

The Relationship between Ghrelin and Copeptin Levels, and Anxiety and Depression Levels in Suicide Attempts

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Objective: It was aimed to detect acylated ghrelin (AG), unacylated ghrelin (UG) and copeptin levels in patients with suicide attempts and to determine if these biomarkers are risk factors for suicide attempts.

Methods: Serum copeptin, AG and GU levels were screened in 128 patients who were admitted to emergency department with suicide attempts and 59 healthy controls. Beck Anxiety Inventory (BAI) and Beck Depression Inventory (BDI) were applied simultaneously, and the data were compared statistically.

Results: AG, UG and copeptin levels were higher in the patient group compared with the healthy control group. BAI scores of patients were found to be positively correlated with BDI scores. While there was a significant difference ($p=0.0064$) between psychiatric and non-psychiatric patients with suicide attempts in terms of BAI scores, there were no differences in BDI scores and levels of biomarkers. We found significantly increased BDI and BAI scores and increased levels of AG, UG and copeptin in psychiatric and non-psychiatric patients compared with healthy individuals. The specificities yielded by receiver operating characteristic curve analysis in patients with suicide attempts were as follows: 91.53% for AG, 72.88% for UG and 94.92% for copeptin.

Conclusion: Serum levels of AG, UG and copeptin increase with increasing anxiety and depression in patients with suicide attempts. Increased levels of AG, UG and copeptin could be considered a risk factor for suicide attempts.

KEY WORDS: Acylated ghrelin; Beck Anxiety Inventory; Beck Depression Inventory; Copeptins; Suicide; Unacylated ghrelin.

INTRODUCTION

Suicide attempts are common in the society and are one of the public health problems with increased mortality rate. Although the prediction of suicide attempts is not exactly possible, some risk factors have been determined.¹ One of the risk factors for suicide attempts is the level of anxiety and/or depression. Also, the risk of suicide attempts has been reported to be increased in psychiatric patients.^{2,3} In clinical and experimental studies, stress and depression have been shown to be associated with increased levels of circulating cytokines and acute phase reactants, and hypothalamic pituitary adrenal (HPA) axis hyperactivity.^{4,5} HPA and sympathetic nervous system are known as two major pathways involved in stress development.⁶ While corticotropin-releasing hormone (CRH) in the HPA axis causes the release of adrenocorti-

cotropic hormone (ACTH), arginine vasopressin (AVP) enables the production of cortisol. In individuals with intact HPA, serum cortisol levels have been reported to be proportional to stress, and also associated with chronic stress that forms the basis of certain psychiatric disorders such as depression.⁷⁻¹⁰ Copeptin, which has been reported to gradually increase with increasing stress, can be tested with ease and is a stable peptide compared with vasopressin; hence it is a reliable AVP surrogate and can demonstrate its plasma concentrations. It is thought to play role in endogenous stress response.^{11,12}

Acylated ghrelin (AG), which was shown to cause increased concentrations of ACTH and corticosterone following intracerebroventricular injection, is a 28-amino acid peptide hormone that is mainly produced by the cells that have endocrine function and are located in the gastric mucosa.¹³⁻¹⁵ It was found that Ghrelin was present in hypothalamus, pituitary gland, brain, kidney, pancreas, heart, stomach and intestinal tissues, and that it acted through growth hormone secretagogue receptor type 1a (GHS-R1a).^{16,17} Ghrelin is thought to contribute to the development of anxiety due to its relation with HPA axis,

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and to cause an increase in suicidal behavior as a result of increased anxiety.¹⁸⁾ In addition, ghrelin has been shown to be associated with depressive disorders in several studies.¹⁹⁻²¹⁾ In previous studies on ghrelin and other markers in patients with suicide attempts, patients who were at psychiatry clinic or who had a diagnosis of psychiatric disorder were recruited. However, all patients who were admitted to the emergency room with a suicide attempt were included in our study. In order to determine the effect of presence or absence of a chronic psychiatric disorder on the levels of AG, unacylated ghrelin (UG) and copeptin in these patients, the patients were divided into two groups as patients with and without a diagnosis of chronic psychiatric disorder.

METHODS

Participant Selection and Assessment

The study was initiated following Firat University Faculty of Medicine, Ethics committee approval (Aproval no: 30.12.2014/22-2). Patients who were admitted to our emergency department with attempted suicide (n=128) and healthy individuals (n=59) were included. The exclusion criteria were as follows: impaired consciousness, requirement for intubation and mechanical ventilation, < 18 years of age, patients who did not consent to participate in the study, hormone therapy, steroid therapy, chronic diseases such as autoimmune, pulmonary or neoplasms, and illiterate patients. The demographic data of the patients were recorded into the standard data form.

