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Plasma Leptin Level, the Adipocyte-Specific Product of the *Obese* Gene, Is Associated with Tumor Progression and Is a Marker of the Nutritional Status of Patients with Gastric Cancer

Michio Maeta, Shinichi Oka*, Hiroaki Saito*, Masahide Ikeguchi*, Shunichi Tsujitani* and Nobuaki Kaibara*

*Department of Fundamental Nursing and *First Department of Surgery, Tottori University Faculty of Medicine, Yonago 683-0826 Japan*

Leptin, a product of the *obese* gene, is synthesized and released into the circulation in response to increased energy storage in adipose tissue. Leptin plays an important role in the regulation of body weight and energy balance. However, leptin levels in patients with malignant tumor have not been fully examined. The purpose of the present study is to clarify the clinical implications of leptin levels in the circulation in patients with gastric cancer. The subjects were 103 patients with gastric cancer at various stages. Levels of leptin in the plasma were determined with a commercially available human leptin-selective quantitative enzyme immunoassay kit. There were clear decreasing trends in leptin levels along with tumor progression in both males and females, and statistically significant differences were observed in males between stages II and IV, and in females between stages I and IV. Plasma leptin levels of females were consistently higher than those of males when we compared them with patients in the same stages. Moreover, statistically significant decreases in leptin levels were observed post-operatively. However, there were no statistically significant relationships between leptin levels and clinicopathological findings. There was a positive correlation between levels of plasma leptin and values of the body mass index. These findings may indicate that plasma leptin levels do not involve factors relevant to specific tumor growth but involve some tumor-related nutritional status due to tumor progression. We conclude that leptin levels are reflected during tumor-bearing status, and these are also useful markers for both indicating tumor progression and discovering the nutritional status of patients with gastric cancer.

Key words: gastric cancer; nutritional marker; plasma leptin level

Leptin, a product of the *obese* gene, is synthesized and released into the circulation in response to increased energy storage in adipose tissue (Zhang et al., 1994; Campfield et al., 1995; Halaas, et al., 1995; Pelleymounter et al., 1995). Administration of recombinant leptin to *obese* mice results in weight loss through both a reduction in food intake and an increase in energy expenditure (Campfield et al., 1995; Halaas et al., 1995; Pelleymounter et al., 1995). Therefore, it has been suggested that leptin plays an important role in the regulation of body weight and energy balance.

In humans, mutations of the leptin gene in adipose tissue (Montague et al., 1997; Strobel et al., 1998) and of the leptin receptor gene in the hypothalamus (Tartaglia et al., 1995; Clement et al., 1998) induce a decreased leptin level in the circulation and result in morbid obesity. Supplementation with recombinant human leptin for such patients with leptin deficiency leads to a sustained reduction in body weight as

Abbreviations: BMI, body mass index; EIA, enzyme immunoassay

a result of a loss of fat (Farooqi et al., 1999), as is the case with *obese* mice. Leptin levels in the circulation, in general, correlate positively with indexes of obesity (Maffei et al., 1995; Considine et al., 1996; Havel et al., 1996; Giannini et al., 1999), such as body fat or the body mass index (BMI) (Kahn et al., 1997).

Although there is a gender difference in leptin levels between males and females, females have higher plasma leptin concentrations than males, which is attributable to the relatively greater body fat mass in females (Maffei et al., 1995; Havel et al., 1996; Giannini et al., 1999). Whatever the initial concentration, weight loss due to food restriction is associated with a decrease in plasma leptin in both obese and normal weight humans (Maffei et al., 1995; Rosenbaum et al., 1997). Therefore, leptin concentrations have been regarded as a simple marker of the extent of obesity in humans. However, the clinical implications of these leptin levels in patients with malignant tumor have not been clearly studied, excluding those of patients with leptin-producing pregnancy-related gynecological placental tumor (non-adipose tissue production of leptin) (Masuzaki et al., 1997).

Is malignancy-modified nutritional status reflected in leptin levels or not? The purpose of the present study is to examine plasma leptin levels in relation to BMI values and to clarify the clinical implications of leptin in patients with gastric cancer.

Patients and Methods

Patients

The subjects were 103 patients with gastric cancer at various stages who had been treated in the First Department of Surgery, Tottori University Hospital, between November 1995 and April 1998. Among them, 97 patients (mean age, 63.3 ± 11.3 years; range, 33 to 85 years; 65 males, 32 females) underwent resection of a primary gastric tumor, and the remaining 6 patients (mean age, 67.3 ± 8.3 years; range, 56 to 78 years; 1 male, 5 females) underwent chemotherapy because of unresectable postoperative recurrent tumor from gastric cancer.

