RESEARCH ARTICLE

Clinical significance of serum leptin level in patients with gastric cancer

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ABSTRACT. Leptin may support the proliferation and hinder the apoptosis of tumor cells. Although leptin expression has been studied in several human tumors, its potential clinical significance remains uncertain in patients with gastric carcinoma. Furthermore, the majority of available findings have been determined from preclinical studies using stomach carcinoma tissue section and, to date, few studies have evaluated the clinical significance of leptin in the serum or plasma of gastric carcinoma patients. In the current study, the serum concentration of soluble leptin was assessed in gastric carcinoma patients, and its contributions to the clinical parameters and prognosis of patients were determined. A total of 63 pathologically confirmed gastric cancer patients and 30 healthy subjects were enrolled in the study and circulating leptin levels in the serum of all subjects were determined by ELISA. The serum leptin concentrations were significantly lower in the gastric cancer patients compared with the healthy control group ($P = 0.009$). In the gastric cancer patients, the clinical features of patient age, sex, lesion localization, histopathology, pathological grade, stage of disease, and serum tumor markers including lactate dehydrogenase, carcinoembryonic antigen, and carbohydrate antigen 19-9 were not correlated with serum leptin concentration. Furthermore, no association was observed between serum leptin concentration and responsiveness to chemotherapy ($P = 0.51$), and leptin level had no apparent prognostic role in clinical outcome ($P = 0.57$). In conclusion, although it was not predictive or prognostic, serum leptin level may be a valuable diagnostic indicator in patients with gastric carcinoma.

Key words: serum, leptin, gastric cancer, diagnosis, prognostic factor

Stomach carcinoma has a number of etiologies, and its immunological and genetic bases remain to be fully elucidated. *In vitro* studies have observed that cultured stomach carcinoma cell lines produce markedly high concentrations of growth factors and cytokines with pleiotropic biological actions. Among them, leptin functions as an autocrine and paracrine agent that influences a range of cellular processes, and in tumors leptin has been associated with the regulation of tumor growth, angiogenesis, invasion, and metastasis [1-5].

Leptin, as an adipocytokine, is mainly secreted by adipocytes, though is also produced by non-adipose tissues, including the gastric mucosa [1-5]. In gastric carcinoma, leptin has been reported to serve a prominent role in carcinogenetic processes by promoting proliferation and inhibiting apoptosis in the tumor cells [1]. Additionally, increased leptin expression in gastric cancer cells has been correlated with tumor aggressiveness and, in some cases, reduced clinical survival rate [2-4]. Furthermore, regarding its mechanism of action, leptin may stimulate the proliferation of gastric carcinoma cells by acting on the extracellular signal-regulated kinase 1/2 and signal transducer and activator of transcription 3 pathways [1].

Although the expression of leptin has been examined in several human malignancies, its potential clinical importance remains uncertain in gastric carcinoma, despite there being a number of previous studies on leptin expression in stomach carcinoma [1-5]. Furthermore, these studies have reported conflicting findings regarding the link between leptin expression and the clinical features of gastric cancer patients [1-5]. Therefore, there is an unsatisfactory understanding of the molecular action of leptin in gastric carcinoma, and its potential clinical importance in patients remains unclear.

The majority of available results regarding leptin expression in gastric cancer have been obtained from preclinical studies using stomach carcinoma tissue sections, and to date, few studies have examined the clinical significance of leptin in the serum or plasma of gastric cancer patients [6-10]. As a result, the significance of serological leptin levels in patients with gastric carcinoma is yet to be fully elucidated. Thus, the present study assessed the concentration of soluble leptin in the serum of gastric carcinoma patients, and defined correlations with the clinical features and prognosis of the patients.

