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• *BRIEF REPORTS* •

Impact of *Helicobacter pylori* infection on ghrelin and various neuroendocrine hormones in plasma

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Abstract

AIM: Ghrelin, an endogenous ligand for growth hormone secretagogue receptor, influences appetite, energy balance, gastric motility and acid secretion. The stomach is the main source of circulating ghrelin. There are inconsistent reports on the influence of *Helicobacter pylori* (*H pylori*) infection on circulating ghrelin levels. We sought to elucidate the relationship between ghrelin and various peptides in plasma, with special reference to *H pylori*.

METHODS: Plasma ghrelin levels were measured by radioimmunoassay in 89 subjects who were referred for upper gastrointestinal endoscopy, consisting of 42 *H pylori* infected and 47 uninfected ones. Plasma gastrin, somatostatin, leptin, insulin-like growth hormone 1 (IGF-1) and chromogranin A concentrations were also measured. Twelve patients were treated with anti- *H pylori* regimen.

RESULTS: Ghrelin circulating levels were greatly decreased in *H pylori*-positive than negative individuals (194.2±90.2 fmol/mL and 250.4±84.1 respectively, $P<0.05$), but did not significantly alter following the cure of infection (176.5±79.5 *vs* 191.3±120.4). There was a significant negative correlation between circulating ghrelin and leptin levels, as well as body mass index, for the whole and uninfected population, but not in *H pylori*-infected patients. Plasma ghrelin concentrations correlated positively with IGF-1 in *H pylori*-negative group and negatively with chromogranin A in the infected group. There were no significant correlations among circulating levels of ghrelin, gastrin and somatostatin irrespective of *H pylori* status.

CONCLUSION: *H pylori* infection influences plasma ghrelin dynamics and its interaction with diverse bioactive peptides involved in energy balance, growth and neuroendocrine function.

Key words: *Helicobacter pylori*; Ghrelin; Leptin; Gastrin; Insulin-like growth factor

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INTRODUCTION

Helicobacter pylori (*H pylori*) is the major cause of chronic gastritis and peptic ulcer disease[1,2]. Chronic infection leads to atrophic gastritis, which increases the risk of gastric adenocarcinoma[2]. It is well- documented that the immune and inflammatory response against *H pylori* affects various cell types in gastric mucosa that are important in acid homeostasis, such as somatostatin-producing, gastrin-producing, chief and parietal cells[3-5]. *H pylori* gastritis causes a reduction of mucosal somatostatin levels and hypergastrinemia[3,4]. Leptin, a protein primarily secreted by the adipose tissue known to suppress appetite and modulate energy expenditure[6], is also produced by the chief and parietal cells[5]. Furthermore, gastric leptin levels are higher in *H pylori*-infected than in uninfected subjects, although serum levels may not be altered[5,7]

Ghrelin is a 28-amino acid peptide recently identified in the stomach as an endogenous ligand for growth hormone secretagogue receptor[8]. In addition to its potent growth hormone releasing activity, ghrelin influences appetite, energy balance, gastric motility and acid secretion[9,10]. This hormone is primarily produced by X/A-like neuroendocrine cells in the oxyntic glands[11] and the stomach is the main source of circulating ghrelin[8-11]. To date, conflicting results have been reported regarding the influence of *H pylori* status on ghrelin dynamics[12,13]. In addition, there is little information on the relationship between circulating ghrelin and other neuroendocrine hormones during the infection.

In the present study, we sought to determine the plasma concentrations of ghrelin, as well as leptin, gastrin and somatostatin, with special reference to *H pylori* infection. In addition, we assessed the relationship between circulating ghrelin and insulin-like growth factor 1 (IGF-1), the principal mediator of growth hormone axis[14], and chromogranin A, a reliable marker of gastric neuroendocrine proliferation[15].

MATERIALS AND METHODS

Patients

The study- subjects were 89 patients referred for upper gastrointestinal endoscopy between June 2003 and October 2003. The study was approved by Nagasaki University Ethics Committee. All samples were obtained with written informed consent of the patients prior to their inclusion, in accordance with the Helsinki Declaration. The exclusion criteria were: age <18 or >80 years, pregnancy, body mass index (BMI) >30 kg/m^2, diabetes mellitus, systemic infection, thyroid and liver diseases, renal impairment, use of medications effective against *H pylori* during the preceding 3 mo, alcohol abuse, drug addiction, and chronic corticosteroid or nonsteroidal anti-inflammatory drug use. None had undergone gastrointestinal surgery.

