunteers, were included in the study. MetS components were investigated according to IDF, NCEP, AHA and NHLBI guidelines. This work was divided into two groups as patient group with MetS and control group without MetS. According to the results of the study, patients diagnosed with MetS were compared with the control group. There was no significant difference between thyroid tests (TSH, T4, and T3) in MetS subjects. Blood samples taken from the control and MetS groups were studied for routine biochemistry tests and their mean values were specified in the reference ranges (Table 1).

Parameters	Control(n=30)	Met S.(n=44)
Total Cholesterol (mg/dl)*	189.21 ± 29.50	198.04 ± 36.29
Triglycerides (mg/dl)	166.10 ± 63.81	175.5 ± 70.3
LDL(mg/dl)*	117.38 ± 28.05	133.97 ± 38.3
HDL(mg/dl)*	38.7 ± 11.5	27.8 ± 4.84
Fasting blood glucose (mg/dl)*	101.6 ± 66.5	110.58 ± 40.38
Insulin(μU/ml)*	5.83 ± 1.7	15.67 ± 3.22
*Control with compared p<0.05		

Table 1. Control and MetS Groups some biochemistry analysis values.

Total cholesterol and LDL values were found significantly higher in the MetS group compared to the control group. On the contrary, HDL levels were found to be significantly lower in the MetS group. Fasting blood glucose and insulin were significantly higher in the MetS group compared to the control group. In the current study, serum copeptin values were significantly higher in patients with metabolic syndrome (0.59 ± 0.47 pmol/l) than those in controls (0.46 ± 0.32 pmol/l) (p=0.007). However, serum ghrelin concentrations were found lower in patients with MetS (65.9 ± 93.0 pmol/l) than those in controls (158.9 ± 48.3 pmol/l) (p=0.002). Serum proBNP values were higher in patients (82.7 ± 67.5 pg/mL) than those in the control group (52.0 ± 20.9 pg/mL) (p=0.028) (Table 2, Figure.1). And also, there were also a negative correlation was found between copeptin and ghrelin values (r=-0.264, p<0.05). Comparing subjects with MetS and healthy controls, we found that total cholesterol, fasting blood glucose,

insulin, LDL values in the MetS group were significantly higher and HDL values were significantly lower than controls. There was no significant difference between thyroid tests (TSH, T4, and T3) in individuals with MetS.

	Control (n=30)		**MetS Individuals (n=44)**			
	Mean ± SD	SEM	Mean ± SD	SEM	p	U
Copeptin (pmol/l)	0.46 ± 0.32	0.05	0.59 ± 0.47	0.07	0.007*	414
Ghrelin (pmol/l)	158.9 ± 48.3	8.81	65.9 ± 93.0	14.02	0.002*	378
ProBNP (pmol/l)	52.0 ± 20.9	3.83	82.7 ± 67.5	10.2	0.028*	460
SD: Std. Deviation, SEM: Std. Error Mean						
U: Mann-Whitney U Test data						
*p<0.05						

Table 2. Descriptive statistics and Mann-Whitney U Test data of Copeptin, Ghrelin and ProBNP values in all groups.

