Is leptin related to systemic inflammatory response in acute pancreatitis?

Andrés Duarte-Rojo, Ana Lezama-Barreda, María Teresa Ramírez-Iglesias, Mario Peláez-Luna, Guillermo Robles-Díaz

Abstract

AIM: To evaluate the relationship between leptin and systemic inflammation in acute pancreatitis.

METHODS: Consecutive patients with acute pancreatitis were included. Body mass index and serum samples were obtained at admission. Leptin, TNF-α, IL-6, -8 and -10 levels were determined by ELISA. Severity was defined according to Atlanta criteria.

RESULTS: Fifty-two (29 females) patients were studied. Overall body mass index was similar between mild and severe cases. Women with low body mass index had lower body mass index (P = 0.04) and men showed higher body mass index (P = 0.05). No difference was found in leptin levels regarding the severity of pancreatitis, but higher levels tended to appear in male patients with increased body mass index and severe pancreatitis (P = 0.1). A multivariate analysis showed no association between leptin levels and severity. The strongest cytokine associated with severity was IL-6. Correlations of leptin with another cytokines only showed a trend for IL-8 (P = 0.058).

CONCLUSION: High body mass index was associated with severity only in males, which may be related to android fat distribution. Serum leptin seems not to play a role on the systemic inflammatory response in acute pancreatitis and its association with severe outcome in males might represent a marker of increased adiposity.

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Key words: Leptin; Cytokines; Obesity; Acute pancreatitis; Prognosis

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INTRODUCTION

Obesity is a risk factor for severe acute pancreatitis (AP) and for the development of both local and systemic complications[1]. A chronic, low grade, pro-inflammatory state has been described in human obesity, including increased plasma levels of IL-6, TNF-α, C-reactive protein and endothelial adhesion molecules[2]. This may be due to the cytokine-synthesizing capabilities of adipose tissue driven by yet unknown signals, giving rise to insulin resistance and the metabolic syndrome[3]. Actually, one third of the circulating IL-6 originates from adipose tissue, especially from the visceral depot[4]. On the other side, the inflammatory cascade evoked by AP has a pivotal role in determining severity of the disease, and many cytokines and other acute reactants are well-established prognostic markers (i.e. IL-6, -8, -10, C reactive protein)[5]. Coincidence of both a chronic and acute inflammatory scenarios (obesity and AP, respectively) with similar cytokine patterns may offer an explanation for the association between severe AP and obesity. Therefore, obese individuals may be prone to develop a more intense inflammatory reaction in response to AP that would lead to a dismal prognosis.

Leptin, the protein derived from the Ob gene, is an adipocyte-derived signal[6], and its circulating concentrations are highly correlated with body fat mass[7,8]. It has also been implied as a modulator of immunity with pro-inflammatory properties[9,10], and it is highly correlated to C-reactive protein, independently of obesity[11]. Research about leptin in AP is scarce. Two experimental...
studies in animal models have suggested a protective role for leptin on AP by suppression of the pro-inflammatory response, reduction of leukocyte infiltration on pancreatic tissue and increased pancreatic repair\textsuperscript{[11,12]}. These results contrast with those found when studying non-pancreatic conditions, suggesting a tissue-specific protective role for leptin. Also, elevated plasma levels of leptin were found in patients with AP when compared to healthy controls with similar body mass index\textsuperscript{[11]}. However, distinction between severe and mild cases was not established.

Whether circulating leptin would have a pro- or anti-inflammatory role in AP, its serum concentrations should correlate with severity in AP, as has been reported for IL-6 and IL-10, respectively. It should also correlate with other cytokines previously found to be increased in severe AP (i.e. TNF-\( \alpha \), IL-6, -8, -10). The purpose of the present work was to measure serum concentrations of leptin and other cytokines in patients with severe and mild AP to identify a possible role of the former in the systemic inflammatory response, and in the prognosis assessment of AP.

**MATERIALS AND METHODS**

All patients with AP diagnosis admitted between March 2002 and August 2004 to a tertiary medical center were included in the study. Diagnosis of AP was based on typical clinical manifestations with at least a 3-fold increase of serum amylase and/or lipase. Whenever uncertainty about diagnosis existed, CT-scan was performed to confirm/rule out AP. Patients were excluded if they had received IV therapy before admission. HIV/AIDS patients were also excluded because of the confounding lipodystrophy and altered leptin levels described in these patients\textsuperscript{[13]}. The etiology of AP was classified as biliary, alcoholic, idiopathic or other. Weight and height from all patients were measured during the first 48 h from admission for body mass index (BMI) calculation. Prognostic severity by modified Ranson’s criteria was recorded for each patient\textsuperscript{[14]}.

