



# New hormones to predict the severity of gallstone-induced acute pancreatitis

## PANCREAS

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### ABSTRACT

**Background/Aims:** Levels of the hormones ghrelin and leptin in rat models of acute pancreatitis (AP) have been investigated in several experimental studies. However, there are very few clinical studies addressing the connection between hormone levels and AP. A few recent studies investigating the changes in ghrelin and leptin levels in patients with AP have been reported; however, our study is the first clinical study to investigate the change of nesfatin-1 levels in patients with gallstone-induced AP.

**Materials and Methods:** Forty patients were enrolled in this study, eight of which presented with severe AP. Two blood samples were obtained from each study patient. The first blood samples were obtained at patient admission to the hospital and the second was obtained at patient discharge. All samples were collected after at least 6 h of fasting. Plasma nesfatin-1, leptin, and ghrelin levels were measured.

**Results:** In all 40 patients, nesfatin-1 and leptin levels were higher at admission and had decreased at discharge. In contrast, the ghrelin levels at discharge were significantly higher than those at admission. Only the changes in these hormones in the mild AP group were significant.

**Conclusion:** Levels of these hormones were altered during the course of gallstone-induced AP. These changes might be associated with the clinical outcomes of the disease. To clarify whether the magnitude of the change in hormone levels at AP onset can be used as a biomarkers to predict the severity of the disease requires further investigation.

**Keywords:** Gallstone-induced acute pancreatitis, nesfatin-1, ghrelin, leptin

### INTRODUCTION

Acute pancreatitis (AP) is a severe disease associated with significant morbidity and mortality. There is no specific treatment for AP other than supportive care. Approximately 80%-85% of AP attacks are mild and can be cured with supportive therapy. However, 10%-20% of patients develop severe AP, which can lead to localized and systemic complications (1). The most common cause of the disease in the United States is excessive alcohol consumption, whereas in Europe, it is gallstones (1,2). The mortality rate of the disease varies between 2%-40% depending on the severity (1-3). An ideal laboratory test for the evaluation of AP should provide an early assessment of its severity. Predictive determination criteria, including APACHE-II, Imrie, Balthazar, and

Ranson scores, are being used for assessment of the severity of AP (1,2). Single biochemical markers such as C-reactive protein are used for determining the severity of the disease. None of these tests, however, have been ideal for determining the severity of the disease (4). Studies are being conducted to find an ideal test for early assessment of AP.

Nesfatin-1 is an 82-amino acid polypeptide found in the circulation and cerebrospinal fluid. Pronesfatin (nucleobindin 2) is cleaved to nesfatin-1, nesfatin-2, and nesfatin-3 by prohormone convertases. Central and peripheral injections of nesfatin-1 have been shown to inhibit feeding in rodents (5). Pronesfatin mRNA was detected mainly in the brain, but recently pronesfatin immuno-

This study was presented at the 48<sup>th</sup> the Congress of the European Society for Surgical Research, 29 May- 1 June 2013, Istanbul, Turkey.

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**Received:** September 20, 2013

**Accepted:** March 23, 2014

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reactivity was detected in the  $\beta$  cells of the pancreatic islets of rats and mice (6,7). Nesfatin-1 was reported to play a role in regulating feeding behavior, carbohydrate metabolism, glucose homeostasis, and gastrointestinal motility (8-10).

Ghrelin is a 28-amino acid polypeptide and was isolated from the endocrine cells in the gastric mucosa and the  $\epsilon$  cells of the pancreatic islets (11). Ghrelin is known to bind the growth hormone secretagogue receptor that affects growth hormone release, gastrointestinal function, feeding behavior, and energy metabolism (12,13). Pancreatic exocrine secretion was found to be inhibited by ghrelin in rat acinar cells (14).

Leptin, a 146-amino acid polypeptide encoded by the *ob* gene, is primarily produced in the peripheral system but also in the brain. It has been implicated in obesity, food intake, and energy homeostasis (15). There are also studies that suggest that leptin plays a role in pancreatic exocrine and endocrine functions (16-18).

On the basis of previous information, several studies have been conducted to evaluate the changes in the levels of leptin and ghrelin during the course of AP. Till date, however, no study has been conducted to investigate the changes in nesfatin-1 levels during the course of gallstone-induced AP.

Although recent studies have shown that nesfatin-1, ghrelin, and leptin have been detected in pancreatic tissue, we investigated changes in the levels of these hormones in patients with gallstone-induced AP in this study.