We treated 12 *H pylori*-positive patients with 7-d triple therapy consisting of lansoprazole, amoxicillin and clarithromycin[16]. Four weeks after cessation of the treatment, fasting plasma samples were also collected.

Plasma ghrelin concentrations

On the day of endoscopy, blood samples were taken between 8 and 10 a.m., after an overnight fast, transferred into chilled tubes containing ethylenediaminetetraacetic acid-2Na and aprotinin, stored on ice during collection, centrifuged, plasma separated and stored at -80 ℃ until assay. Plasma ghrelin concentrations were measured in-house in duplicate by radioimmunoassay (RIA), as described previously[11]. This RIA system employs a rabbit polyclonal antibody raised against the C-terminal fragment of human ghrelin, and can measure both the acylated and des-acyl forms. The intra-assay coefficient of variation was 2.8% and inter-assay coefficient of variation was 3.1%[11].

Circulating anti-H pylori antibody and other peptide concentrations

Plasma anti-*H pylori* immunoglobulin (Ig) G antibody was assessed by an enzyme-linked immunosorbent assay kit (HEL-p TEST, AMRAD Co., Melbourne, Australia). The cut-off value was determined according to the protocol provided by the manufacturer. Fasting plasma concentrations of gastrin, somatostatin, leptin, IGF-1 and chromogranin A were commercially determined by RIA (Mitsubishi Chemical Co., Tokyo, Japan).

Detection of H pylori Infection

H pylori status was assessed by anti-*H pylori* Immunoglobulin G antibody, ^{13}C-urea breath test (UBiT, Otsuka Pharmaceutical Co., Tokushima, Japan) or rapid urease test (Helicocheck, Otsuka Pharmaceutical Co.) using biopsy specimens endoscopically taken from the antrum within 2 cm of the pyloric ring and the corpus along the greater curvature. Patients were considered positive for *H pylori* infection when two of these examinations yielded positive results. On the other hand, patients were defined as *H pylori*-negative if all test results were negative[17]. Eradication of *H pylori* was considered successful when ^{13}C-urea breath test became negative[16].

Statistical analysis

Statistical analyses were performed using Fisher's exact, χ^2, Student's *t*, Mann-Whitney *U*, Kruskal-Wallis, Spearman rank and Wilcoxon signed ranks tests, as appropriate. A *P* value of less than 0.05 was accepted as statistically significant. Data were expressed as mean±SD.

RESULTS

Patient demographics

The study population consisted of 22 patients with chronic gastritis, 12 benign gastric polyps, 10 with gastric ulcer, 8 duodenal ulcer, 5 reflux esophagitis and 32 subjects with normal mucosa of the upper gastrointestinal tract at endoscopy. They included 44 men and 45 women, with a mean age of 53 years (range, 19-80). They consisted of 42 *H pylori*-infected and 47 uninfected subjects. The two groups were matched for age, sex, alcohol intake, smoking habit and BMI (Table 1).

Table 1 Baseline characteristics of the two groups (mean±SD)

Parameters	*H pylori* positive (*n* = 42)	*H pylori* negative (*n* = 47)
Age (yr)	52.7±16.9	53.4±14.3
Sex (Male:Female)	20:22	24:23:00
Alcohol intake	20	21
Smoking habit	18	21
Body mass index	23.1±3.3	22.8±3.2

Plasma concentrations of other peptides and H pylori status

As shown in Table 2, plasma ghrelin was significantly lower in *H pylori*-positive than negative subjects ($P<0.05$), whereas gastrin concentrations were significantly higher in *H pylori*-positive than negative subjects ($P<0.005$). There were no significant differences in plasma leptin, somatostatin, IGF-1 and chromogranin A levels with reference to *H pylori* status.

