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Plasma Leptin Levels in Rats with Pancreatitis

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Department of General Surgery, Cerrahpasa Faculty of Medicine, Istanbul University, [1]Department of General Surgery, Haydarpasa Numune Teaching Hospital, [2]Department of General Surgery, PTT Erenkoy Teaching Hospital, [3]Department of Biophysics, Cerrahpasa Faculty of Medicine, Istanbul University, [4]Department of Pathology, Haydarpasa Numune Teaching Hospital, Istanbul, Turkey

Yavuz, N., Unal, E., Memisoglu, K., Krand, O., Kiziler, A.R., Aydemir, B., Kusaslan, R., Dogan, M., Gunes, P. and Titiz, I. *Plasma Leptin Levels in Rats with Pancreatitis*. Tohoku J. Exp. Med., 2004, **204** (4), 243-248 —— Diagnosis of pancreatitis is based on the determination of serum amylase and lipase levels. However, recent identification of specific leptin receptors in the pancreas suggests that this peptide may also play some roles in the modulation of pancreatic function. The objective of the present study was to investigate the relationship between serum leptin levels and pancreatitis. Thirty male Wistar rats were divided into 3 groups: the control group, acute pancreatitis group and chronic pancreatitis group. Pancreatitis was induced by injection of ethyl alcohol into the common biliary duct. A sham laparotomy was performed in the control group. Control and acute pancreatitis groups were sacrificed 24 hours later, and chronic pancreatitis group was sacrificed on postoperative day 7. Blood was taken by cardiac puncture for the determination of plasma leptin levels, and the pancreatic tissue was excised for histopathologic confirmation of pancreatitis. Plasma leptin rose significantly from the median of 0.78 ± 0.12 ng/ml in the control group to 1.92 ± 0.10 ng/ml and 1.86 ± 0.13 ng/ml in acute and chronic pancreatitis groups, respectively ($p < 0.001$, for both). There was no significant difference in the plasma leptin levels between the acute pancreatitis group and the chronic pancreatitis group ($p > 0.05$). These findings confirm that leptin has a role in pancreas inflammation, and the inflamed tissue can be the source of local production of leptin. ——— leptin; acute pancreatitis; chronic pancreatitis

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Pancreatitis is an inflammatory disorder of the pancreas associated with edema, various amounts of autodigestion, necrosis, and, in some cases, hemorrhage. Clinically, it is defined by a typical symptom complex associated with elevated serum amylase and lipase levels. However, in recent years, specific biochemical markers like catalase, protease inhibitors, and complements have been used for diagnostic criteria.

Leptin is known mainly for its role in the regulation of food intake, body composition and energy expenditure through a central feedback mechanism (Konturek et al. 2001). In several recent studies, it has also been shown that leptin has a profound inhibitory influence upon insulin secretion by acting directly on the pancreatic beta-cells (Jaworek et al. 2003; Perez et al. 2004). In most overweight individuals, however, physiological regulation of body weight by leptin seems to be disturbed, representing "leptin resistance." This leptin resistance at the level of the pancreatic beta-cell is suggested to contribute to the development of hyperinsulinemia and manifest type 2 diabetes in overweight patients (Jaworek et al. 2003).

There are also some data in the recent literature showing that leptin plays a role in the inflammatory process. It has been shown that leptin receptor expression on T lymphocytes modulates chronic intestinal inflammation in mice (Jaworek et al. 2002). Leptin has also been used as an inflammatory marker protein to predict the cardiovascular disease in patients with end-stage renal disease (Siegmund et al. 2004). Recent detection of specific leptin receptors in the pancreas suggests that this peptide may also play some roles in the modulation of pancreatic function (Zoccali et al. 2004). Leptin may modulate inflammatory responses in pancreatitis. However, the involvement of leptin in pancreatitis is unknown.

In the present study, we aimed to detect any possible change in the plasma leptin levels in rats with alcohol-induced acute and chronic pancreatitis.

Materials and Methods

Thirty male Wistar Albino rats weighing 200-300 g were divided into 3 groups: the control group (n = 10), acute pancreatitis group (n = 10) and chronic pancreatitis group (n = 10). The animals were fed on standard laboratory diet and water ad libitum before and after surgery. The study was approved by Cerrahpasa Medical Faculty Laboratory Animals Ethics Committee, and all procedures with animals were performed in accordance with the guide of the Committee on Care and Use of Laboratory Animals (CCULA 1985).

All animals were anesthetized with ether to undergo a midline laparotomy. Acute pancreatitis was induced by injection of 48% ethyl alcohol, in a volume of 1 cm^3, into the common biliary duct using an insulin injector. A sham laparotomy was performed in the control group.

Control and acute pancreatitis groups were sacrificed 24 hours later, and chronic pancreatitis group was sacrificed on postoperative day 7. Under ether anesthesia, 4 cm^3 (3-7 cm^3) of blood was taken by cardiac puncture. Then, a laparotomy was done and pancreatic tissue was excised and fixed in 10% formol solution for histopathologic confirmation of pancreatitis. Histopathological evaluation was done under light microscopy, after sectioning and staining with hematoxylin and eosin (H & E).