Beck Anxiety Inventory (BAI)

It is an anxiety rating scale that determines the frequency of anxiety symptoms experienced by the individuals.²²⁾ BAI consists of 21 questions and is a Likert-type scale based on a 0-3 point scale. The scores from the scale are calculated in the range of 0-63 points. Increased scores are an indication of increased levels of anxiety. The validity and reliability study for Turkey was performed by Ulusoy *et al.*²³⁾

Beck Depression Inventory (BDI)

It is a self-report inventory and determines the level of depression according to the scores.²⁴⁾ BDI consists of 21 items, 15 psychological and 6 somatic symptoms. Each item in BDI is scored between 0 to 3 points. The depression score was calculated by summing these scores. The highest score is 63. Increased scores indicate high depression levels or severity. The validity and reliability study for Turkey was performed by Hisli.²⁵⁾

Collection and Preparation of Blood Samples

In order to measure the levels of AG, UG and copeptin, 3 ml blood samples were collected in aprotinin-containing tubes within the first hour of patient admission. The blood samples were then centrifuged at 4,000 rpm for 5 minutes at 4°C. The obtained sera were transferred to Eppendorf tubes and stored at -80°C until analysis.

Copeptin Levels

Serum samples were analyzed via Human Copeptin ELISA kit (catalog number: YHB0830Hu; YhBiosearch Laboratory, Shanghai, China) in accordance with the analysis procedure. This kit is based on the principle of double-antibody sandwich technique. Absorbance was read spectrophotometrically via ELx800™ Absorbance Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA) at 450 nm. ELx50™ Microplate Strip Washer (BioTek Instruments, Inc.) was used as an automatic microplate washer. Results were expressed in ng/ml. Detection range was 0.05-20 ng/ml and sensitivity was 0.024 ng/ml. Intra- and interassay coefficients of variation (CVs) were < 10% and < 12%, respectively.

Acylated Ghrelin (AG) Levels

Serum samples were analyzed via Human Acylated Ghrelin ELISA kit (catalog number: YHB0089Hu; YhBiosearch Laboratory) in accordance with the analysis procedure. This kit is based on the principle of double-antibody sandwich technique. Absorbance was read spectrophotometrically via ELx800™ Absorbance Microplate Reader at 450 nm. ELx50™ Microplate Strip Washer was used as an automatic microplate washer. Results were expressed in ng/ml. Detection range was 5-1,500 ng/ml and sensitivity was 2.52 ng/ml. Intra- and interassay CVs were < 10% and < 12%, respectively.

Unacylated Ghrelin (UG) Levels

Serum samples were analyzed via Human Unacylated Ghrelin ELISA kit (catalog number: YHB3507Hu; YhBiosearch Laboratory) in accordance with the analysis procedure. This kit is based on the principle of double-antibody sandwich technique. Absorbance was read spectrophotometrically via ELx800™ Absorbance Microplate Reader at 450 nm. ELx50™ Microplate Strip Washer was used as an automatic microplate washer. Results were expressed in ng/ml. Detection range was 0.5-100 ng/ml and sensitivity was 0.25 ng/ml. Intra- and interassay CVs were < 10% and < 12%, respectively.

Table 1. The data of patients with and without suicide attempts

| Variable | Suicide attempts group | Healthy controls | <i>p</i> value |
|--------------------------------------|--------------------------|------------------------|----------------|
| Subjects (female/male) | 128 (77/51) | 59 (34/25) | 0.744 |
| Age (yr) | 28±11.95 | 30.22±8.58 | 0.428 |
| Body mass index (kg/m ²) | 22.65 (20.70-25.71) | 23.39 (21.36-26.60) | 0.229 |
| Acylated ghrelin (ng/ml) | 1,021.29 (718.03-282.09) | 432.00 (294.73-786.15) | <0.001 |
| Unacylated ghrelin (ng/ml) | 256.35 (124.17-354.25) | 117.59 (66.38-217.09) | <0.001 |
| Copeptin (ng/ml) | 21.27 (10.43-57.04) | 9.44 (5.14-15.6) | <0.001 |
| Beck Depression Inventory (score) | 24 (16-34.5) | 14 (6.25-19.75) | <0.001 |
| Beck Anxiety Inventory (score) | 20 (13-27.75) | 12 (7/23) | <0.001 |

Values are presented as number only, mean±standard deviation, or median (interquartile range).