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MATERIALS AND METHODS

Patients and therapy

The study enrolled 63 consecutive patients admitted to the Department of Medical Oncology, Istanbul University (Istanbul, Turkey). All patients had histopathologically confirmed stomach carcinoma and had not received any form of chemotherapy or radiation therapy. The staging of disease was based on the American Joint Committee on Cancer and Union for International Cancer Control staging systems. A baseline evaluation included detailed patient medical history and physical examination with a series of complete blood cell counts and biochemical tests including measurement of serum lactate dehydrogenase and tumor markers [carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 19-9]. Those with good performance status (Eastern Cooperative Oncology Group score $\leq$2) and appropriate blood test results received chemotherapy with fluorouracil, leucovorin, cisplatin, docetaxel, capecitabine, epirubicine with/without radiotherapy based on the staging of disease, which was received on an outpatient basis and on different schedules. The response to chemotherapy was performed at 8-12 week intervals during chemotherapy. The assessment of chemotherapy responsiveness by clinical, laboratory, and radiological investigations was based on the revised Response Evaluation Criteria in Solid Tumors version 1.1.

To comparatively evaluate the serum leptin of patients, 30 healthy subjects were included in the analysis. Informed consent was obtained from all participants and the study was approved by the ethics committee of the Institute of Oncology, Istanbul University.

Measurement of leptin concentration

Serum samples were obtained on the first admission of the patients and prior to any therapy. Blood samples were collected from patients and controls by venipuncture and clotted at room temperature. Following centrifugation, the samples were collected and immediately frozen at -20°C until analysis. A Human Leptin ELISA kit (Assaypro LLC, St. Charles, MO, USA) was used to measure the concentration of leptin in the serum samples, according to the manufacturer's protocol. The results were presented as ng/mL.

Statistical analysis

As a reference point, continuous parameters were classified as the median and range. Comparisons between clinicopathological variables and the serum concentration of leptin were performed using Mann-Whitney U tests. Survival analysis was performed by using the Kaplan-Meier method, and differences in clinical outcomes were evaluated using log-rank statistics. Statistical analyses were performed using SPSS 21.0 software (IBM Corp., Armonk, NY, USA), and $P \leq 0.05$ was considered to indicate statistical significance.

RESULTS

Patient characteristics

A total of 63 gastric carcinoma patients were enrolled in study. The demographic features and clinicopathological characteristics of the patients are summarized in *table 1*. The median age of the patients was 62 years (range, 28-82 years), and the male/female ratio was 0.67.

Serum leptin levels

The baseline serum leptin levels of the patients with gastric carcinoma were significantly lower than those in the healthy subjects (median values, 4.02 *versus* 8.06 ng/mL, respectively, $P = 0.009$; *figure 1*). A similar pattern was observed for body mass index (BMI) between the patients and controls (median values, 24.3 *versus* 27.3, respectively, $P = 0.008$; data not shown). In the gastric cancer patients, clinicopathological features including patient age, sex, lesion localization, histopathology, pathological grade, stage of disease and serum concentrations of LDH, CEA and CA 19-9 were not correlated with serum leptin levels (*table 2*). Furthermore, no association was observed between the circulating leptin level and chemotherapy responsiveness ($P = 0.51$; *table 2*).

Prognostic value of serum leptin and other factors

At the end of the observation period (median follow-up time, 25 weeks), 35 patients (55.6%) were deceased. The median survival time was 42.0 ± 4.2 weeks, and the 1-year survival rate was 42.2%. The presence of metastasis ($P = 0.03$), antrum localization ($P = 0.04$), increased erythrocyte sedimentation rate ($P = 0.02$), elevated serum CEA concentration ($P = 0.01$), high serum CA 19-9 concentration ($P = 0.04$) and chemotherapy unresponsiveness ($P = 0.05$) were significantly poor prognostic factors of survival outcome (*table 3*). However, serum leptin levels were not correlated with survival ($P = 0.57$; *table 3* and *figure 2*).

DISCUSSION

Leptin, an adipocyte-derived cytokine, connects nutritional status with neuroendocrine and immune functions by proinflammatory T helper 1 immune responses; the decrease in leptin plasma concentration during food deprivation leads to impaired immune function [11, 12]. Therefore, it regulates numerous signaling proteins involved in a range of biological processes, including carcinogenesis. The roles of leptin in tumor cells result in complex and uncertain effects on the cellular microenvironment.

