Nesfatin-1, leptin and ghrelin as a biomarker for early diagnosis and prognosis of spontaneous subarachnoid hemorrhage.

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Abstract

Objectives: The diagnosis of spontaneous subarachnoid hemorrhage is extremely important for the earliest medical interventions. For this purpose, the present study was designed to investigate the serum nesfatin-1, leptin and ghrelin levels and to evaluate whether the said molecules could serve as a biomarker for severity and prognosis of subarachnoid hemorrhage.

Material and methods: Sixty-six patients with spontaneous subarachnoid hemorrhage admitted to the emergency department and 23 healthy adults as the control were included in this study. Brain computed tomography was used to diagnose subarachnoid hemorrhage. Nesfatin-1, leptin and ghrelin levels in the blood circulation were measured in these patients. Clinical severity and prognosis of subarachnoid hemorrhage was evaluated according to Glasgow Coma Scales using clinical and neurological status on admission.

Results: Nesfatin-1 and ghrelin levels were measured significantly lower, however leptin concentrations significantly higher determined in patients with subarachnoid hemorrhage when compared to healthy control ($p<0.05$). In the other hand, we could not observe a significant correlation among serum levels of nesfatin-1, leptin and ghrelin with respect to clinical severity and prognosis ($p>0.05$).

Conclusions: The results of this clinical study suggest that nesfatin-1 and ghrelin concentrations decrease but leptin level increases in patients with spontaneous subarachnoid hemorrhage. Serum nesfatin-1, leptin and ghrelin values in patients are no correlated with severity and prognosis of subarachnoid hemorrhage.

Introduction

Non-traumatic (or spontaneous) Subarachnoid Hemorrhage (SAH) is a neurologic emergency potentially life-threatening defined by mostly arterial and rarely venous bleeding into the subarachnoid space [1]. The diagnosis of SAH currently relies on clinical symptoms and Computerized Tomography (CT) imaging; however, molecular biomarkers that could improve diagnosis are required to decrease SAH morbidity/mortality in the early stage [2].

Nesfatin-1 is a polypeptide which is found in the different regions of the brain that play a role in the feeding and metabolic regulation. Also, it has anti-inflammatory and antiapoptotic properties [3]. Ghrelin is a 29-amino acid polypeptide that is secreted in substantial amount into the blood circulation by gastrointestinal tract, whereas the central nervous system, kidneys, pancreas and pituitary all produces small amounts of ghrelin. In addition to its well-known effects on appetite regulation, ghrelin alleviates SAH-induced

oxidative brain injury, and exerts neuroprotection by inhibiting proinflammatory mediators in traumatic brain injuries [4,5]. Leptin "anorexigenic hormone" made by adipose cells helps to regulate energy balance by inhibiting hunger. Leptin is opposed by the actions of the ghrelin "hunger hormone", as leptin inhibits and ghrelin stimulates nutrient intake. These hormones act on receptors in the arcuate nucleus of the hypothalamus to regulate appetite to achieve energy homeostasis. Leptin concentrations are also known to be associated with cerebrovascular diseases [6,7]. The primary aim of the present study was to investigate the serum nesfatin-1, ghrelin and leptin concentrations in patients with spontaneous SAH and to investigate the correlation of nesfatin-1, ghrelin and leptin according to clinical severity and prognosis in relation to Glasgow Coma Scale (GCS) in patients with SAH on admission.

Materials and Methods

This study was conducted in the Emergency Department (ED) of Faculty of Medicine, Ondokuz Mayıs University. After ethical approval from Local Ethics Committee (OMU KAEK 2013/228), 23 healthy subjects as control group and 66 consecutive patients older than 18 y admitted to our ED due to the clinical findings of SAH were recruited to this study. Detailed physical examination findings of the patients on admission, age, gender, complaints, vital signs, Glasgow Coma Scale (GCS), complete blood count (CBC) levels (white blood cell count, hemoglobin and thrombocyte levels), biochemical test results (AST, ALT, blood urea nitrogen, creatinine), imaging studies (X-ray, CT, ECG, angiography), length of hospital stay and outcomes (discharge or death) were recorded. The GCS is to use widely as a representative tool for evaluating the severity of neurological patients. Therefore, with respect to the scores ranged from 3 to 15 and the GCS classifies less than 9 as severe, 9-13 points as moderate, and 14-15 as mild brain damage. The present study is based on the scores noted at admission. Inclusion criteria were as follows; patients older than 18 y and patients with CT-confirmed non-traumatic SAH. Exclusion criteria were as follows; patients with heart, kidney and liver failure on medical history, pediatric patients and those to whom CT was not performed.