The clinicopathological findings were determined according to the rules of the Committee on the Japanese Classification of Gastric Carcinoma (Japanese Research Society for Gastric Cancer, 1993).

Fasting blood samples (early morning before breakfast) were obtained before surgery or chemotherapy and stored at –80˚C prior to analysis. Postoperative samples were obtained 3 weeks after surgery.

Methods

Levels of leptin in the plasma were determined with a commercially available human leptin-selective quantitative enzyme immunoassay (EIA) kit (Immuno-Biological Laboratories Co., Ltd., Fujioka, Japan) according to the manufacturer's instructions. In brief, a 100-μL sample was added to each well of a microtiter plate, which had been precoated with rabbit monoclonal antibody specific for human leptin, and incubated at 37˚C for 1 h. After washing, a 100-μL solution of horseradish peroxidase-conjugated antibodies, raised in the rabbit against human leptin, was added to each well and incubation was continued at 37˚C for 30 min. After further washing, color was developed by incubating each well with 100 μL of the chromogenic substrate solution. The colored reaction product was quantified with a spectrometer at 450 nm. Quantification was achieved by the construction of a standard curve using a known concentration of purified, recombinant human leptin. The limit of detection using this kit was 0.78 ng/mL.

The BMI of each patient was calculated by the following formula :

[BMI = body weight (kg)/height (m^2)].

Statistical analysis

Data were expressed as mean ± SD. The mean values were compared with Student's *t*-test and two-way analysis of variance. *P* values of < 0.05 were considered statistically significant.

Table 1. Levels of preoperative plasma leptin and body mass index (BMI)

Stage		Leptin (ng/mL) (mean ± SD)	BMI (mean ± SD)
Stage I	[60]	4.3 ± 5.1	22.4 ± 2.5
Stage II	[10]	4.3 ± 4.9	23.7 ± 2.3
Stage III	[12]	3.8 ± 3.9	21.1 ± 2.7
Stage IV	[15]	1.3 ± 0.9	21.2 ± 3.2
Recurrence	[6]	1.2 ± 0.7	19.5 ± 1.6

$P < 0.03$ (Stage I vs Stage IV)
$P < 0.03$ (Stage II vs Stage IV)
$P < 0.03$ (Stage III vs Stage IV)
$P < 0.02$ (Stage I vs Stage II)
$P < 0.05$ (Stage I vs Stage III)
$P < 0.008$ (Stage I vs Recurrence)
$P < 0.008$ (Stage II vs Recurrence)

[ ], number of patients.

A Macintosh personal computer system and StatView software (Abacus Concepts, Inc., Berkeley, CA) were used for all statistical analyses.

Results

Preoperative levels of plasma leptin and BMI in patients with gastric cancer are shown in Table 1. Both the mean level of leptin and BMI, in general, were associated with the tumor progression of the cancer and decreased along with stage progression. Statistically significant decreases in leptin levels were found in patients in stage IV, as compared to patients in stages I, II and III, respectively. The differences of mean value among cancer stages were greater in leptin than in BMI, although values of SD were lower in BMI than those in leptin.

Since levels of plasma leptin have been reported to indicate gender and age differences (Maffei et al., 1995; Havel et al., 1996; Giannini et al., 1999), these levels were analyzed in terms of gender and age in each stage (Table 2). There was no age difference in patients between males and females in each stage. However, leptin levels of females were consistently higher than

Table 2. Levels of preoperative plasma leptin and body mass index (BMI) analyzed in terms of age and gender

Stage/gender		Age (year) (mean ± SD)	Leptin (ng/mL) (mean ± SD)	BMI (mean ± SD)
Stage I				
Male	[38]	62.0 ± 10.0	2.6 ± 2.2	22.4 ± 2.5
Female	[22]	67.9 ± 12.9	7.2 ± 7.1	22.4 ± 2.6
Stage II				
Male	[7]	58.9 ± 10.3	3.8 ± 4.5	23.8 ± 2.6
Female	[3]	69.0 ± 6.9	5.8 ± 6.4	23.5 ± 1.8
Stage III				
Male	[7]	63.9 ± 10.0	2.4 ± 2.2	20.9 ± 3.4
Female	[5]	59.0 ± 18.5	5.7 ± 5.5	21.5 ± 1.6
Stage IV				
Male	[13]	62.4 ± 9.8	1.2 ± 0.8	21.5 ± 3.1
Female	[2]	57.5 ± 16.2	1.5 ± 0.7	19.3 ± 3.6
Recurrence				
Male	[1]	63.0	0.78	19.5
Female	[5]	68.2 ± 9.0	1.3 ± 0.7	19.5 ± 1.8

$P < 0.001$ (Stage I Male vs Female)
$P < 0.02$ (Stage II Male vs Stage III Male)
$P < 0.05$ (Stage I Female vs Stage III Female)
$P < 0.03$ (Stage II Female vs Stage IV Female)
$P < 0.03$ (Stage I Female vs Stage IV Female)

[ ], number of patients.

