

CLINICAL STUDY

***Helicobacter pylori* has no effect on plasma ghrelin levels**

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Abstract

Objective: *Helicobacter pylori* is the major etiologic agent for chronic active gastritis, and it also plays a crucial role in gastric and duodenal ulcer disease, as well as in gastric carcinoma. *H. pylori* infection has been shown to decrease plasma somatostatin (SST) and increase plasma gastrin concentrations. Ghrelin is a recently discovered peptide produced mostly in the stomach of rodents and humans and is secreted into the bloodstream. There is no data in the literature about the relationship between *H. pylori* and ghrelin.

Design: Thirty-nine age- and BMI-matched *H. pylori* infection positive and negative women, from whom biopsy specimens were taken during gastric endoscopy, were included in the study.

Methods: Total ghrelin was measured by enzyme immunoassay (EIA) in Medistek. All samples were measured in duplicate and averaged; results differing by more than 20% were re-assayed. Two biopsy specimens from antrum, corpus and fundus were obtained.

Results: Fifteen of the subjects were *H. pylori* negative and 24 were *H. pylori* positive. Age, BMI, lipid profile and insulin sensitivity indices of the groups were similar. Plasma ghrelin levels (375.92 ± 7.10 vs 370.00 ± 4.14 pmol/l; $P > 0.05$) of *H. pylori* negative and positive groups did not differ significantly.

Conclusion: *H. pylori* has no effect on plasma ghrelin concentration.

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Introduction

Helicobacter pylori is the major etiologic agent of chronic active gastritis, and it also plays a crucial role in gastric and duodenal ulcer disease, as well as in gastric carcinoma (1, 2). *H. pylori* infection can alter acid secretion in both directions. Corpus gastritis and subsequent development of mucosal atrophy induced by *H. pylori* infection results in a decrease of acid secretion. Gastric acid secretion is also regulated by many factors involving the autonomic nervous system and gut hormones. Among several hormones affecting gastric acid secretion directly or indirectly, the most important regulatory peptides in the gastric mucosa include gastrin and somatostatin (SST) (3). SST has an inhibitory effect on growth hormone (GH) release, and the presence of SST-containing D cells in the canine antral mucosa has been shown by Fujita and Kobayashi (4). Kaneko *et al.* reported that antral SST concentrations were decreased in *H. pylori*-infected patients (5) and Moss *et al.* demonstrated that eradication of *H. pylori* caused approximately twofold increases in SST mRNA

in antral biopsies (6). *H. pylori* infection has been shown to increase plasma gastrin concentrations during fasting and after meals (3).

Ghrelin is a recently discovered peptide produced mostly in the stomach of rodents and humans and secreted into the bloodstream (7). It is produced by approximately 20% of the neuroendocrine cell population of oxyntic glands, by cells different from enterochromaffin-like, enterochromaffin or D cells and probably of the X-like cells (7, 8). Ghrelin potentially stimulates GH release (9, 10) and it may also play a role in different aspects of food intake and energy balance control (11, 12). Ghrelin also stimulates gastric motility and acid secretion in rats (13, 14). A recent study has shown that central ghrelin administration decreases gastric acid secretion, but peripheral administration does not modify gastric acid secretion in rats (15). Peripheral ghrelin administration also has no effect on gastric emptying in humans (12). Ghrelin secretion is not affected by gastrin (8). There are no data in the literature about the relationship between *H. pylori* and ghrelin. In the present study, we measured

serum ghrelin concentrations in age- and BMI-matched, *H. pylori* negative and positive women to assess the relationship between *H. pylori* and ghrelin.

Materials and methods

This prospective study was conducted at Baskent University Adana Hospital. Thirty-nine age- and BMI-matched *H. pylori* infection positive and negative women, from whom biopsy specimens were taken during gastric endoscopy, were included in the study. After informed consent was obtained, a complete medical history and physical examination were carried out to exclude endocrine disorders, gastrointestinal diseases and operations, pregnancy, interfering medications, or any other condition known to affect endocrine and gastric function. The study was approved by the Baskent University Ethics Committee.