Severe AP was considered when patients developed one or more local (i.e. necrosis, infected necrosis, abscess, pseudocyst) or systemic (i.e. renal or respiratory failure, cardiovascular collapse, coagulopathy, gastrointestinal bleeding, sepsis or multiple organ failure) complications according to the Atlanta classification of AP\textsuperscript{[15]}. Patients were managed by the medical house staff who were blinded as to the aims and methodology of this study.

During the first 24 h from admission, aliquots of a serum sample obtained from all patients were stored at \(-70^\circ\)C, until laboratory determinations were performed. Serum leptin levels were measured in duplicate in the same run using a commercial ELISA kit for human leptin (Linco Research, MO, USA), with a sensitivity of 0.5 ng/mL. According to protocol insert, mean serum leptin levels from normal individuals (BMI: 18.25 kg/m\(^2\)) are 3.8 \(\pm\) 1.8 ng/mL for men, and 7.4 \(\pm\) 3.7 ng/mL for women. TNF-\( \alpha \), IL-6, -8 and -10 concentrations were quantified using commercially available ELISA kits (Beckton Dickinson, NJ, USA), according to the manufacturer’s instructions.

BMI was expressed as mean \(\pm\) SD, while serum determinations were expressed as medians and ranges. Comparisons were carried out by means of the \(t\) test and Mann-Whitney U-test, as appropriate. A multivariate analysis was constructed to test the independent association of circulating leptin and severity, adjusted for potential confounders (gender and BMI). Correlations among cytokines and any other non-parametric variable were done using the Spearman rank-order test. All statistical analysis was performed using SPSS statistical software (Chicago, Illinois, USA). \(P < 0.05\) was considered statistically significant.

Informed consent was obtained from all patients. This study was approved by the local Institutional Board for Human Research and designed according to international guidelines.

**RESULTS**

A total of 58 patients with AP were studied. Six patients were excluded from the analysis, four of them because of HIV/AIDS and two because of unavailability of serum for complete leptin and/or cytokines determinations (2 patients had severe AP, 33%). There were 29 women (56%) and mean age of all patients was 42 \(\pm\) 16 years. The most frequent AP etiology was biliary (\(n = 19\)), followed by hypertriglyceridemia (\(n = 7\)), alcoholic (\(n = 6\)) and post-ERCP (\(n = 4\)). Other causes included: acute episodes of chronic pancreatitis (\(n = 4\)), pancreas divisum (\(n = 2\)), drug-induced (\(n = 2\)) and hypercalcemia (\(n = 1\)); while 7 cases were considered idiopathic. First time AP was present in 31 patients (60%). Severity was identified in 14 cases (27%). Local complications occurred in 8 (15\%) patients, whereas systemic complications developed in 11 (21\%); presence of both complications was noted only in 5 cases. Six patients (12\%) were admitted to the ICU and 4 had lethal AP (8\%).

BMI according to severity status and gender is shown in Table 1. Although there was no difference between severe and mild cases in all patients, females with severe AP had significantly lower BMI when compared to mild

| Table 1  Body mass index (BMI) and leptin levels in acute pancreatitis (AP) according to severity status and gender |
|---------------------------------------------------------------|
| | BMI | BMI \(\geq 25\) | BMI \(\geq 30\) | Leptin (ng/mL) |
| | \(n\) | mean \(\pm\) SD | \(n\) | \(n\) | Median (range) |
| All patients | 52 | 27 \(\pm\) 5 | 32 (63) | 13 (25) | 8.6 (0.5-215) |
| Severe AP | 14 | 27 \(\pm\) 5 | 7 (54) | 2 (15) | 10.3 (0.5-61) |
| Mild AP | 38 | 27 \(\pm\) 5 | 25 (66) | 11 (29) | 7.7 (0.5-215) |
| Women | 29 | 27 \(\pm\) 5 | 18 (64) | 9 (32) | 11 (0.5-215) |
| Men | 23 | 27 \(\pm\) 4 | 14 (61) | 4 (17) | 3.7 (0.5-32) |
| Women & severe AP | 7 | 24 \(\pm\) 3 | 2 (33) | 0 | 10.4 (1.39 - 61) |
| Women & mild AP | 22 | 28 \(\pm\) 6 | 16 (73) | 9 (41) | 11.7 (0.5-215) |
| Men & severe AP | 7 | 29 \(\pm\) 6 | 5 (71) | 2 (29) | 7 (0.5-32) |
| Men & mild AP | 16 | 26 \(\pm\) 3 | 9 (65) | 2 (13) | 3.4 (0.5-19) |