Although leptin expression and localization have been examined in several human tumors, its possible function remains obscure in human stomach cancer. Thus, the potential clinical importance of leptin expression in stomach carcinoma remains unknown, as to date, there have been few studies into the clinical importance of leptin expression in gastric carcinoma [1-5].

In previous studies of leptin expression in gastric cancer tissues [1-5], the expression levels of leptin were observed to vary in the different trials; in one study, leptin

Table 1
Characteristics of the patients and diseases

Characteristics	n (%)
No. of patients	63 (100)
Age of patient, years	
≥60	35 (56)
<60	28 (44)
Sex	
Male	25 (40)
Female	38 (60)
Body mass index (BMI)	
Underweight	5 (8)
Normal	34 (54)
Overweight and obese	24 (38)
Localization of tumor	
Cardia	21 (33)
Antrum	27 (43)
Undetermined	15 (24)
Histopathology	
Adenocarcinoma	42 (67)
Signet-ring cell	21 (33)
Grade of histology	
I-II	10 (16)
III	44 (70)
Undetermined	9 (14)
Tumor (T) stage	
1-3	14 (22)
4	22 (35)
Unknown	27 (43)
No. of lymph node metastasis	
0-2	12 (19)
≥3	13 (21)
Unknown	38 (60)
Stage of disease	
Nonmetastatic	32 (51)
Metastatic	31 (49)
Liver metastasis (in metastatic disease)	
Yes	14 (45)
No	17 (55)
Curative surgery (in nonmetastatic disease)	
Yes	17 (53)
No	9 (28)
Unknown	6 (19)
Serum hemoglobin level	
Low (<12 g/dL)	35 (56)
Normal (≥12 g/dL)	28 (44)
Serum white blood cell count	
Normal (<10,000)	52 (83)
Elevated (≥10,000)	11 (17)
Serum platelet count	
Normal (<350,000)	54 (86)
Elevated (≥350,000)	9 (14)
Serum lactate dehydrogenase level	
Normal (<450 U/L)	43 (68)
Elevated (≥450 U/L)	10 (16)
Unknown	10 (16)
Erythrocyte sedimentation rate/h	
Elevated (≥50)	16 (25)
Normal (<50)	10 (16)
Unknown	37 (59)

Table 1
(*Countinued*)

Characteristics	n (%)
Serum CEA level	
Normal (<10 ng/mL)	44 (70)
Elevated (≥10 ng/mL)	13 (21)
Unknown	6 (9)
Serum CA 19.9 level	
Normal (<40 IU/Ml)	32 (51)/
Elevated (≥40 IU/mL)	25 (40)
Unknown	6 (9)
Response to chemotherapy	
Yes (stable + partial + complete response)	13 (43)
No (progressive)	17 (57)
Last status	
Alive	28 (44)
Dead	35 (56)

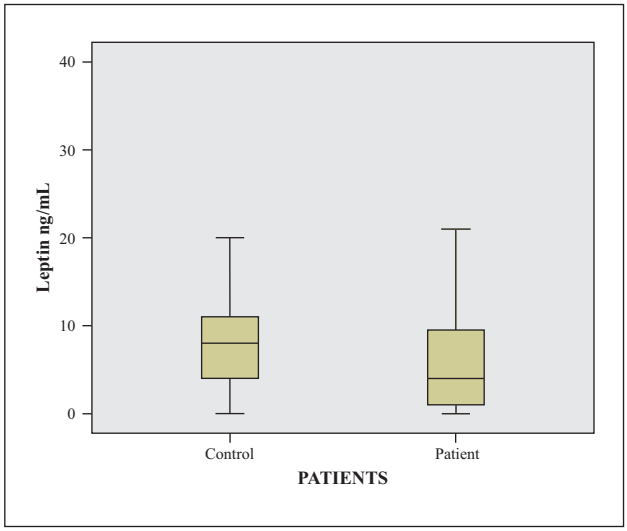

Figure 1
Serum leptin levels in patients with gastric cancer and control group.