Body weight and height were measured while subjects wore light clothing without shoes. Samples for the measurement of lipid profile, plasma glucose, insulin and ghrelin levels were drawn after an overnight fast. Levels of plasma glucose, total cholesterol, high-density lipoprotein cholesterol, and triglycerides were determined by the calorimetric method using a Cobas Mira Plus autoanalyzer (Roche Diagnostics, Mannheim, Germany). Low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol levels were calculated by the Friedwald formula. Insulin was measured in an AXSYM autoanalyzer (Abbott Laboratories, Abbott Park, IL, USA) using the microparticle enzyme immunoassay (EIA) method. Insulin sensitivity was calculated using the homeostasis model assessment index (HOMA; formula: fasting glucose (mmol/l) \times fasting insulin (μ u/ml)/22.5) and the quantitative insulin sensitivity check index (QUICKI; formula: $1/(\log \text{fasting insulin } (\mu\text{u/ml}) + \log \text{fasting glucose } (\text{mg/dl}))$). Total ghrelin (Phoenix Pharmaceuticals, Belmont, CA, USA) was measured by EIA in Medistek. Intra-assay variation was less than 5% and interassay variation was less than

14%. All samples were measured in duplicate and averaged; results differing by more than 20% were re-assayed.

Two biopsy specimens from antrum, corpus and fundus were obtained. The specimens were stained with hematoxylin and eosin and Giemsa. Gastritis was described according to the Sydney classification (16).

Data are expressed as means \pm S.E.M. The Student's *t*-test or Mann-Whitney *U* test were used to assess significant differences between values in various groups of patients where appropriate. Homogeneity of variances were calculated by Levene's test. A value of $P < 0.05$ was considered statistically significant. Data were analyzed using SPSS for Windows (version 10.0; SPSS, Inc., Chicago, IL, USA).

Results

Characteristics of 39 subjects are summarized in Table 1. Fifteen of the subjects were *H. pylori* negative and 24 were *H. pylori* positive. Age, BMI, lipid profile and insulin sensitivity indices of the groups were similar (Table 1). Ghrelin (375.92 ± 7.10 vs 370.00 ± 4.14 pmol/l; $P > 0.05$) of *H. pylori* negative and positive groups did not differ significantly.

Discussion

H. pylori infection has been found to decrease the expression of antral SST and to increase the release of the acid-stimulating hormone gastrin (3). *H. pylori* infection increases plasma gastrin concentrations during fasting, after meals, and after the infusion of bombesin or gastrin-releasing peptide (17–19). SST deficiency had been proposed in duodenal ulcer patients before the discovery of *H. pylori* (3). Recent studies have clearly demonstrated that antral SST levels were decreased in *H. pylori* infection (20, 21). Like SST and gastrin, it was likely that ghrelin could

Table 1 Characteristics of *H. pylori* negative and positive subjects. No statistically significant differences were found between the two groups of patients. Values are means \pm S.E.M.

Characteristics	<i>H. pylori</i> negative subjects (n = 15)	<i>H. pylori</i> positive subjects (n = 24)	Total (n = 39)
Age (years)	44.53 \pm 2.89	42.83 \pm 1.79	43.49 \pm 1.55
BMI (kg/m ²)	23.23 \pm 0.52	24.38 \pm 0.70	23.94 \pm 0.48
Ghrelin (pmol/l)	375.92 \pm 7.10	370.00 \pm 4.14	372.96 \pm 2.96
Fasting blood glucose (mmol/l)	5.00 \pm 0.07	4.91 \pm 0.07	4.95 \pm 0.005
Fasting insulin (pmol/l)	52.60 \pm 6.47	64.11 \pm 6.49	59.69 \pm 4.74
HOMA	1.66 \pm 0.22	1.95 \pm 0.19	1.84 \pm 0.14
QUICKI	0.36 \pm 0.006	0.35 \pm 0.006	0.36 \pm 0.005
Total cholesterol (mmol/l)	4.84 \pm 0.21	5.26 \pm 0.31	5.10 \pm 0.21
HDL cholesterol (mmol/l)	1.32 \pm 0.08	1.25 \pm 0.06	1.28 \pm 0.05
LDL cholesterol (mmol/l)	2.95 \pm 0.17	3.09 \pm 0.21	3.04 \pm 0.14
Triglyceride (mmol/l)	1.10 \pm 0.11	1.32 \pm 0.14	1.23 \pm 0.09