Table 2 Plasma concentrations of various peptides with respect to *H pylori* (mean±SD)

Peptide	*H pylori* positive (*n* = 42)	*H pylori* negative (*n* = 47)	*P*
Ghrelin (fmol/mL)	194.2±90.2	250.4±84.1	<0.05
Gastrin (pg/mL)	133.5±309.1	66.7±130.2	<0.005
Somatostatin (pg/mL)	11.3±5.7	9.7±6.2	Not significant
Leptin (ng/mL)	5.2±3.9	4.6±2.9	Not significant
Insulin-like growth factor 1 (ng/mL)	176.1±71.6	204.3±61.9	Not significant
Chromogranin A (ng/mL)	21.4±29.0	13.8±7.2	Not significant

Student's *t*-test was employed in statistical analyses.

Correlations between plasma ghrelin levels and plasma concentrations of other peptides

As shown in Table 3, there was a significantly negative correlation between plasma ghrelin and leptin levels for the

Table 3 Correlations of ghrelin with other peptides in plasma in terms of *H pylori* status

	Gastrin	Somatostatin	Leptin	IGF-1	Chromogranin A
H pylori positive	0.015 (NS)	0.076 (NS)	-0.239 (NS)	0.064 (NS)	0.285 (P<0.05)
H pylori negative	0.025 (NS)	0.100 (NS)	-0.353 (P<0.05)	0.272 (P<0.05)	0.073 (NS)
Total	0.018 (NS)	0.086 (NS)	-0.318 (P<0.05)	0.097 (NS)	0.200 (NS)

Data are correlation coefficients and (P values) of the correlations. IGF-1: insulin-like growth factor-1, NS: not significant.

whole and the uninfected cohort (P<0.05 for each). Within *H pylori* positive cohort, however, no such correlation was observed. In the whole series, there were no significant correlations between plasma ghrelin concentrations and gastrin, somatostatin, IGF-1 and chromogranin A levels. However, circulating levels of ghrelin correlated positively with those of IGF-1 within the uninfected population and negatively with chromogranin A within the *H pylori*-infected group (P<0.05 for each). There was a significant positive correlation between plasma gastrin and chromogranin A levels (r = 0.293, P<0.05), possibly reflecting gastrin-induced enterochromaffin-like cell proliferation[15].

Relationship between plasma ghrelin levels and baseline parameters

For the whole group, plasma ghrelin concentrations correlated negatively with BMI (r = -0.286, P<0.05). The same correlation was also noted in the uninfected (r = -0.562, P<0.005), but not infected group (r = 0.018). Plasma leptin levels correlated positively with BMI (r = 0.543, P<0.0001), irrespective of *H pylori* status (r = 0.473, P<0.01 for *H pylori*-infected group and r = 0.585, P<0.0001 for uninfected group). Other baseline characteristics including age, sex, alcohol intake and smoking habit were not associated with plasma ghrelin concentrations or circulating values of other peptides.

Changes in plasma levels of ghrelin and gastrin following eradication of H pylori

Successful eradication of the organism was confirmed in 10 of 12 patients treated with anti-*H pylori* regimen. Post-cure plasma gastrin levels tended to decrease although insignificantly (from 131.9±107.6 pg/mL to 100.8±78.0 pg/mL). There was no significant change in plasma concentrations of ghrelin after cure of the infection (from 176.5±79.5 fmol/mL to 191.3±120.4 fmol/mL).

DISCUSSION

Ghrelin is produced in a variety of human tissues[9,10], but its messenger ribonucleic acid (mRNA) is most highly expressed in the stomach[18]. Plasma ghrelin levels decrease by as much as 65% after gastrectomy[18], and this is consistent with the findings of decreased plasma levels after gastric bypass surgery[19]. Thus, the stomach is the major source of circulating ghrelin[9,10,18,19]. Our results showed that plasma ghrelin concentrations were significantly lower in *H pylori*-positive than negative group, in contrast to the Turkish study[12]. There are several possible explanations for this disparity, including differences in radioimmunoassay protocols for ghrelin, inadequate assessment of *H pylori* status in their series, i.e., only by histology, leading to underestimation of infection, differences in study populations with respect to baseline diseases, race, nutrient status and dietary habits and sample size. However, Nwokolo *et al*[20], reported that plasma ghrelin levels significantly increased following cure of the infection. In addition, ghrelin mRNA expression and peptide production are significantly decreased in *H pylori*-colonized gastric mucosa of chronically infected Mongolian gerbils[21], which is a good rodent model for various aspects of *H pylori*-associated pathogenesis[22]. These findings and our results indicate that *H pylori* status has a negative impact on gastric and plasma ghrelin dynamics.