On admission; CBC and biochemistry analyses were performed from venous blood samples of the patients. Additionally, 8 ml of venous blood samples were obtained in order to determine nesfatin-1, leptin and ghrelin levels of the patients. These samples were centrifuged at 3000 g for 5 min, and then serum separated. Serum specimens were stored in polypropylene tubes in refrigerator (Nuaire Ultra-Low Freezer, Nu-6420E) at -80°C until analyses. The same procedures were performed for blood samples of control group. Leptin, ghrelin and nesfatin-1 measurements were performed by Sunrise Elisa Reader Device® in the laboratory of biochemistry. Serum leptin, nesfatin-1 and ghrelin concentrations were measured by leptin Elisa Kit (Diasource, Catalogue No: KAP22819), Human Nesfatin-1 (NES1) Elisa Kit (Eastbiopharm Catalogue No: CK-E90098) and Human Ghrelin Elisa Kit (Eastbiopharm Catalogue No: CK-E90121), respectively.

SPSS version 15 was used for the statistical analyses. In comparison of the groups, Student T test was used for normally distributed data and Mann-Whitney U test was used for non-normally distributed data. In comparison of groups more than two, Anova was used for normally distributed data, and Kruskal-Wallis and Bonferoni-corrected Mann-Whitney U tests were used for non-normally distributed data. For normally distributed leptin values, Prearson's correlation analysis was used while for non-normally distributed values, Spearman's correlation analysis was used. $P<0.017$ was considered statistically significant for Bonferoni-corrected Mann-Whitney U and $p<0.05$ was considered statistically significant for other tests. The data were reported as median (min-max) or as frequency (%).

Results

During the study period, a total 66 consecutive non-traumatic SAH patients were initially evaluated in the ED. Laboratory data, baseline demographic and clinical characteristics are summarized in Table 1. The most frequent complaint of patients on admission was mental status alterations, and then followed by headache. Following patients' discharge from the hospital, we performed follow-up evaluation according to the GCS; the majority of the patients discharged from the hospital were in GCS 14-15. In relation to the severity and prognosis or mortality, 20.9% of GCS 14-15 point, 57.1% of GCS 9-13 point and 88.9% of GCS<9 point in patients with SAH have been exitus. Rates of mortality in GCS<9 point and GCS 9-13 points were determined to be higher statistically when compared to GCS 14-15 points ($\chi^2=17.1$, df=1, $p<0.05$). Upon measuring the serum nesfatin-1, leptin and ghrelin levels in non-traumatic SAH, nesftin-1 and ghrelin were higher, but leptin was lower in patients with SAH compared to control subjects ($p<0.05$). Also, WBC count was significantly higher in patients with SAH compared with healthy control group ($p<0.05$). To determine whether Glasgow Coma Scales in patients is associated with the observed changes in nesftin-1, leptin, and ghrelin levels, Spearman's correlation was used. However, no statistical correlation was observed for nesftin-1, leptin and ghrelin levels in patients with SAH according to the patients' GCS as shown in Table 2 ($p>0.05$).

Table 1. Laboratory data, baseline demographic and clinical features of study population.

Variables		SAH	Control	P value
Age (y) (median (min-max))		54.9 (27-71)	38.7 (30-48)	
Sex (n, %)	Female	39 (59.1%)	13 (56.5%)	
	Male	27 (40.9%)	10 (43.5%)	
Initial symptoms (n, %)				
Mental status alteration		30 (45.5%)	----	
Headache		24 (36.4%)	----	
Vomiting		7 (10.6%)	----	

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Focal neurological findings/seizure	5 (7.5%)	----	
Glasgow coma score at admission (n, %)			
GCS 14-15	43 (65.2%)	----	
GCS 9-13	14 (21.2%)	----	
GCS<9	9 (13.6%)	----	
Final outcome			
Discharged	41 (62.1%)	----	
Exitus	25 (37.9%)	----	
Laboratory findings (median (min-max))			
WBC count (/mm^3)	11450 (3380-23400)	7200 (5200-11200)	<0.05
AST (U/L)	25 (12-109)	22 (16-32)	>0.05
ALT (U/L)	20 (7-128)	18 (10-32)	>0.05
Creatine (mg/dL)	0.7 (0.3-2.6)	0.8 (0.6-1.1)	<0.05
Leptin (ng/ml)	4.7 (1.1-10.9)	2.4 (1.0-4.7)	<0.05
Ghrelin (ng/ml)	155.3 (60.9-3148)	978.7 (119.5-9402.0)	<0.05
Nesfatin-1 (μg/dL)	1.2 (0.9-4.7)	1.7 (1.0-4.5)	<0.05

Data were presented as median (min-max), SAH; Subarachnoid Hemorrhage.

Table 2. Nesfatin-1, leptin, and ghrelin levels according to Glasgow coma scales.

Parameters	GSC 14-15 point	GSC 9-13 point	GSC<9 point	P value
Leptin (ng/ml)	4.7 (1.1-10.9)	4.4 (1.1-9.9)	5.1 (2.1-9.5)	>0.05
Ghrelin (ng/ml)	158.5 (67.4-3148)	174.8 (60.9-3088)	139 (61-614)	>0.05
Nesfatin-1 (μg/dl)	1.2 (1-4)	1.4 (1.0-4.7)	1.1 (0.9-2.4)	>0.05

Data were presented as median (min-max), GSC; Glasgow Coma Scale.

