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Significance of appetite hormone ghrelin and obestatin levels in the assessment of the severity of acute pancreatitis

PANCREAS

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Original Article

ABSTRACT

Background/Aims: Due to risk of morbidity and mortality, various tests and scoring systems used in the assessment of the diagnosis and severity of acute pancreatitis disease are gaining more importance every day. Most of the current scoring systems, validated by various parameters, have a sophisticated and complex structure. Research is ongoing to establish a method to diagnose the disease and determine the severity by using different and simple parameters. In this trial, we aimed to investigate the role of the orexigenic "ghrelin" and anorexigenic "obestatin" hormones, if any, on the diagnosis and assessment of the severity of acute pancreatitis.

Materials and Methods: A total of 30 patients hospitalized between September 2009 and September 2010 with a diagnosis of acute pancreatitis (AP) and 25 healthy volunteers were enrolled in the trial with a prospective and randomized design. The patients were classified in two groups, mild (Ranson ≤3 and / or Apache II ≤8) and severe (Ranson >3 and/or Apache II >8) cases, as per the Ranson and Apache-II criteria; the ghrelin and obestatin levels in blood samples obtained from the patients were measured using the ELISA method.

Results: Twenty-two of the 30 patients (73%) were regarded as mild pancreatitis cases, while 8 cases (27%) were diagnosed as severe pancreatitis. Comparison of the mild and severe pancreatitis groups did not reveal a statistical difference between the two groups in terms of acylated and de-acylated ghrelin values on presentation and following the initiation of oral feeding. Similarly, no significant difference was found in the comparison of the patient and the control groups in terms of acylated and de-acylated ghrelin values on presentation (p=0.863). On the other hand, acylated and de-acylated ghrelin values after initiation of oral feeding were observed to be higher in the patient group (p=0.001, p=0.000). Comparison of these two groups revealed a significant difference in obestatin values, both on presentation and after initiation of oral feeding (p=0.002 and p=0.000).

Conclusion: Consistently high serum ghrelin values during pancreatic inflammation suggest that ghrelin may be used as an adjunctive parameter in the monitoring of the course of the disease. On the other hand, high obestatin values in patients on presentation indicate that this hormone is a more significant parameter in terms of diagnosis. However, no correlation was established between these two peptide hormones and the severity of AP.

Keywords: Acute pancreatitis, ghrelin, obestatin

INTRODUCTION

The pancreas is probably the most notorious organ in the human body, which is frequently ignored and refrained from touching by surgeons. Acute pancreatitis (AP) is the most common pancreatic disease in clinical practice and was first described by the French surgeon Ambrose Pare in 1579; it has been defined as early activation of pancreatic enzymes in the pancreas, leading to auto-digestion of the pancreas (1). Despite developments in the medical field, AP is still a significant source of morbidity and mortality; it has a complex etiology, and no specific causative factor has been designated

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in some cases, although a number of factors play a role in the development of the disease (2). Similar to the etiology, the clinical course of the disease is also complex. Patients may present with different manifestations, varying from clinical states of mild abdominal pain to multiple organ failure and death (3). The main indicator in the clinical course is the severity of the disease. Therefore, early prediction of disease severity by various methods plays a key role in the provision of appropriate and efficient treatment (4). In this regard, various scoring systems have been recommended to determine the disease severity in the early stages. These systems may be stated as Ban's, Agarwal-Pitchumoni, Ranson, Glasgow (Imrie), and the acute physiology and chronic health evaluation (APACHE-II). However, these scoring systems are rather complex and difficult for use in clinical practice (5,6).

Ghrelin hormone was first discovered by Kojima et al. (7) in 1999 in the stomach and named as the hunger (appetite) hormone, although ghrelin is known to possess many characteristics, including anti-inflammatory activities. It is found in two major forms in biological tissues and fluids. A different form, which has fatty acid bound to the third serine or threonine (only in frogs) of the N-terminus, is referred to as acylated ghrelin. The breaking off of the bond of the fatty acid and peptide generates a different form, called de-acylated ghrelin (8).