## MATERIALS AND METHODS

Forty patients who were admitted to Dicle University Hospital from June 2010 to January 2011 with gallstone-induced AP were enrolled in this study. This provided a homogeneous group of patients with regard to the etiology of the disease. Written informed consent was obtained from all patients. The patients were diagnosed with acute pancreatitis if they presented with two of the three following features: 1) abdominal pain characteristic of acute pancreatitis (acute epigastric pain that continues for more than 24 h), 2) serum amylase and/or lipase  $\geq 3$  times the upper limit of the normal, and 3) characteristic findings of acute pancreatitis on a computed tomography (CT) scan (19). Patients who had AP caused by etiologic factors other than gallstones were excluded from the study. The patients were divided into mild or severe AP groups according to the presence of local complications or organ failure, respectively (20). Organ failure was defined as hypotension (systolic blood pressure  $\leq 90$  mmHg), respiratory system failure (arterial  $pO_2 < 60$  mmHg), renal failure (blood creatinine  $> 2$  mg/dL), or gastrointestinal hemorrhage ( $> 500$  mL in 24 h). Local complications were pancreatic necrosis, abscess, or pseudocysts. Ranson and APACHE II scores from all patients were calculated using the defined criteria for these scoring systems.

## Ülger et al. Nesfatin - 1, ghrelin, leptin in acute pancreatitis

Only the patients who were admitted to the hospital within 24 h after the onset of abdominal pain and diagnosed with gallstone-induced AP were enrolled in this study. We attempted to standardize the parameters for the patients who were in same stages of the disease.

The first set of blood samples were obtained during the first 24 h after the onset of the abdominal pain and hospital admission. The second set of blood samples were obtained on the day of discharge. The collected blood samples were centrifuged and the separated plasma was frozen at  $-70^\circ\text{C}$  for subsequent analysis.

After reaching the target number of patients, plasma from the patients was thawed and the nesfatin-1 (Uscn Life Science, Wuhan, China), ghrelin (RayBio, Norcross, GA, USA), and leptin (DRG Diagnostics, Marburg, Germany) levels were measured by ELISA. Serum nesfatin-1, ghrelin, and leptin levels are expressed as nanograms per milliliter (ng/mL).

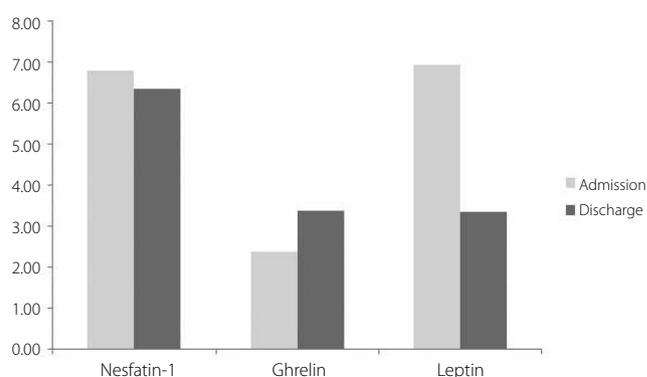
For all statistical analyses, SPSS software (version 12; SPSS Inc., Chicago, IL, USA) was used and a p-value of  $< 0.05$  was considered statistically significant. The Kolmogorov-Smirnov test was used to assess whether the results fit a normal distribution. A paired-samples t-test was used for statistical analyses of the normally distributed data. When the result did not satisfy normality, we analyzed the results using Wilcoxon signed-rank tests.

This study was approved by the Ethics Committee of the Faculty of Medicine of Dicle University.

## RESULTS

Twelve of the 40 patients (30%) enrolled were males, and eight of 40 patients had severe AP.

The mean age of the patients with mild AP was  $42.67 \pm 15.25$  years and the mean hospital stay was  $7.93 \pm 2.62$  days. The mean nesfatin-1 level was  $6.80 \pm 0.77$  ng/mL at admission and  $6.36 \pm 0.74$  ng/mL at discharge ( $p < 0.05$ , Figure 1). The mean



**Figure 1.** Mean nesfatin-1, ghrelin, and leptin levels at admission and at discharge (ng/mL) in patients with mild acute pancreatitis.

ghrelin level was  $2.37 \pm 0.63$  ng/mL at admission and  $3.39 \pm 1.99$  ng/mL at discharge ( $p < 0.05$ , Figure 1). The mean leptin level was  $6.93 \pm 4.35$  ng/mL at admission and  $3.36 \pm 1.56$  ng/mL at discharge ( $p < 0.05$ , Figure 1).

The mean age of the patients with severe AP was  $79.00 \pm 3.66$  years and the mean hospital stay was  $11.75 \pm 3.66$  days. The mean nesfatin-1 level was  $6.50 \pm 0.55$  ng/mL at admission and  $6.20 \pm 0.75$  ng/mL at discharge ( $p > 0.05$ , Figure 2). The mean ghrelin level was  $2.49 \pm 1.60$  ng/mL at admission and  $2.60 \pm 2.11$  ng/mL at discharge ( $p > 0.05$ , Figure 2). The mean leptin level was  $7.63 \pm 6.27$  ng/mL at admission and  $5.09 \pm 3.51$  ng/mL at discharge ( $p > 0.05$ , Figure 2).