Discussion

The results of the current study mainly showed that serum nesfatin-1 and ghrelin values were found to be lower in patients when compared to healthy controls; however serum leptin level was determined to be higher statistically in patients with spontaneous SAH as compared to controls. In addition, the levels of these biomarkers were no correlated with disease severity and prognosis in relation to patients' GCS points.

Usually, the initial symptom of SAH is the severe headache. The patients generally describe this headache as the worst headache ever before. Additionally, nausea/vomiting, confusion and mental distresses, focal neurological deficits, hypertension and/or memory loss might also be seen in patients related to SAH [8-13]. The most common findings in our study were observed for mental status alteration and headache in the rates of 45.5% and 36.4%, respectively. In a similar way, there are various studies reporting different frequencies on mental status alteration in SAH [14-16]. The reason for variety in different reports may be the difference of periods between onset of SAH and arrival in the ED as mental status is known to be affected by the duration of vasospasm occurs after SAH.

The GCS has been used to judge the level of consciousness with worldwide acceptance, is used for grading patients with an SAH. Gotoh et al. have reported that there is a strong correlation between higher GCS and better outcome for the prognostic strength of the GCS in patients with SAH [17]. In this manner, patients' mortality rates based on the GCS<9 and GCS 9-13 points checked at admission were raised in accordance with poor prognosis in our study. However, the admission GCS in connection with severity and prognosis probability of the patients is usually known to influence by many factors such as age, pre-existing hypertension, the amount of blood present on admission CT, time of admission after SAH, aneurysm location and size, presence of intracerebral or intraventricular hemorrhage, and blood pressure at admission [17,18].

In general, biomarkers have been thought to be reliable tools for diagnosis, early medical intervention, therapeutic decision making and prognosis in many pathophysiological states or

disease process. By the way, biomarkers such as central nervous system-specific markers, inflammatory biomarkers, molecular adhesion and extracellular matrix markers, and the other markers (leptin, ghrelin, nesfatin-1, etc.) in SAH could aid in the identification of high-risk patients, guide to treatment/management and improve outcome [19-21].

Leptin is mainly produced by adipose tissue and regulates energy balance. Elevated leptin levels have been reported in patients with intracerebral hematomas, SAH, ischemic and hemorrhagic stroke. Also, it may imply clinical severity of the initial bleeding and have prognostic value for clinical outcomes in SAH [22-24]. Similarly, Zhoa et al. [25] reported that leptin levels were higher in patients with SAH than healthy controls. They also reported that leptin level might be used as an independent prognostic marker. In our study, leptin levels were found to be higher in patients with SAH as compared to controls. However, we could not determine a relationship between serum leptin value and mortality in SAH in connection to patients' GCS. This issue needs further investigations with larger patient groups.

Ghrelin secreted in a pulsated manner as its level increase up to the onset of meal and decrease after feeding. Wang et al. [26] have reported that ghrelin attenuates inflammation that plays a significant role which contributes to brain injury in hours to days after ischemic events. Additionally, ghrelin treatment also alleviates neurological deficits, SAH-induced oxidative brain damage and early brain injury after SAH in rats [4,27]. In the present study, serum ghrelin level was measured lower in patients with SAH compared to controls. Also, serum ghrelin concentrations are not associated with prognosis in SAH patients according to patients' GCS at the admission. Although the molecular signals that regulate leptin and ghrelin secretion are not exactly known, the present results might show that appetite loss in SAH patients can be induced by higher serum leptin and lower serum ghrelin levels based on high blood glucose and insulin concentrations due to a catecholamine surge following SAH in the acute stage of SAH patients as indicated by Kubo et al. [28]. Usually, in patients with SAH, appetite loss might be induced by not only headache, but also higher leptin and lower ghrelin concentrations.

Nesfatin-1, a novel anorexigenic neuropeptide appears to play a substantial role in hypothalamic pathways regulating food intake and energy homeostasis, is expressed in several neurons of central nervous system. Recently, plasma or serum nesfatin-1 concentrations were estimated to be elevated and associated with the severity and prognosis in patients with traumatic brain injury and non-traumatic SAH [20,29]. In contrast to the latest reports, serum nesfatin-1 levels in the current study were measured lower in patients with non-traumatic SAH when compared to controls. No significant difference was observed for nesfatin-1 in association with the severity and prognosis in SAH according to patients' GCS.

Conclusion

In the literature, there is no a scientific report comparing leptin, ghrelin and nesfatin-1 levels in SAH patients in association with GCS. Our results do not support the hypothesis that the said serum parameters are useful for predicting early diagnosis and prognosis or mortality in patients with non-traumatic SAH. Further research in connection with leptin, ghrelin and nesfatin-1 levels in early diagnosing and prognosis of SAH patients should be evaluated with future clinical studies on larger subjects prior to the presentation of neurological deterioration.

Acknowledgement

Conflicts of Interest

The authors declare no conflict of interest.

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