In a trial conducted by Cetinkaya et al. (9), ghrelin levels in the preoperative serum and saliva samples of appendectomy patients were reported to be lower compared to values in the postoperative period and in healthy controls. The authors suggested that the observed decrease in ghrelin levels may be due to the loss of appetite in acute appendicitis. On the other hand, the number of trials investigating the correlation between acute pancreatitis and obestatin, the product of the same gene with ghrelin, is limited. Yet, obestatin is synthesized in a co-localized fashion with ghrelin in all organs producing ghrelin, and studies indicate that it has the reverse activity of ghrelin, regarded as anorexigenic, although a consensus has not yet been established regarding this standpoint (10).

Therefore, this trial was designed to determine the role of the orexigenic "ghrelin" and anorexigenic "obestatin" hormones, if any, on the diagnosis and assessment of the severity of AP, in addition to conventional methods.

MATERIALS AND METHODS

The trial was initiated upon approval of the protocol by the Ethical Committee of Fırat University Medical Faculty, dated 22.02.2007 and designated number 2007-02/08. The patients and relatives/caretakers were informed as required, followed by completion of signed informed forms by the patients and volunteers. All the data were recorded prospectively. The trial was conducted on a total of 30 patients who had presented to and were hospitalized with a diagnosis of acute pancreatitis in the Emergency Unit of Fırat University Medical Faculty between September 2009 and September 2010, in addition to 25 healthy volunteers.

Cases presenting to the Emergency Unit with an acute attack of chronic pancreatitis, patients diagnosed with an acute pancreatitis state following ERCP, and patients undergoing an operation where bile or pancreatic duct flow was diverted were excluded from the trial. Furthermore, patients hospitalized and fully monitored by the Department of Gastroenterology, cases initially followed up in the Emergency Unit with a different diagnosis and diagnosed as acute pancreatitis after 48 hours, and patients referred from a different medical center were not enrolled in the trial. Pancreatitis was diagnosed by epigastric/periumbilical abdominal pain or abdominal pain radiating to the lumbar region/back, high amylase levels in the serum or urine (at least 3 times the upper limit of normal in serum), or by verification of pancreatitis on computerized tomography (5,11,12). In order to determine the appetite level, the visual analog scale (VAS) test was performed, in addition to information obtained from the patient's medical history.

The Ranson and APACHE-II scores of all patients were calculated based on the parameters on presentation. The patient group in the trial was classified into two groups per the Ranson and APACHE-II criteria (13).

1. mild acute pancreatitis: Ranson ≤3 and/or Apache II ≤8
2. severe acute pancreatitis: Ranson >3 and/or Apache II >8

Venous blood samples were obtained from patients on presentation and after initiation of oral feeding. Morning fasting and postprandial blood samples were obtained from volunteers in the control group. In order to prevent denaturation of the investigated peptide hormones by proteases, 500 KIU aprotinin (protease inhibitor) was added to each millimeter of blood. These biological fluids were stored at -20°C until the trial was completed.

After completion of the number of patients, the ghrelin and obestatin levels in serum samples were measured by the ELISA method. Specific ghrelin and obestatin kits were used for this purpose. The SPI-BIO kit (A05119: Unacylated Ghrelin (human) EIA kit produced by Bertin Pharma and A05106: Acylated Ghrelin (human) EIA kit) was used for acylated and de-acylated ghrelin (SPI Bio Bertin Pharma, Bretonneux, France), while the USCN kit (Catalog Number 92039) (Uscn Life Science, Wuhan, China) was used for obestatin.

Statistical analysis

All prospective data were recorded in the Microsoft® Office Excel 2003 program and analyzed by the SPSS 11.5 Version for Windows Excel program (SPSS Inc. Software Chicago, IL, USA). At the end of the trial, the correlation of severity of pancreatitis and ghrelin and obestatin values in each group was assessed using the Mann-Whitney U-test. A p value of <0.05 was regarded as significant.