Table 2. The data of psychiatric and non-psychiatric patients with suicide attempts

| Variable | Psychiatric | Non-psychiatric | <i>p</i> value |
|--------------------------------------|----------------------------|----------------------------|----------------|
| Subjects (female/male) | 40 (23/17) | 88 (54/34) | 0.679 |
| Age (yr) | 30.07±13.0 | 28.28±11.48 | 0.125 |
| Body mass index (kg/m ²) | 23.03 (20.61-25.92) | 22.54 (20.83-25.71) | 0.6979 |
| Acylated ghrelin (ng/ml) | 1,094.01 (651.22-1,334.23) | 1,011.60 (727.99-1,235.06) | 0.6253 |
| Unacylated ghrelin (ng/ml) | 274.01 (118.42-372.38) | 286.09 (147.85-380.08) | 0.7190 |
| Copeptin (ng/ml) | 14.75 (10.49-57.47) | 22.96 (10.53-56.14) | 0.5510 |
| Beck Depression Inventory (score) | 22 (12-28) | 20 (13-27) | 0.8390 |
| Beck Anxiety Inventory (score) | 29.5 (21.5-38.5) | 22.5 (14.5-30) | 0.0064 |

Values are presented as number only, mean±standard deviation, or median (interquartile range).

Statistical Analysis

Data were analyzed by IBM SPSS Statistics ver. 21.0 (IBM Co., Armonk, NY, USA). Kolmogorov-Smirnov and Shapiro-Wilk tests were used as tests of normality for continuous variables. Parametric data, non-parametric data and categorical data were expressed as mean±standard deviation, median (interquartile range, 25-75 percentile) and %, respectively. Mann-Whitney *U* test was used for pairwise comparison of data without normal distribution. Spearman's correlation test was used for assessment of the relationship between numerical parameters. A *p* value less than 0.05 was considered significant in all analysis.

RESULTS

A total of 187 participants, 128 patients with suicide attempts and 59 healthy controls, were enrolled in our study.

There were no differences between patients with and without attempted suicide in terms of age, gender and body mass index. However, AG, UG and copeptin levels were found to be significantly higher in patients with attempted suicide compared to the control group (Table 1). Besides, a statistically significant difference was detected between two groups in terms of anxiety scores ($p < 0.001$; Table 1).

Of all patients with suicide attempts, 40 patients had a

Table 3. The Spearman's correlation data of BAI, BDI and AG, UG and copeptin levels

| | | AG | UG | Copeptin |
|-----|----------|--------|--------|----------|
| BAI | <i>r</i> | 0.655 | 0.798 | 0.756 |
| | <i>p</i> | <0.001 | <0.001 | <0.001 |
| BDI | <i>r</i> | 0.479 | 0.565 | 0.625 |
| | <i>p</i> | <0.001 | <0.001 | <0.001 |

BAI, Beck Anxiety Inventory; BDI, Beck Depression Inventory; AG, acyated ghrelin; UG, unacylated ghrelin.

previous psychiatric disorder diagnosis and 88 patients had no previous psychiatric disorder diagnoses. Psychiatric diagnoses of patients were as follows: major depressive disorder in 19 patients, schizophrenia in 8 patients, schizoaffective disorder in 5 patients, bipolar disorder in 7 patients and personality disorder in one patient. While there was a significant difference ($p=0.0064$) between psychiatric and non-psychiatric patients with suicide attempts in terms of BAI scores, there were no differences in BDI scores and levels of biomarkers (Table 2).

Positive correlations were found between BAI and AG ($r=0.655$, $p < 0.001$), UG ($r=0.798$, $p < 0.001$) and copeptin ($r=0.756$, $p < 0.001$) levels. Positive correlations were also detected between BDI and AG ($r=0.479$, $p < 0.001$), UG ($r=0.565$, $p < 0.001$) and copeptin ($r=0.625$, $p < 0.001$) levels (Table 3).

The sensitivity and specificity yielded by receiver operating characteristic curve analysis in patients with suicide attempts were as follows: 51.81% and 91.53% for AG, 69.53% and 72.88% for UG, and 50.78% and 94.92% for copeptin (Fig. 1, Table 4).