HOMA, homeostasis model assessment index; QUICKI, quantitative insulin sensitivity check index; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

be affected by *H. pylori* infection. We measured plasma ghrelin levels in patients with *H. pylori* activation in the fundus and compared them with the ghrelin levels of age- and BMI-matched subjects without *H. pylori* infection. We were expecting to find lower plasma ghrelin levels in subjects with *H. pylori* infection, because of the *H. pylori*-induced direct or cytokine-dependent mucosal damage. Contrary to our expectations, we did not find any statistically significant difference between the groups.

It is well known that SST has an inhibitory effect on GH, insulin and glucagon release, as well as on many other gastroenteropancreatic hormonal secretions and functions (22, 23). SST infusion inhibits ghrelin secretion in humans (23, 24). At present, the mechanisms underlying the inhibitory effect of SST on ghrelin secretion are unknown but they likely reflect direct activation of SST receptors in the gastric mucosa (22, 23). A previous study has shown that ghrelin inhibits SST release in rats (25). It has been thought that SST and ghrelin systems are linked by a functional relationship (23). This functional relationship between SST and ghrelin may be relevant in the control of the endocrine pancreas (22, 23, 26). The inhibitory effect of SST on insulin secretion is independent of ghrelin (23). Ghrelin also reduces insulin secretion in isolated rat pancreas and humans (25, 27). Tschop *et al.* found a negative correlation between plasma ghrelin and insulin concentrations (28). In contrast, we did not observe any relation between plasma ghrelin and insulin concentrations. Shiiya *et al.* did not find any significant difference in plasma ghrelin concentrations between diabetic and non-diabetic subjects (29). The administration of glucose and insulin does not suppress ghrelin levels (30).

Ghrelin-producing endocrine cells have been found mainly in oxyntic mucosa of the stomach, but ghrelin is also released from other tissues, including small and large intestine, the arcuate nucleus of the hypothalamus, and α cells of the pancreatic islet, lung and kidney (7, 8, 31–35). This unchanged plasma ghrelin concentration may be the result of diminished inhibitory effect of SST or compensatory increased ghrelin release from other tissues. In fact, plasma ghrelin levels in the gastrectomized patients still remain about one-third of those in normal subjects, suggesting that tissues other than stomach, such as duodenum, jejunum, kidney and lung, contribute to a certain amount of circulating ghrelin (32, 34). Plasma ghrelin concentration may not directly reflect ghrelin release from the oxyntic mucosa of the stomach. The study by Breidert *et al.* has shown that *H. pylori* infection was associated with a significant increase of leptin levels in the corpus mucosa compared with *H. pylori* negative subjects, but circulating leptin levels did not differ between groups (36). We need to measure gastric fluid ghrelin concentration or expression of ghrelin mRNA in the stomach to investigate whether

H. pylori infection might have an effect on the expression of fundic ghrelin.

As a conclusion, *H. pylori* infection has no effect on plasma ghrelin concentration. Plasma ghrelin level may not directly reflect ghrelin release from the oxyntic mucosa of the stomach because it is also released from other tissues. Further studies are needed to evaluate the effect of *H. pylori* infection on gastric ghrelin secretion.

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