RESULTS

Among a total of 30 patients, 8 were males and 22 were females. The mean age was specified as 61.9±14.6 (23-85). While 22 of 30 patients (73%) were mild pancreatitis cases, 8 (27%) were designated severe pancreatitis. Comparison of the mild and severe pancreatitis groups revealed normal acylated (octanate) ghrelin levels on presentation, while the values in the severe pancreatitis group were slightly over normal limits (Figure 1). However, this difference was statistically insignificant (p=0.500). The acylated ghrelin values in both groups were observed to be increased following initiation of oral feeding, but the difference was not significant (p=0.764) (Figure 1). Both groups exhibited increased de-acylated ghrelin values on presentation, with no significant difference between the groups (p=0.614) (Figure 2). The de-acylated ghrelin values in the two groups increased to 3X normal levels following initiation of oral feeding, but the comparison did not reveal a significant difference (p=0.746) (Figure 2). The obestatin values in both groups followed a similar course, with no significant difference between the groups (p=0.323 and p=0.287) (Figure 3).

Comparison of the patient and the control groups did not reveal a significant difference between the two groups in terms of acylated ghrelin values on presentation, which were determined to be within normal limits (p=0.863). After initiation of oral feeding, a significant difference was observed between the acylated ghrelin values (p=0.001), showing high ghrelin values in the patient group in contrast to the decreased levels in the control group (Figure 1). No significant difference was found between the patient and the control groups in terms of de-acylated ghrelin values on presentation (p=0.851). However, de-acylated ghrelin values were also determined to be higher in the patient group, while decreased levels were observed in the control group (Figure 2). This difference was statistically significant (p=0.000).

Comparison of the obestatin values in the two groups revealed a significant difference in terms of obestatin values, both on presentation and following oral feeding (p=0.002 and p=0.000) (Figure 3). There was no correlation between amylase levels and peptide concentrations. ERCP was done for only three patients after hospitalization. Those ERCP results showed no effects on peptide levels.

DISCUSSION

Currently, there is no specific biochemical parameter available to be used in the diagnosis and severity assessment of acute pancreatitis (14). Three hormones encoded by the ghrelin gene have been discovered in recent years, namely obestatin, de-acylated ghrelin, and acylated ghrelin, also known as bioactive peptides. In previous trials, it was well established that ghrelin displays anti-inflammatory activities (15). Furthermore, the data obtained in these trials showed that hormones encoded by the ghrelin gene are synthesized

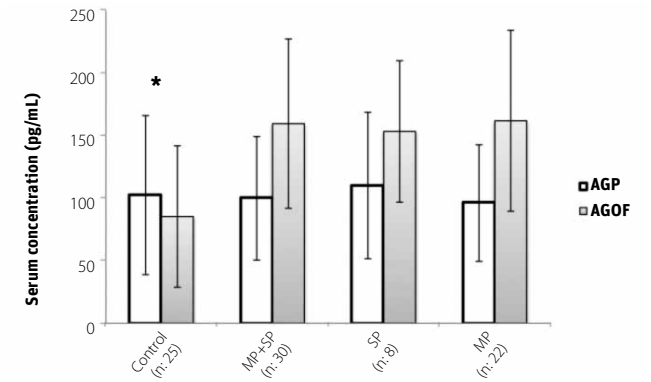

Figure 1. Comparison of acylated ghrelin changes in patients with and without pancreatitis. AGP: Acylated ghrelin on presentation; AGOF: Acylated ghrelin after oral feeding. MP: Mild pancreatitis; SP: Severe pancreatitis. (Mean±standard deviation. Statistical significance *p<0.05).