Table 3. Relationship between preoperative plasma leptin levels and clinicopathological findings

Variable	Leptin (ng/mL)				*P*-value
	Male [65]	*P*-value (vertical)†	Female [32]	*P*-value (vertical)†	(horizontal)‡
Gross finding					
Early cancer	2.6 ± 2.3 [33]	NS	7.6 ± 7.1 [17]	NS	< 0.001
Invasive cancer					
Localized	2.2 ± 2.2 [10]		7.8 ± 7.9 [5]		NS
Infiltrative	1.7 ± 2.0 [22]		4.0 ± 3.9 [10]		< 0.04
Histology					
Intestinal type	2.7 ± 2.8 [33]	NS	6.9 ± 7.6 [14]	NS	< 0.02
Diffuse type	2.0 ± 1.9 [32]		6.2 ± 5.7 [18]		< 0.001
Depth of cancer invasion					
T1	2.6 ± 2.3 [34]	NS	7.4 ± 7.1 [18]	NS	< 0.001
T2	2.7 ± 3.3 [9]		5.7 ± 7.4 [5]		NS
T3,4	1.9 ± 2.2 [22]		5.3 ± 5.3 [9]		< 0.02
Nodal involvement					
Negative	2.6 ± 2.4 [41]	NS	7.3 ± 7.0 [24]	NS	< 0.001
Positive	2.1 ± 2.4 [24]		4.3 ± 4.6 [8]		NS
Lymphatic vessel invasion					
Negative	2.7 ± 2.7 [42]	NS	7.5 ± 7.1 [18]	NS	< 0.001
Positive	1.8 ± 1.7 [23]		5.2 ± 5.7 [14]		< 0.02
Blood vessel invasion					
Negative	2.5 ± 2.5 [45]	NS	7.2 ± 6.8 [26]	NS	< 0.001
Positive	2.2 ± 2.3 [20]		3.5 ± 4.7 [6]		NS
Peritoneal metastasis					
Negative	2.5 ± 2.5 [61]	NS	6.7 ± 6.6 [31]	NS	< 0.001
Positive	2.2 ± 2.3 [4]		3.5 ± 4.7 [1]		NS
Liver metastasis					
Negative	2.5 ± 2.5 [61]	NS	6.5 ± 6.6 [32]	NS	< 0.001
Positive	1.6 ± 1.5 [4]		—		

[ ], number of patients.
NS, not significant.
† Vertical analysis: statistical difference between specific variables in the same gender.
‡ Horizontal analysis: statistical difference in each specific variable between males and females.

those of males when we compared them in the same stage, and there was a statistically significant difference in patients in stage I. There were still clear decreasing trends in leptin levels along with stage progression in both males and females, and statistically significant differences were observed in males between stages II and IV, and in females between stages I and IV. With respect to BMI values, there were no gender differences in each stage. The decreasing trend in the value of BMI along with stage progression was observed in both males and females; however, it was not so consistent compared with that in cases of leptin. In females, there were statistically significant differences in BMI between recurrent cases and stage I or II, respectively.

Relationships between the preoperative levels of plasma leptin and clinicopathological findings of gastric cancer are shown in Table 3. In both males and females, although the same gender showed no statistically significant differences between variables (vertical analysis), plasma leptin had a decreased trend accompanied with tumor progression in each variable. However, gender differences in levels of leptin were obvious in each variable (horizontal analysis), as shown in Table 3.