Plasma leptin was measured using diagnostic system laboratories (DSL) kit by radioimmunoassay (RIA) method. The concentrations were expressed as ng/ml.

All values are expressed as the mean ± S.E.M. Statistics were done by SPSS program at 11.5 version. One-way ANOVA and Scheffe's F post-hoc tests were used for multiple comparison. $P < 0.05$ was considered as statistically significant.

Results

Histopathological studies confirmed alcohol-induced pancreatitis. Inflammatory infiltration of neutrophils and mononuclear cells, interstitial

edema and focal necrotic areas were seen in the pancreatic tissues of acute pancreatitis group (Fig. 1), and interstitial fibrosis, lymphocyte infiltration, ductal and ductular dilation, acinar cell atrophy, periductal ductular hyperplasia were seen in chronic pancreatitis group (Fig. 2).

Plasma leptin rose significantly from the median of 0.78 ± 0.12 ng/ml in the control group to 1.92 ± 0.10 ng/ml and 1.86 ± 0.13 ng/ml in acute and chronic pancreatitis groups, respectively ($p < 0.001$, for both) (Fig. 3). However, there was no significant difference in the plasma leptin levels

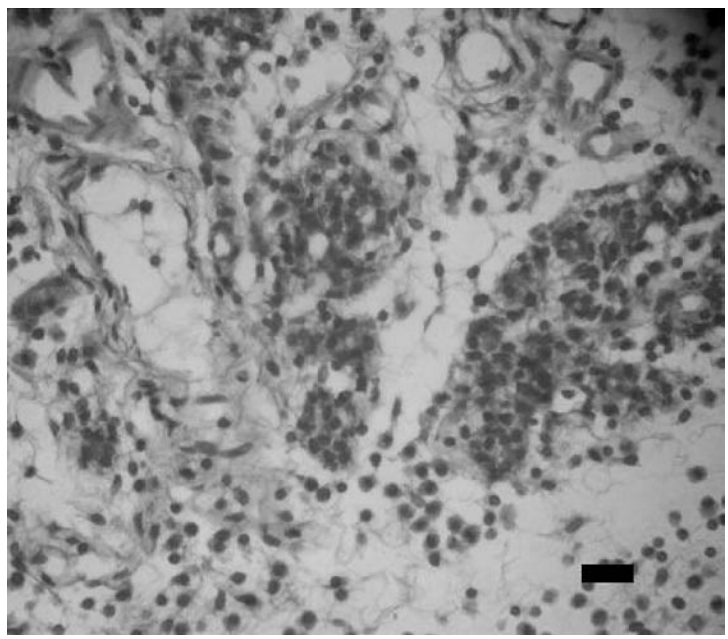

Fig. 1. Interstitial edema, neutrophil and mononuclear cell infiltration showing acute pancreatitis. Bar, 5 μm.

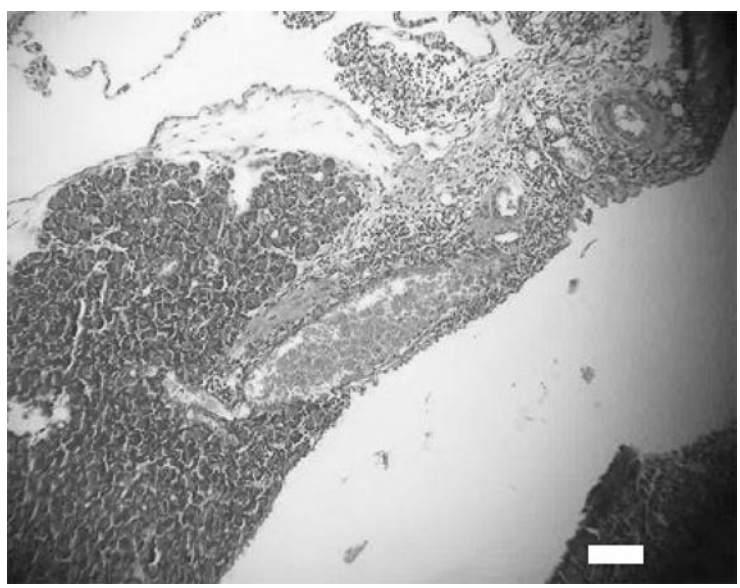

Fig. 2. Interstitial fibrosis, lymphocyte infiltration, ductal dilatation and aciner cell atrophy showing chronic pancreatitis. Bar, 25 μm.