However, we did not observe any significant post-cure rise in fasting plasma ghrelin levels following the same post-treatment period as the British study[20]. One possibility of the inconsistent results is that they assessed six-hour integrated plasma ghrelin (between 8:00 and 13:00) while we evaluated the value at only one point after an overnight fast. Another plausible explanation includes age-related differences in the severity or extent of gastritis, as the mean age of their population (36 years) was substantially lower than that of our population sample (53 years). Also, the likely possibility that there was a type 2 statistical error related to the small sample size might explain the negative results. Further larger study to compare the long-term effects of anti-*H pylori* therapy and placebo are warranted to elucidate the reversibility of ghrelin production.

Ghrelin has metabolic effects opposite to leptin. It stimulates food intake, enhances the use of carbohydrates and reduces fat utilization, contributing to weight gain[9,10]. Plasma ghrelin levels showed a diurnal rhythm over a 24-h period that was exactly in phase with that of leptin[9,10]. Consistent with these reports, we demonstrated here the significant negative associations between plasma ghrelin concentrations and leptin values and BMI within the *H pylori*-negative cohort. However, plasma ghrelin concentrations did not correlate with those of leptin, as well as BMI, within *H pylori*-positive group. Since ghrelin is predominantly secreted from the stomach[9,10,18,19], its products derived from other tissues are unlikely to be sufficient to compensate for the altered plasma ghrelin dynamics affected by inflammatory events associated with *H pylori* infection[13]. On the other hand, plasma leptin levels had strongly positive correlations with BMI, irrespective of *H pylori* status, as the primary contributor of circulating leptin is exclusively the adipose tissue[5,6].

Likewise, plasma ghrelin concentrations correlated positively with the circulating levels of IGF-1, which mediates most of the anabolic effects of growth hormone[14], within the uninfected population only. In line with this finding,

gastric ghrelin expression was found to depend on circulating IGF-1 levels in mouse models[23]. Muller *et al*[24], reported positive correlations among ghrelin, growth hormone and IGF-1 secretion in normal human volunteers, suggesting that ghrelin is potentially associated with linear growth, through the growth hormone-IGF-1 axis. Our results regarding the relationship between plasma ghrelin and IGF-1 levels, particularly in *H pylori*-positive cohort, may be relevant, since there have been conflicting data on the relationship between *H pylori*-infection and growth retardation in children[25-27].

In our study, there was no significant correlation between plasma ghrelin and gastrin levels, in agreement with the recent observation in human subjects of unaltered circulating gastrin concentrations following ghrelin administration[28]. In addition, increased levels of gastrin caused by omeprazole treatment failed to raise the level of ghrelin mRNA in oxyntic mucosa and circulating ghrelin concentrations in a rat model[29]. Based on these findings, we suggest that ghrelin production/release does not seem to operate under gastrin control.

Recent evidence has revealed that systemic infusion of somatostatin suppresses plasma ghrelin concentrations[30]. In the present study, however, there were no correlations between ghrelin and somatostatin levels both in *H pylori*-infected and uninfected settings. Although this hormone is released postprandially from somatostatin-containing cells into the blood stream[31,32], we assessed the values in fasting plasma, which might explain the lack of relationship between circulating levels of ghrelin and its potential regulator, somatostatin. In conclusion, we have demonstrated in the present study that circulating ghrelin levels were more decreased in *H pylori*-positive than in negative persons, albeit they did not alter after eradication of the organism. There were significant correlations of ghrelin with leptin, IGF-1 and chromogranin A in plasma, depending on *H pylori* status. Our results indicate *H pylori* infection may affect plasma ghrelin dynamics and be involved in energy homeostasis, growth and neuroendocrine function through the interaction with ghrelin.

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