expression was significantly higher in the gastric cancer tissues compared with normal tissues [2], while a different study reported no change in expression from normal tissues [1, 4]. Leptin expression has also been associated with gastric cancers with more aggressive behavior [2-4]. Additionally, leptin expression was significantly higher in cases with greater invasion depth, lymphovascular invasion, lymph node metastasis and of advanced stage [2-4]. However, it has previously been reported that leptin expression and cancer invasiveness were not significantly associated [1]. Nevertheless, leptin may be expressed to a higher level in more advanced tumors associated with poorer survival rate [2, 3, 5]. Furthermore, tumor leptin expression has been correlated with resistance to chemotherapy and therapy-independent prognosis in gastro-esophageal adenocarcinomas [5].

The variation in results regarding leptin expression possibly reflects the differences in tissue source, regulation and/or degradation kinetics, and potentially marks consist of their expression and release. Furthermore, the discordant results of these investigations may be accredited to

Table 2
Comparisons between serum leptin levels and various clinical parameters in patients with gastric cancer

Parameters	Leptin level (ng/mL) median (range)	p
Age of patients, years		
<60	5.32 (0.41-31.86)	0.27
≥60	2.83 (0.14-29.73)	
Sex		
Male	4.52 (0.14-28.22)	0.50
Female	4.02 (0.39-31.86)	
Body mass index (BMI)		
High (>median, 24.3)	5.92 (0.14-31.86)	0.35
Low (<median, 24.3)	2.54 (0.41-28.22)	
Localization of tumor		0.99
Cardia	4.13 (0.31-31.86)	
Antrum	6.15 (0.14-26.60)	
Histopathology		0.50
Adenocarcinoma	4.90 (0.14-31.86)	
Signet ring	2.35 (0.41-29.73)	
Grade of histology		0.95
I-II	5.58 (0.14-28.22)	
III	5.04 (0.31-31.86)	
Tumor (T) stage		0.99
1-3	5.04 (0.39-28.22)	
4	6.38 (0.34-31.86)	
Lymph node metastasis		0.85
0-2	6.50 (0.97-28.22)	
≥3	5.50 (0.34-29.73)	
Curative surgery		0.07
Yes	6.69 (0.97-29.73)	
No	4.02 (0.34-24.21)	
Stage of disease		0.23
Metastatic	2.83 (0.14-31.86)	
Nonmetastatic	5.22 (0.34-29.73)	
Liver metastasis		0.77
Yes	3.73 (0.14-28.22)	
No	2.83 (0.31-31.86)	
Serum hemoglobin level		0.15
Low	2.40 (0.14-31.86)	
Normal	5.90 (0.39-26.60)	
Serum white blood cell		0.87
Elevated	2.40 (0.39-24.71)	
Normal	4.89 (0.14-31.86)	
Serum platelet count		0.06
Elevated	4.91 (0.34-21.86)	
Normal	1.15 (0.14-24.71)	
Erythrocyte sedimentation rate		0.66
Elevated	3.43 (0.14-20.89)	
Normal	2.88 (0.41-14.56)	
Serum lactate dehydrogenase level		0.66
Elevated	3.43 (0.14-20.89)	
Normal	2.88 (0.41-14.56)	
Serum CEA level		0.44
Elevated	6.15 (1.08-31.86)	
Normal	3.65 (0.31-29.73)	
Serum CA 19.9 level		0.32
Elevated	2.83 (0.31-31.86)	
Normal	5.02 (0.34-29.73)	
Response to chemotherapy		0.51
Yes	2.54 (0.14-29.73)	
No	1.64 (0.41-24.21)	

Table 3
Effects of clinicopathological parameters on overall survival in patients with gastric cancer