\(P = 0.008, \ ^{\mathrm{a}}P = 0.04, \ ^{\mathrm{b}}P = 0.05, \ ^{\mathrm{c}}P = 0.1\) (Mann-Whitney U-test).
Leptin and AP severity | Overall | Gender adjusted | BMI adjusted
--- | --- | --- | ---
P | 0.766 | 0.5 | 0.272

Table 3 Levels of cytokines (other than leptin) in acute pancreatitis (AP) according to severity status and gender

| Cytokine | All patients | Women | Men |
|---|---|---|---|
| TNF-α | 0 (0-579) | 13 (0-385) | 0 (0-579) |
| IL-6 | 52 (1-560) | 206 (10-590) | 40 (1-537) |
| IL-8 | 4 (0-4596) | 99 (0-4596) | 4 (0-1322) |
| IL-10 | 4 (0-560) | 6 (0-54) | 2 (0-560) |

Table 4 Leptin correlation with other cytokines according to gender

| Cytokine | All patients | Females | Males |
|---|---|---|---|
| Leptin | NS | NS | 0.487 (0.019) |
| TNF-α | NS | NS | 0.265 (0.058) |
| IL-6 | NS | NS | 0.488 (0.007) |
| IL-8 | NS | NS | 0.397 (0.061) |
| IL-10 | NS | NS | 0.397 (0.061) |

Leptin and AP severity | Overall | Gender adjusted | BMI adjusted
--- | --- | --- | ---
P | 0.766 | 0.5 | 0.272

1 pg/ml, all results expressed as median (range).

Table 2 Independent association between leptin levels and severity of acute pancreatitis (AP) adjusted by gender and body mass index (BMI)

| Leptin and AP severity | Overall | Gender adjusted | BMI adjusted |
|---|---|---|---|
P | 0.766 | 0.5 | 0.272

Discussion

Severe AP has a mortality around 24%-50%[1]. Therefore, major efforts have focused on the discovery of prognostic markers for an early identification of severe cases, to anticipate a more aggressive diagnostic and therapeutic approach. The recognition of obesity as a risk factor for severe AP[3] lead to the identification of other markers of obesity, such as android fat distribution and higher waist circumference, as more strongly associated with severity[17]. These findings provided a possible link between AP and the so-called metabolic syndrome, which among certain criteria is characterized by a chronic pro-inflammatory condition[3].

Particularly since the identification of human leptin on 1994, adipose tissue has been increasingly recognized as an endocrine organ capable of coordinating a variety of biological processes including not only energy metabolism, but neuroendocrine and immune functions[10]. IL-6, TNF-α and leptin are among the principal immune-related

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signals produced by adipose tissue[3,4]. In vitro and in vivo experimental studies have shown that leptin is capable of promoting neutrophils activation and chemotaxis, inducing TNF-α and IL-6 from monocytes, promoting monocytes/macrophages activation and phagocytosis, enhancing lymphocytes proliferation and favouring a Th1 cytokine response[5,8]. Also, it has been demonstrated that leptin can restore immune function under critical conditions such as starvation and it has been linked to autoimmunity[9,10]. Consequently, adipose tissue and leptin seem to play a major role on immunological status, being the latter a pro-inflammatory signal.