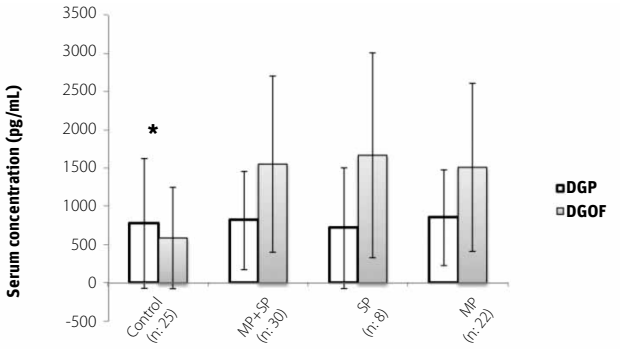

Figure 2. Comparison of de-acylated ghrelin changes in patients with and without pancreatitis. DGP: De-acylated ghrelin on presentation; DGOF: De-acylated ghrelin after oral feeding. MP: Mild pancreatitis; SP: Severe pancreatitis. (Mean±standard deviation. Statistical significance *p<0.05).

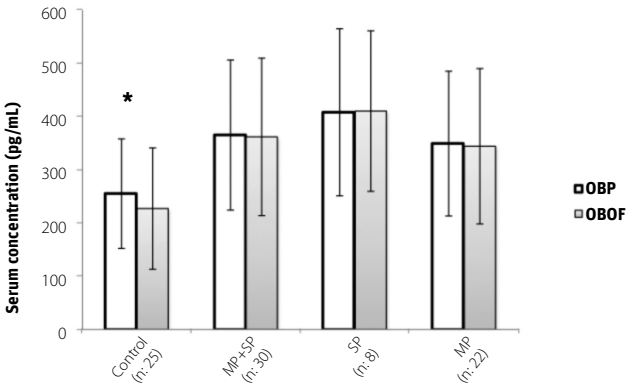

Figure 3. Comparison of obestatin changes in patients with and without pancreatitis. OBP: Obestatin on presentation; OBOF: Obestatin after oral feeding. MP: Mild pancreatitis; SP: Severe pancreatitis. (Mean±standard deviation. Statistical significance *p<0.05).

in the pancreas, emphasizing the protective activity on the pancreas in general (16).

In the current trial, comparison of the serum ghrelin and obestatin values in mild and severe AP patients did not reveal a statistically significant difference. Hence, our results indicate

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that serum ghrelin and obestatin levels are not significant in terms of the severity assessment of pancreatitis. However, the general evaluation of the data obtained in two groups indicates that the values in the severe group were somewhat higher than the levels in the mild group.

On the other hand, comparison of the obestatin values on presentation and after oral feeding in the patient and the control groups revealed a significant difference. Moreover, we observed a significant difference in the acylated and de-acylated ghrelin values in these two groups after initiation of oral feeding. Based on these findings, we suggest that serum ghrelin and obestatin values play a role in the diagnosis of AP. Consistently high obestatin values both on presentation and after subsiding of the clinical course, compared to the control group, indicate that this parameter may be significant in terms of diagnosis.

Increased acylated and de-acylated ghrelin levels were determined in both groups in the comparison of the control and the pancreatitis groups. On the other hand, decreased levels were observed in the control group following initiation of oral feeding. It is obvious that ghrelin, also known as the hunger hormone, would display such a course during satiety in the control group. However, the lack of a decrease in the levels in the patient group shows that ghrelin may have been increased due to the inflammation observed in pancreatitis. Accordingly, increased ghrelin levels were determined throughout the course of pancreatitis. The exact source of ghrelin levels on presentation in the diagnosis of AP has not been confirmed; nevertheless, consistently high ghrelin levels after subsiding of the clinical course is a supportive parameter for the diagnosis of the disease.