Parameters	**Survival median weeks (±SD)**	**1-year (%) (±SD)**	**p**
Age patients, years			
<60	44.0 (8.4)	43.0 (10.5)	0.61
≥60	42.0 (9.3)	41.1 (9.8)	
Sex			0.56
Male	44.0 (19.8)	47.3 (9.2)	
Female	42.0 (7.6)	35.0 (10.9)	
Body mass index (BMI)			0.29
>median	46.7 (12.3)	85.0 (8.0)	
<median	37.7 (11.4)	70.0 (10.2)	
Localization of tumor			0.04
Cardia	NR	65.1 (11.9)	
Antrum	37.0 (8.2)	31.9 (9.7)	
Histopathology			0.22
Adenocarcinoma	44.0 (18.6)	48.5 (8.9)	
Signet ring	42.0 (11.0)	31.3 (11.2)	
Grade of histology			0.10
Good-int.	NR	75.0 (15.8)	
Poor	37.0 (9.7)	36.2 (8.5)	
Tumor (T) stage			0.06
1-3	NR	85.7 (9.4)	
4	37.0 (16.8)	41.5 (11.2)	
Lymph node metastasis			0.21
0-2	NR	65.5 (16.7)	
≥3	68.0 (24.8)	60.6 (13.8)	
Curative surgery			0.36
Yes	NR	64.8 (16.5)	
No	68.0 (28.9)	57.6 (13.6)	
Stage of disease			0.03
Metastatic	30.0 (11.0)	21.1 (8.9)	
Nonmetastatic	82.0 (13.5)	61.3 (9.5)	
Liver metastasis			0.11
Yes	42.0 (13.7)	35.7 (14.0)	
No	23.0 (3.3)	NR	
Serum hemoglobin level			0.34
Low	42.0 (12.4)	33.8 (9.5)	
Normal	82.0 (40.2)	51.7 (10.4)	
Serum white blood cell count			0.30
Elevated	24.0 (13.0)	NR	
Normal	42.0 (3.4)	40.8 (7.9)	
Serum platelet count			0.51
Elevated	25.0 (1.3)	NR	
Normal	42.0 (4.9)	43.4 (7.7)	
Erythrocyte sedimentation level			0.02
Elevated	25.0 (6.4)	NR	
Normal	NR	67.5 (15.5)	
Serum lactate dehydrogenase level			0.11
Elevated	21.0 (10.9)	NR	
Normal	44.0 (4.4)	43.2 (8.8)	
Serum CEA level			0.01
High	23.0 (12.4)	NR	
Normal	44.0 (17.0)	49.4 (8.6)	
Serum CA 19.9 level			0.04
High	39.0 (10.2)	29.4 (10.5)	
Normal	NR	56.3 (10.0)	

Table 3
(*Countinued*)

Parameters	Survival median weeks (±SD)	1-year (%) (±SD)	p
Response to chemotherapy			0.05
Yes	71.0 (23.5)	60.2 (15.9)	
No	42.0 (9.6)	NR	
Serum leptin level			0.57
<median (4.02 ng/mL)	42.0 (11.8)	39.0 (9.9)	
>median (4.02 ng/mL)	46.0 (49.5)	45.7 (10.3)	

NR: not reached.

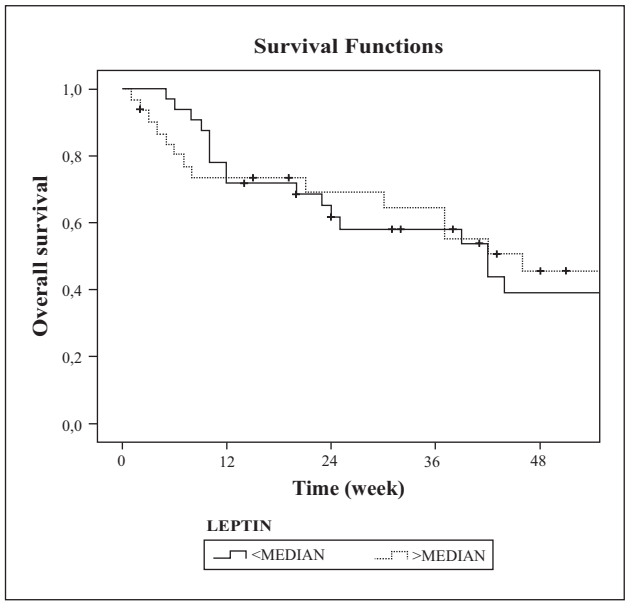

Figure 2
Survival graphs in stomach carcinoma patients according to soluble leptin concentrations, median value 4.02 ng/mL ($P = 0.57$).