On the pancreatic view, TNF-α, IL-1, -8, -10, and especially IL-6, have a major role on the pathophysiology of AP, as part of the inflammatory network evoked systemically[3,4]. Experimental studies of AP in rats have shown that leptin levels increase after cerulein-induced pancreatitis, presumably from pancreatic origin (increase in leptin mRNA from the pancreas), and that exogenous administration is associated with diminished histological manifestations of pancreatitis and reduced plasma TNF-α[11]. Similarly, administration of leptin reduced the severity of ischemia/reperfusion-induced pancreatitis by diminishing morphological features of pancreatic damage and serum IL-1β levels, while accelerated pancreatic repair by increasing pancreatic blood flow and DNA synthesis[12]. These results are contrary to what would be expected according to other experimental studies evaluating the effect of leptin in non-pancreatic tissues[9,10,14], and point-out a possible tissue-specific anti-inflammatory role in AP.

The absence of relationship between BMI and severity in the present study may be related to our reduced sample size, or to the predominance of female patients. Most reports on obesity as a prognostic marker of AP (including one meta-analysis) have not addressed whether this association may be modified by gender. However, two previous studies by our group have found that the association between obesity and severity is mostly seen in male patients[11,12]. A clear explanation for this dichotomy on the risk of obesity on AP is unavailable yet, although it may be related to the higher waist to hip ratio or waist circumference (android fat distribution) found in males[17].

Our results on the positive correlation of leptin with BMI, as well as the higher levels in females are in agreement with what has been previously described[9]. Leptin levels did not differ between severe and mild cases, although male patients with severe AP had higher leptin levels in association with increased BMI, which may only represent a marker of increased adiposity[9]. Moreover, these results were supported by the lack of association between leptin levels and severity in a multivariate analysis adjusted for gender and BMI. Similar findings occurred in patients with inflammatory bowel disease in whom leptin differences were entirely explained by variations in BMI, rather than disease activity[18]. Furthermore, leptin did not correlate with any other cytokine in the whole group, while in the correlation by gender, leptin was neither correlated with the cytokines increased in severe cases (TNF-α and IL-6 for females, IL-6 and IL-8 for males, IL-10 for both). Although there was a positive association between TNF-α and leptin levels for males, all the evidences above make it difficult to think that leptin plays a major role in the inflammatory response of AP; whether as a pro- or anti-inflammatory cytokine.

TNF-α, IL-6 and IL-10 were increased in the whole group of patients with severe AP, although IL-6 was the only cytokine that remained significantly higher in women and men with severe episodes, either in presence of local or systemic complications, or when admitted to the ICU. However, mortality was associated with elevation in serum levels of TNF-α and IL-8. Both cytokines, TNF-α and IL-8, were associated with Ranson’s prognostic scale for mortality. Many studies have consolidated IL-6 as the cytokine with the most important prognostic utility for severity within the initial 24 h, although it seems not to be useful for prediction of death[19,21-23]. This cytokine has also demonstrated use for identification of infected necrosis[24].

Similarly, IL-8, TNF-α and IL-10 have early prognostic utility in AP: all of them are increased early in severe cases[25,26] and IL-8 has been previously found increased in fatal AP[27].

Konturek et al, found that leptin levels were increased in patients with AP when compared to BMI- and age-matched controls, however this study did not distinguish mild from severe cases[28]. It is well known that acute inflammatory conditions (i.e. AP) are associated with hyperglycemia, insulin resistance and increased levels of leptin, IL-6 and TNF-α[3,4], and it is also recognized that insulin resistance leads to leptin resistance and further elevation of leptin concentrations[29].

Therefore, this could be the explanation for previously reported increased leptin levels in AP that would result in even further increased levels if superimposed on a chronic inflammatory condition (i.e. obesity), as was observed in male patients with AP and higher BMI. Since our findings do not support leptin as being associated with a more severe inflammatory response and worse prognosis (i.e. severity, mortality or admission to ICU), it could represent just a bystander of the inflammatory reaction in AP; and unlike IL-6, IL-10 and TNF-α, not play a role on its pathophysiology.

The role of leptin as an anti-inflammatory and protective signal as was demonstrated by some experimental models in rats[11,12], should have lead to significantly increased or decreased levels in severe cases, which was not demonstrated in our results; therefore, not ascertaining leptin as a protective signal in AP. This difference could only represent species differences in the actions for leptin, as has been shown with respect to leptin effects on glucocorticoids (these are decreased by leptin in mice, while not affected in humans)[30].

In summary, our results do not support human leptin as a major pro-inflammatory signal involved in AP, nor as a protective and anti-inflammatory mediator. It seems neither to be the link between obesity and a higher rate of complications in AP; nor a prognostic marker.

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