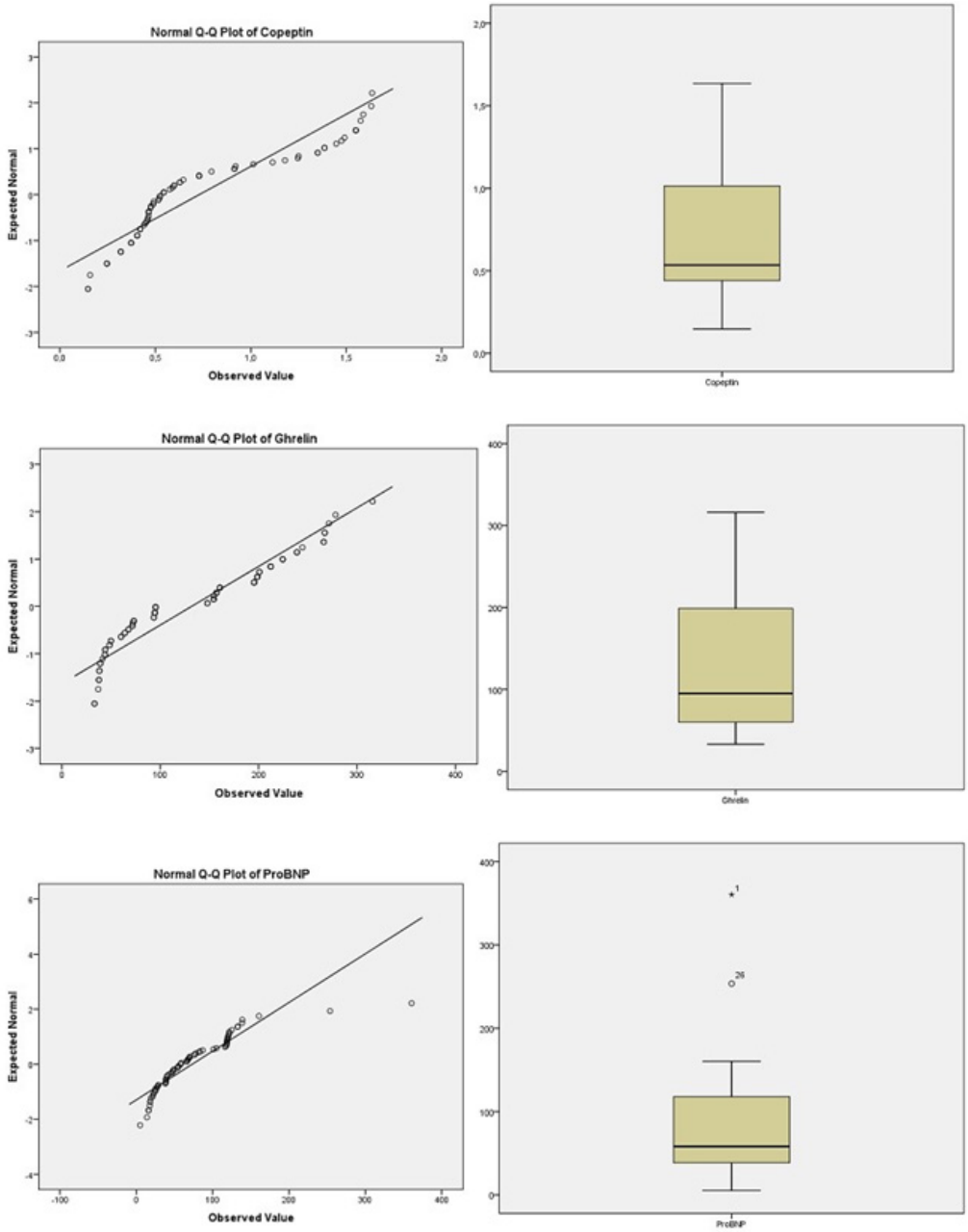

Figure 1: Plots of Copeptin, Ghrelin and ProBNP

Discussion

MetS is an important cause of mortality and morbidity affecting more people by becoming one of the most important pandemics of our age in the modern world which is formed by the combination of severall factors. MetS is a medical condition that needs to be seriously addressed but avoidable. Environmental factors that may cause negative imbalance in energy metabolism such as inadequate physical activity and unhealthy eating habits pave the way for MetS. Iqbal et al. reported that MetS is associated with rised risk for both type 2 diabetes and cardiovascular disease in studies of MetS, dyslipidemia, and regulation of lipoprotein metabolism. They also found a decline in the levels of high-density lipoproteins and an rise in the values of LDL particles in the remaining small density.[19] Recent findings suggest alternative hypotheses in which natriuretic peptides can act as markers or for insulin-dependent conditions, obesity, metabolic syndrome and diabetes.[20]