### DISCUSSION

Till date, none of the available tests can be used as the "gold standard" for the assessment of the severity of AP. Early detection of severe AP is essential because early diagnosis of patients who may require intensive care may improve mortality. Thus, we investigated the changes of the plasma levels of the hormones nesfatin-1, ghrelin, and leptin in patients with gallstone-induced AP.

Only patients with gallstone-induced AP were enrolled in this study in order to standardize the study group. The etiology of AP varies across countries. Alcohol abuse was reported to be the most common etiologic reason leading to AP in the United States, whereas bile stones were the most common reason in Europe (1,3,21). Our group of study patients did not consume excessive alcohol; thus, bile stones were the most common etiologic cause for AP (22).

In the mild AP group, the mean nesfatin-1 level was higher at admission than at discharge ( $p < 0.05$ ), whereas in the severe AP group, it was higher at admission than at discharge, but the difference was not significant ( $p > 0.05$ ). Gonzales et al reported that nesfatin-1 was a regulator of blood glucose levels as well as an appetite-regulating peptide with weight-reducing effects (23). It also caused a reduction in gastrointestinal movements

as reported previously (10). The findings suggest that impaired glucose levels, anorexia, and slowing of gastrointestinal motility, seen at the onset of the disease, might be associated with high levels of nesfatin-1.

In the mild AP group, the mean ghrelin level was higher at discharge than at admission ( $p < 0.05$ ), whereas in the severe AP group, it was higher at patient discharge than at admission, but the difference was not significant ( $p > 0.05$ ). In a group of 53 patients with AP, Lee et al found that the overall ghrelin level was significantly lower at patient admission than at discharge (24). A regulatory function of ghrelin in insulin secretion has been demonstrated by many studies (25-27). Several studies have also been conducted on the effects of ghrelin on anorexia and gastrointestinal motility (13,25). Low levels of ghrelin at the onset of the disease might be associated with the impaired regulation of insulin secretion and anorexia. In the mild AP group, the mean leptin level was higher at admission than at discharge ( $p < 0.05$ ), and additionally, in the severe AP group, it was higher at patient discharge than at admission, but the difference was not significant ( $p > 0.05$ ). Kerem et al found that leptin levels were higher at 12, 24, and 48 h in rats with AP compared to the control group (12). In a prospective observational study on 45 patients with AP, Konturek et al. (28) found that median plasma leptin levels were significantly higher in patients with AP than those of the controls. Our results are similar to the results of these studies. Ahren et al found that leptin inhibited insulin secretion induced by cellular cAMP in a pancreatic  $\beta$  cell line (17). According to our findings, the inhibition of insulin secretion by high levels of leptin at the onset of the disease might be associated with impaired glucose regulation in patients with AP.

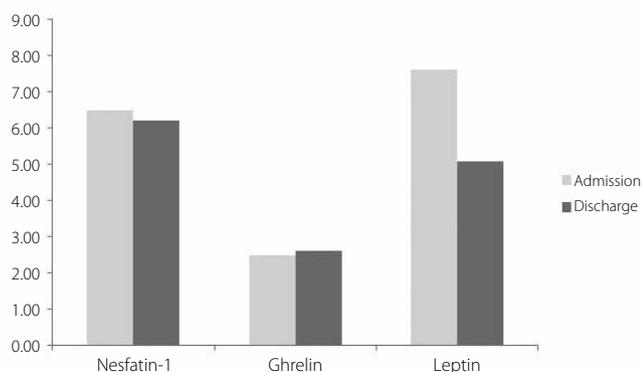
In our study, gallstone-induced AP altered the levels of nesfatin-1, ghrelin, and leptin. The changes in the levels of these hormones might play a role in glucose regulation, feeding behavior, and gastrointestinal movement in the patients with AP. Although levels of these hormones were altered in all patients with AP, only the changes in patients with mild AP were significant. This result led us to question regarding the usefulness of measuring these three hormones as a means of predicting the severity of gallstone-induced AP. Further studies are warranted to determine the usefulness of measuring these hormones in gallstone-induced AP.

**Ethics Committee Approval:** Ethics committee approval was received for his study from the Ethics Committee of the Faculty of Medicine of Dicle University.

**Informed Consent:** Written informed content was obtained from patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author contributions:** Concept - B.V.U., A.B.; Design - A.B., Z.B.; Supervision - A.O., O.U.; Resources - B.V.U., M.G.; Materials - M.G., H.Y.; Data Collection and/or Processing - B.V.U., A.B.; Analysis &/or Interpretation: A.O., A.B.; Literature Search - B.V.U., A.B.; Writing - B.V.U., M.G.; Critical Reviews - A.B., O.U., H.G.



**Figure 2.** Mean nesfatin-1, ghrelin, and leptin levels at admission and at discharge (ng/mL) in patients with severe acute pancreatitis

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Financial Disclosure:** This study was financially supported by the Coordinating Authority of Scientific Research Projects of Dicle University (DUBAP; Project no. 10-TF-49).

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