several other factors. During recent years, immunohistochemistry (IHC) has become a useful integrated process in diagnostic histopathology. There are several obstacles with IHC, in that the most significant of which being a lack of standardization in the assay procedure and interpretation of staining. Furthermore, the aforementioned studies were performed with relatively small patient groups, which may have been inadequate in identifying significant differences. A uniform procedure remains to be developed and approved for larger samples of patients in prospective trials.

Similar to the previous tissue trials, leptin expression has been investigated in the serum and plasma of stomach carcinoma patients in a number of studies. Bolukbas *et al.* [6] studied 25 Turkish metastatic gastric cancer patients with >5% weight loss, and observed that serum leptin level in the patients was significantly lower than that in healthy subjects ($P = 0.002$). However, no differences were detected between male and female patients. Similarly, in another study from Turkey consisted of 39 patients with stage IV gastrointestinal malignancies who had mostly gastric cancer ($n = 21$) serum leptin levels were lower in both patients with cachectic and non-cachectic cancer than that in control subjects ($P < 0.001$) [7]. Conversely, Capelle *et al.* [8] observed that median leptin levels were significantly higher in 119 gastric cancer cases (73% were Dutch origin) than those in controls ($P = 0.01$). Meanwhile, serum leptin concentrations were increased with age and in females ($P < 0.001$). A study by Kemik *et al.* [9] of 31 cachectic Turkish stomach carcinoma patients detected significantly elevated serum leptin levels in patients with gastric cancer compared with controls ($P < 0.001$). However, no gender difference was determined. Serum leptin levels have also been determined in 48 Korean stomach carcinoma patients who underwent curative radical gastrectomy with standard lymph node dissection [10]; no significant difference was observed in leptin levels between the patients and a control group ($P = 0.14$). Similarly, there was no significant difference in leptin levels between early and late stage disease.

In the current study, the clinical significance of serum leptin concentrations in patients with gastric carcinoma was investigated. A total of 63 patients who had significantly lower BMIs compared with normal controls, and who presented with different stages of stomach carcinoma, were evaluated. Serum leptin levels were detected by using ELISA, from which a significant decrease in the serum leptin concentration of cancer patients was identified compared with healthy subjects. It has been proposed that low levels of circulating serum leptin may be linked with decreased body fat mass in patients. These results indicated that the assessment of serum leptin was able to differentiate between the stomach carcinoma patients and healthy subjects, thus suggesting that serum leptin level may be an effective serological diagnostic marker for stomach carcinoma. However, no significant correlations were observed between serum leptin level and tumor characteristics, including disease stage, histopathology, pathological grade and soluble tumor indicators. Additionally, circulating leptin levels were not associated with patient outcome. Thus, in the present study, serum leptin level was implicated to be inefficient as a prognostic indicator for predicting the prognosis of patients with stomach carcinoma. Furthermore, the lack of association between serum leptin level and chemotherapy sensitivity indicates the insufficiency of serum leptin as a predictor of chemotherapy responsiveness for patients undergoing chemotherapeutic regimens.

In conclusion, the present study identified that serum leptin level may serve a diagnostic role in stomach carcinoma patients. However, its prognostic and predictive values were not indicated. The small sample size and short follow-up times of the current study should be noted as restrictions that may have affected the results. Nevertheless, the results were in accordance with previous reports, possibly due

to the inclusion of all disease stages and predictive and prognostic analyses that have been preliminarily investigated in previous studies. Future studies in larger patient populations are required to conclusive determine any clinical significance of serum expression in patients with stomach carcinoma.

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