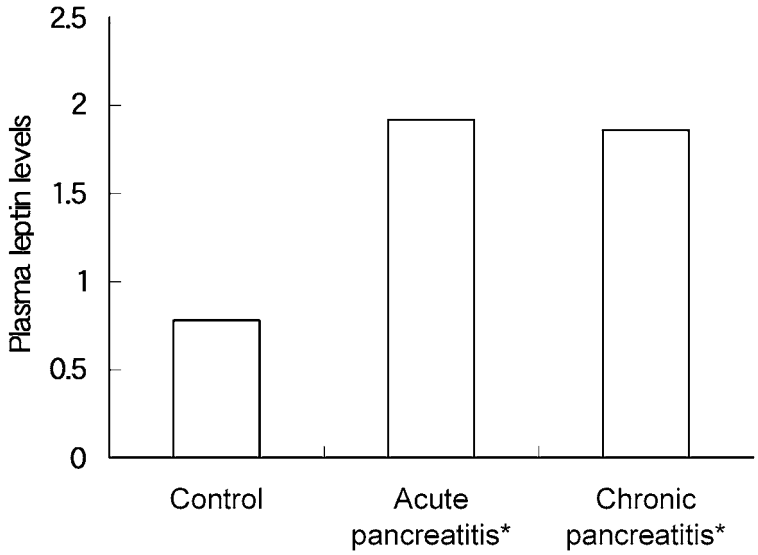

Fig. 3. Schematic representation of plasma leptin levels.
* $p < 0.001$.

between the acute pancreatitis group and the chronic pancreatitis group ($p > 0.05$).

Discussion

Pancreatitis is an inflammatory process in the pancreas, and its diagnosis is made on the basis of the clinical presentation combined with appropriate laboratory determinations, such as serum amylase and lipase levels, and radiologic findings. However, regarding an experimental study, histopathological confirmation is the most important step for precise diagnosis of both acute and chronic pancreatitis. Therefore, in the present study, histopathological evaluation of pancreatic tissues made it unnecessary to determine the serum amylase and lipase levels.

The hormone leptin is secreted from white adipocytes, and plasma levels of leptin correlate with adipose tissue mass (Seufert 2004). Leptin was first described to act on the satiety center in the hypothalamus through specific receptors to restrict food intake and enhance energy expenditure (Konturek et al. 2001). It has also been demonstrated that leptin has some peripheral effects such as inhibition of insulin biosynthesis and secretion in pancreatic beta-cells (Jaworek et al. 2003; Benomar et al. 2004; Perez et al. 2004). In most overweight individuals, however, physiological regulation of body weight by leptin seems to be disturbed, representing "leptin resistance." This leptin resistance at the level of the pancreatic beta-cell may contribute to dysregulation of the adipo-insular axis and promote the development of hyperinsulinemia and manifest type 2 diabetes in overweight patients (Jaworek et al. 2003).

Leptin has also been shown to elicit a number of immunoregulatory effects, including the promotion of T cell proliferative responses, and the induction of proinflammatory cytokines (Siegmund et al. 2004; Zarkesh-Esfahani et al. 2004). Leptin deficiency was shown to be associated with an increased susceptibility to infection (Goren et al. 2003; Zarkesh-Esfahani et al. 2004). As polymorphonuclear neutrophils (PMN) play a major role in innate immunity and host defense against infection, the influence of leptin on PMN activation was studied, and the presence of leptin receptor in human PMN was determined (Caldefie-Chezet et al. 2001). These findings provide an additional link among the obesity-derived hormone leptin, innate immune function, and infectious disease.

Recent identification of specific leptin receptors in the pancreas suggests that this peptide may

also play some role in this gland (Zoccali et al. 2004). However, the involvement of leptin in pancreatitis remains unknown. Therefore, the objective of this study was to investigate the relationship between serum leptin levels and pancreatitis.

In a recent study, Konturek et al. (2002) have shown that acute pancreatitis in rats and in humans is associated with a marked increase in the plasma level of leptin. The authors claimed that inflamed pancreas could be the source of local production of leptin. They also showed that exogenous leptin administration protects the pancreas against development of acute pancreatitis. In another study, Warzecha et al. (2002) examined the influence of leptin administration on the development and the course of acute ischemic pancreatitis. They concluded that leptin reduces the pancreatic damage in the course of ischemic pancreatitis and accelerates the pancreatic tissue repair. According to the authors, the beneficial effects of leptin appear to be dependent on the improvement of pancreatic blood flow, the increase in pancreatic cell growth, and the limitation of pro-inflammatory interleukin-1beta release.

Jaworek et al. (2003) have also examined the effect of exogenous leptin on pancreatic enzyme secretion in vitro using isolated pancreatic acini, and they concluded that leptin could take a part in the inhibition of postprandial pancreatic secretion and this effect could be related to the direct action of this peptide on pancreatic acini.

For the first time in the literature, we assessed plasma leptin concentrations in both acute and chronic pancreatitis, and our findings showed a significant rise in plasma leptin level in both conditions ($p < 0.001$). However, there was no significant difference between acute and chronic pancreatitis groups ($p > 0.05$). These findings confirm that leptin has a role in pancreas inflammation, and the inflamed tissue can be the source of local production of leptin, because while the inflammation process continues, leptin level seems to remain elevated.

In conclusion, leptin has both central and peripheral effects, such as restriction of food intake, enhancement of energy expenditure, inhibition of insulin biosynthesis, and secretion in pancreatic beta-cells. Its immunoregulatory role has also been documented. The present study indicates that leptin is involved in the inflammatory processes of pancreas.

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