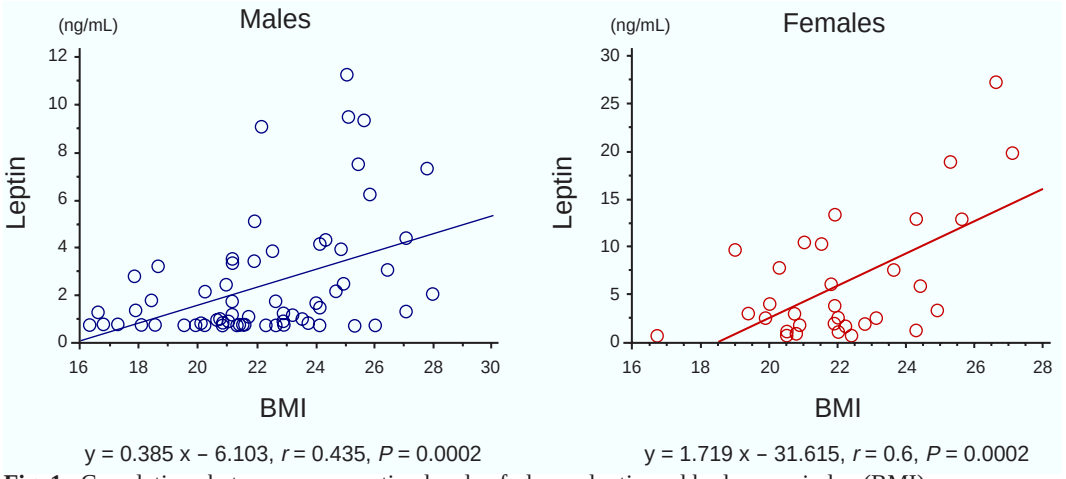

Fig. 1. Correlations between preoperative levels of plasma leptin and body mass index (BMI).

There was a positive correlation in both males and females between preoperative levels of plasma leptin and the BMI, as shown in Fig. 1.

Table 4 compares plasma leptin levels before and after surgery. Statistically significant decreases were observed postoperatively.

Discussion

Circulating leptin, which is a product of the *obese* gene, provides feedback information on the size of fat stores to central *obese* receptors that control food intake and body weight homeostasis. Recent studies of plasma leptin levels in patients with obesity or metabolic disorder have given us a wealth of novel information; however, little knowledge was given about plasma leptin levels of patients with malignant tumors. Masuzaki et al. (1997) reported that plasma leptin levels were markedly elevated in patients with hydatidiform mole or choriocarcinoma and were reduced after surgical treatment or chemotherapy, suggesting nonadipose tissue production of leptin; namely, placental trophoblasts and amnion cells from the uteri of pregnant women. Since breast cancer has been associated with obesity and reproductive hormones, leptin levels of patients with carcinoma in situ of the breast were examined and analyzed for putative involvement of leptin in the etiology of breast cancer (Mantzoros et al., 1999). A possible association has been reviewed between the cancer-associated anorexia-cachexia syndrome and leptin (Inui, 1999).

Table 4. Comparisons of pre- and postoperative plasma leptin levels

Stage		Leptin (ng/mL)		*P*-value
		Preoperative	Postoperative	
I and II				
Male	[45]	2.8 ± 2.7	1.3 ± 1.0	0.0010
Female	[25]	7.0 ± 6.9	3.9 ± 3.9	0.0387
III and IV				
Male	[20]	1.7 ± 1.7	0.9 ± 0.5	0.0225
Female	[7]	4.6 ± 5.0	1.8 ± 1.3	0.1059

[ ], number of patients.

However, systematic analyses of leptin levels in patients with malignant tumors and its clinical implications have not yet been studied.

In this series, plasma leptin levels, as a whole, including both males and females, decreased along with stage progression in gastric cancer patients (Table 1). As reported previously, there is a gender difference in leptin levels (Maffei et al., 1995; Havel et al., 1996; Giannini et al., 1999), and therefore, these levels were analyzed separately for males and females. Leptin levels were consistently higher in females than males when we compared them in the same stage. There was a clear reduction in leptin with stage progression in both males and females, as shown in Table 2.

Patients with recurrent tumors from gastric cancer have a serious nutritional disorder due to gastrointestinal passage failure of oral food intake. In this study, patients with recurrent cancer showed the lowest leptin levels among groups of patients including those who underwent gastrectomy of the primary tumor. Moreover, significant postoperative decreases in leptin levels were observed. However, there were no statistically significant relationships between leptin levels and specific clinicopathological findings. These findings may indicate that plasma leptin levels do not involve factors relevant to specific tumor progression but involve tumor-related nutritional status due to tumor progression and/or due to limitation of oral food intake during a relatively short period after gastrectomy. Thus, leptin is not only a simple marker of obesity in humans but also a marker of tumor progression and/or nutritional status in patients with malignant tumor.

BMI is an index of obesity and there is no gender difference in BMI, as shown in Table 2. Leptin levels correlated positively with BMI when data were analyzed separately between males and females (Fig. 1).

We conclude that leptin concentrations in the circulation have been indicated as a simple marker of the obesity; however, the role of leptin is exerted in not only metabolic disorders but also in tumor-bearing status, and it is also a useful nutritional marker for patients with malignancy. We notice that we have little adequate markers for evaluating postoperative nutritional status and/or quality of life when surgeons analyze the superiority of operative procedures from various reconstruction methods of the alimentary tract for patients with gastric cancer. Postoperative long-term follow-up of the plasma leptin level as a marker may be useful for these analyses.

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Corresponding author: Prof. Michio Maeta