In a trial conducted on 90 rats by Kerem et al. (17), experimentally generated acute edematous pancreatitis and acute necrotizing pancreatitis groups were compared. The results showed that serum ghrelin levels increased within 24 hours following the development of pancreatitis, reaching maximum values after 48 hours. It was concluded that pancreatitis caused an increase in the serum ghrelin levels during the first 48 hours and that the increase in values obtained in the acute necrotizing pancreatitis group was determined to be higher than values in the acute edematous pancreatitis group. Hence, these parameters were suggested to be beneficial in terms of determination of the severity of pancreatitis. Our trial differs from this study, both in technical terms and in terms of the duration of hormone testing. The timings of the measurement of ghrelin levels in the patient group in the current trial were on presentation and following the initiation of oral feeding. Initiation of oral feeding is parallel to subsiding of the clinical course, which was longer than 48 hours in many of the patients. It is possible to suggest that we could have obtained results similar to the trial of Kerem et al. (17), provided that blood sampling was performed after 48 hours following the diagnosis.

The correlation between serum ghrelin concentrations and the severity of acute pancreatitis was investigated in a trial performed by Lee et al. (18) on 53 patients. The patients were classified as the risky group and the group with no risk, as per CRP >150 and CT index >4, in addition to the Atlanta criteria. Blood samples were obtained on presentation, after 48 hours, and on discharge from the clinic, and the serum ghrelin levels were measured. Ghrelin levels were determined to be higher in the risky group on presentation; however, no significant difference was found between the two groups after 48 hours and on discharge from hospital. Similar to our trial, monitoring of the ghrelin levels revealed higher values or no decrease, compared to the values obtained on presentation. The authors concluded that it was not possible to clearly show the significance of serum ghrelin levels in the assessment of the severity of pancreatitis; however, they indicated that the serum ghrelin concentrations in severe acute pancreatitis cases were higher.

In a trial conducted by Liu et al. (19), the changes in serum ghrelin levels were investigated. Similar to our trial, the blood samples were obtained from patients on presentation and after improvement of the disease and reported higher levels following subsiding of the clinical course. The ghrelin values in the control group of our trial also showed a significant decrease after the initiation of oral feeding, while consistently high levels were found in the patient group. The results of the trial above and the current trial are supportive of this, and the common conclusion is that the ghrelin levels in acute pancreatitis follow a consistently high course after improvement of the clinical symptoms.

In the light of these literature data and the results of the current trial, we may indicate that ghrelin levels display consistently high levels throughout the course of pancreatitis. The exact source of ghrelin levels on presentation in the diagnosis of AP has not been confirmed; nevertheless, consistently high ghrelin levels after subsiding of the clinical course is a supportive parameter for the diagnosis. The number of trials conducted on obestatin is limited in the literature, while relatively few trials are available investigating the correlation between acute pancreatitis and obestatin (20).

Ceranowicz et al. (20) conducted a trial on rats with acute pancreatitis, experimentally generated by administration of cerulein and investigated the protective effect of obestatin on pancreatitis by intraperitoneal injection of obestatin. They concluded that obestatin decreased the severity of acute pancreatitis induced by cerulein. In the current trial, the serum obestatin level in the patient group was determined to be higher than that of the control group. This finding indicates that secretion of obestatin is increased in pancreatitis patients, and this supports the view that it may be released to exert a protective effect on the pancreas. Once more, it may be regarded as a useful parameter for the diagnosis, perhaps for the differential diagnosis, rather than assessment of the severity.

In conclusion, consistently high serum ghrelin values throughout the course of inflammation indicate that ghrelin increases parallel to the inflammation and suggest that monitoring of this consistent increase may be regarded as a useful parameter in the monitoring of the course of the disease. Taken together, these results also show that there is a direct relationship between inflammation and ghrelin in patients with acute pancreatitis. On the other hand, high obestatin levels among patients on presentation and during inflammation indicate that it is a rather more significant parameter in terms of diagnosis. Nevertheless, the correlation between the severity of AP and these two peptide hormones could not be established.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethical Committee of Fırat University.

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

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