DISCUSSION

In our study, we found that levels of HPA axis-associated biomarkers, AG, UG and copeptin, were increased in patients with suicide attempts. Also, AG, UG and copeptin levels were found to show a positive correlation with BDI and BAI. Difficulties in coping with stress, and stressful life events are among the risk factors for suicide attempts. Because cortisol is associated with anxiety and depression, HPA axis hyperactivity is thought to be effective in suicide attempts.¹⁰⁾

Intracerebroventricular CRH administration leads to inhibition of eating in people. This situation causes anxiety behavior and stimulates the release of glucocorticoids from the adrenal glands.²⁶⁾ In addition, intracerebroventricular ghrelin injection leads to increased plasma levels of CRH, thus ACTH and corticosterone concentrations.¹⁵⁾ It could be considered that ghrelin might indirectly con-

tribute to suicide attempts by leading to the development of anxiety through stimulation of the HPA axis.¹⁸⁾ In several studies, it was reported that low serotonin activity could cause many psychological disorders such as impulsivity, aggression and suicidal ideation and that there was an interaction between serotonergic system and ghrelin.²⁷⁻²⁹⁾ Different results were obtained in studies on ghrelin levels in psychiatric disorders. Nakashima *et al.*¹⁹⁾ reported that panic disorder was not associated with serum ghrelin levels, but related with depressive disorders. However, Ishitobi *et al.*²¹⁾ reported higher serum ghrelin levels in patients with panic disorders compared with healthy controls. In two different studies comparing patients with suicide attempts and healthy controls, it was reported that serum ghrelin levels increased significantly in patients with suicide attempts.^{18,20)} In these studies, the basic mechanism in ghrelin increase was evaluated as the stimulation of the HPA axis. In our study, consistent with earlier studies, ghrelin levels were found to be increased in patients with suicide attempts.

Copeptin is another gradually increasing biomarker with increasing stress.¹¹⁾ Akinladel *et al.*³⁰⁾ reported significantly increased copeptin levels in schizophrenic patients compared to healthy subjects. The main reason for the increase in copeptin levels in psychiatric diseases is thought to be associated with an increased level of cortisol, which is the basic molecule of stress following HPA axis stimulation.⁸⁾ In our study, copeptin levels were significantly higher in patients with suicide attempts than in anxiety patients without suicide attempts. One of the striking results of our study is the presence of increased levels of AG, UG and copeptin in non-psychiatric patients with suicide attempts. Increased biomarkers in case of suicide attempts due to acute stress suggest that HPA axis could have a role in both pathophysiology of chronic psychiatric diseases, and rapid and impulsive stress conditions.

In conclusion, levels of AG, UG and copeptin increase in patients with suicide attempts. BAI and BDI scores have a positive correlation with AG, UG and copeptin levels. Moreover, the levels of these biomarkers and BAI and BDI scores are also high in psychiatric and non-psychiatric patients. Increased levels of AG, UG and copeptin could be

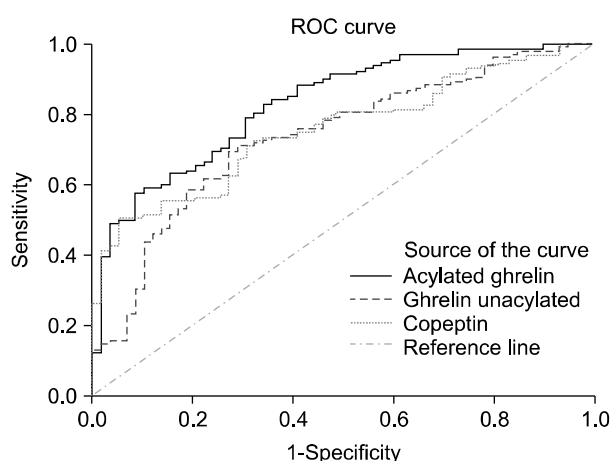


Fig. 1. Receiver operating characteristic (ROC) curve analysis graph. Diagonal segments are produced by ties.

Table 4. ROC curve analysis results

| | Cut-off | AUC | Sensitivity | Specificity | p value | 95% confidence interval |
|--------------------|----------|-------|-------------|-------------|---------|-------------------------|
| Acylated ghrelin | >959.905 | 0.831 | 57.81 | 91.53 | <0.0001 | 0.771-0.892 |
| Unacylated ghrelin | >193.55 | 0.737 | 69.53 | 72.88 | <0.0001 | 0.662-0.813 |
| Copeptin | >19.85 | 0.757 | 50.78 | 94.92 | <0.0001 | 0.689-0.825 |

ROC, receiver operating characteristic; AUC, area under the curve.

considered among the risk factors for suicide attempts.

The limitations of this study are as follows: The parameters such as sampling time, nutritional status and drugs that can affect the levels of copeptin, AG and UG were not utilized in our study as they cause serious decrease in the number of study participants. The small number of participants is the main limitation of our study. Also, cortisol levels could have contributed to the determination of stress status of patients.

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