Metabolic syndrome is an important public health concern affecting approximately one quarter of people in the world.[21] It is strongly associated with increased nearly three fold higher risk of developing CVD and five fold higher risk of diabetes.[3,21] The increasing prevalence of MetS and its consequences including cardiovascular diseases and Type 2 diabetes, has promoted to make researches for new risk factors.[15] The MetS pathophysiology is not well defined, and many researchers have tried to identify a unifying factor that could explain all the MetS components.[21] There is growing evidence of the relevance of cardiac natriuretic peptides in the pathophysiology of metabolic diseases.[2,22] BNP and its N-terminal counterpart, NT-proBNP are globally supported in clinical guidelines as diagnostic and prognostic biomarkers of varied CVD, such as coronary artery disease (CAD) and heart failure.[6,8,23,24] Reduced NP levels were demonstrated in metabolic diseases including type 2 diabetes, obesity and heart failure.[4,10,25,26] Data regarding the relationship between natriuretic peptides and metabolic syndrome are controversial, results demonstrate both decreased and increased NP levels.[10] Some studies found decreased NT-proBNP concentrations in MetS than those without MetS.[26-29] Previous studies mostly noted that individuals with MetS had lower NT-proBNP levels compared to those without the MetS, however, Bruno et al. determined higher NT-proBNP values in people with MetS than without any component of the MetS.[22] In the present study, it was found that proBNP levels were higher in subjects with MetS compared to healthy controls. It may be hypothesized that higher levels of proBNP might be associated with the onset of the MetS in the examined population. The relationship found between the levels of NT-proBNP and obesity, data on relations with other metabolic risk factors remains unclear.[7,30] During the last years, wide range of studies reported an inverse relationship between BNP concentrations and BMI.[30-35] With respect to plasma NT-proBNP, negative associations with plasma glucose, serum total cholesterol and triglycerides were found in the previous study.[29] It was postulated that BNP concentrations were positively associated with age, higher blood pressure, and female gender but did not correlate with CVD risk factors such as serum lipids.[36] Wang et al. indicated an opposite relationship between serum NT-proBNP and MetS in elderly persons. In addition, NT-proBNP levels were positively associated with age, while inversely associated with body fat mass, height and triglyceride values in these individuals.[24] In our study, we find negative correlations between proBNP levels and MetS components.

Copeptin has been recently suggested as one of the new promising diagnostic biomarkers for various diseases. [14,37] During the past years, copeptin measurement has been demonstrated to be of interest in various metabolic diseases, including diabetes, obesity, CVD. Several reports showed that higher copeptin values were associated with cardiovascular diseases.[14,38] obesity.[39] And diabetes.[40,41] On the contrary, copeptin reduced in diabetes insipidus, hyponatremia and other conditions associated with decreased AVP concentration.[37] Serum copeptin levels were determined significantly lower in gestational diabetes mellitus as compared to healthy pregnant.[42] In the previous study, copeptin concentrations were found higher in MetS as compared to controls. In addition, a positive correlation between increased copeptin and MetS or its components was also demonstrated.[12] Saleem et al. reported that individuals with MetS had higher copeptin levels than those without MetS. Moreover, they indicated that plasma levels of copeptin positively correlated with fasting glucose and insulin, homeostasis model assessment of insulin resistance (HOMA-IR), BMI, triglycerides, and inversely correlated with HDL-cholesterol.[21] Similar to previous reports, it was shown that copeptin was related to components of the MetS such as BMI, waist, hypertension, HDL, triglycerides and diabetes.[43] We found that copeptin values were higher in MetS compared to controls probably because of our study population in this study. Our findings indicated that high values of copeptin in MetS might reflect the early step of the MetS development and copeptin values may be used to monitoring progression of the disease. Furthermore, the combined measurement of copeptin and NT-proBNP may be provide more valuable knowledge than the just one biomarker measure.

Ghrelin induces appetite, controls energy balance, plays significant roles in cardiovascular system and has anti-inflammatory effects, and therefore, it is one of the candidate peptide hormone for the treatment of obesity and T2DM.[17,44] Studies during the last years, have shown relationship among ghrelin gene and obesity, diabetes or metabolic syndrome. A wide range of evidence suggested that ghrelin is implicated in the development of MetS and T2DM, and decreased ghrelin levels are related to higher MetS prevalence with progressively lower ghrelin concentrations in relation to the number of MetS components.[16,17] Ukkola et al. found that ghrelin values were lower in obese subjects and reduced ghrelin levels have been associated with MetS and T2DM.[45] Previous studies reported that individuals with MetS had reduced plasma ghrelin levels compared to controls.[16,46] The results found in our patients are in agreement with those of many other authors regarding decreased ghrelin values.

Conclusion

In conclusion, our results demonstrated that copeptin, ghrelin and proBNP levels are directly related not only in itself but also to various metabolic syndrome components such as obesity and high triglyceride concentration. Our findings support the view that these parameters are a guide in the follow-up of high risk patients and monitoring of the disease.

Conflict of interest statement

The authors declare that they have no conflicts of interest for this work.

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