

## Observational Study

# Relationship between ghrelin, *Helicobacter pylori* and gastric mucosal atrophy in hemodialysis patients

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## Abstract

### AIM

To investigate the relationship between plasma ghrelin level, *Helicobacter pylori* (*H. pylori*) infection status and the severity of atrophy in hemodialysis patients.

### METHODS

One hundred eighty patients who received hemodialysis and 13 non-hemodialysis *H. pylori*-negative controls

underwent gastroduodenoscopy to evaluate the severity of gastric atrophy. Serum levels of pepsinogen (PG) were measured as serum markers of gastric atrophy. *H. pylori* infection was evaluated by anti-*H. pylori* IgG antibody, rapid urease test and culture test. We classified *H. pylori* infection status as non-infection, present infection and past infection. In addition, plasma acyl-ghrelin and desacyl-ghrelin levels were measured by enzyme-linked immunosorbent assay.

## RESULTS

Infection rate of *H. pylori* was 45.4% (49/108). Acyl-ghrelin level in the non-infection group ( $39.4 \pm 23.0$  fmol/mL) was significantly higher than in the past ( $23.4 \pm 19.9$  fmol/mL,  $P = 0.005$ ) and present infection groups ( $19.5 \pm 14.0$  fmol/mL,  $P < 0.001$ ). Furthermore, desacyl-ghrelin level in the non-infection group ( $353.2 \pm 190.2$  fmol/mL) was significantly higher than those in the past ( $234.9 \pm 137.5$  fmol/mL,  $P = 0.008$ ) and present infection groups ( $211.8 \pm 124.2$  fmol/mL,  $P < 0.001$ ). Acyl-ghrelin was positively correlated with the PG I level and PG I / II ratio ( $|R| = 0.484$ ,  $P < 0.001$  and  $|R| = 0.403$ ,  $P < 0.001$ , respectively). Both ghrelins were significantly decreased in accordance with the progress of endoscopic atrophy (both  $P < 0.001$ ) and acyl-ghrelin was significantly lower in patients with mild, moderate and severe atrophy ( $24.5 \pm 23.1$  fmol/mL,  $20.2 \pm 14.9$  fmol/mL and  $18.3 \pm 11.8$  fmol/mL) than in those with non-atrophy ( $39.4 \pm 22.2$  fmol/mL,  $P = 0.039$ ,  $P = 0.002$  and  $P < 0.001$ , respectively).

## CONCLUSION

In hemodialysis patients, plasma ghrelin level was associated with the endoscopic and serological severity of atrophy related to *H. pylori* infection.

**Key words:** Acyl-ghrelin; Desacyl-ghrelin; *Helicobacter pylori*; Hemodialysis; Pepsinogen

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**Core tip:** Ghrelin enhances the orexigenic effect, protein anabolism, anti-inflammatory actions and cardiovascular protection in hemodialysis patients. *Helicobacter pylori* (*H. pylori*) infection and gastric mucosal atrophy affect ghrelin level in subjects with normal renal function. However, it is unclear whether ghrelin in hemodialysis patients relates to *H. pylori* status or gastric mucosal atrophy. This study demonstrated that plasma ghrelin level was associated with endoscopic and serological severity of atrophy related with *H. pylori* infection in hemodialysis patients. Therefore, eradication therapy of *H. pylori* before the progression of atrophy might improve ghrelin level associated with nutrition status, such as protein-energy wasting.

gastric mucosal atrophy in hemodialysis patients. *World J Gastroenterol* 2016; 22(47): 10440-10449 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v22/i47/10440.htm> DOI: <http://dx.doi.org/10.3748/wjg.v22.i47.10440>

## INTRODUCTION

The number of end-stage kidney disease patients receiving hemodialysis is more than 1.1 million worldwide, and the size of this population is expanding at a rate of 7% per year with the progress in medical and hemodialysis machine technique<sup>[1]</sup>. Hemodialysis patients often suffer from gastrointestinal conditions such as bleeding, peptic ulcer, gastric cancer and other severe abdominal symptoms during their long treatment period. *Helicobacter pylori* (*H. pylori*) infection causes atrophic gastritis, peptic ulcer and gastric cancer<sup>[2-5]</sup>, and is thought to be one of the major risk factors for gastrointestinal morbidity in hemodialysis patients. However, a recent report noted that the prevalence of *H. pylori* infection in hemodialysis patients was significantly lower than in subjects with normal renal function<sup>[6]</sup>. It is therefore necessary to investigate whether the actual condition of gastroduodenal diseases in hemodialysis patients relates to *H. pylori* infection.

Hemodialysis patients have many risk factors that affect mortality, such as traditional cardiovascular risk factors, chronic inflammation and nutritional disturbance<sup>[7]</sup>. Among these risk factors, the state of metabolic and nutritional derangement, called protein-energy wasting (PEW), has a major impact on mortality in these patients<sup>[8,9]</sup>. PEW is defined as a state of decreased body stores of protein and energy fuels (body protein and fat masses) and is diagnosed if three features are present: (1) abnormal nutrition markers (*i.e.*, low serum levels of albumin, transthyretin or cholesterol); (2) reduced body mass (*i.e.*, low or reduced body or fat mass or weight loss with reduced intake of protein and energy); and (3) reduced muscle mass (*i.e.*, muscle wasting or sarcopenia, and reduced mid-arm muscle circumference)<sup>[10]</sup>. Recently, it was suggested that ghrelin, an orexigenic peptide released primarily from endocrine cells in the stomach, is important in the pathogenesis of PEW in hemodialysis patients<sup>[7,11,12]</sup>. Ghrelin has multiple favorable functions including enhancement of orexigenic effect, protein anabolism, anti-inflammatory actions, and cardiovascular protection<sup>[11,13,14]</sup>. In general, plasma ghrelin level increases after fasting and decreases after eating and plasma ghrelin level was found to associate with nutritional disorder in patients with advanced-stage cancer and anorexia nervosa<sup>[14]</sup>. A low ghrelin level increases the risk of cardiovascular mortality and morbidity in hemodialysis patients<sup>[15]</sup>, and in recent clinical trials, administration of ghrelin improved appetite and food intake in dialysis patients<sup>[16,17]</sup>.

Ichikawa H, Sugimoto M, Sakao Y, Sahara S, Ohashi N, Kato A, Sugimoto K, Furuta T, Andoh A, Sakao T, Yasuda H. Relationship between ghrelin, *Helicobacter pylori* and

The usefulness of monitoring plasma ghrelin at fixed intervals as a biomarker for mortality in hemodialysis patients has been proven<sup>[7]</sup>. Therefore, we think that it is important to know what factors could influence the ghrelin level in dialysis patients.

*H. pylori*-positive patients have low levels of gastric mucosal and/or plasma ghrelin and a small number of ghrelin-positive cells in the gastric mucosa<sup>[18]</sup>. Although, in subjects with normal renal function, the relationship between plasma ghrelin level and the severity of gastric mucosal atrophy is observed<sup>[18]</sup>, the association between ghrelin level and *H. pylori* infection and between ghrelin level and severity of gastric mucosal atrophy in hemodialysis patients is unknown.

Clarifying the factors associated with PEW in hemodialysis patients is important in improving prognosis. We therefore investigated the relationship among plasma ghrelin level, *H. pylori* status and endoscopic and serological gastric mucosal atrophy in hemodialysis patients.

## MATERIALS AND METHODS

Approval for the study protocol was given in advance by the Institutional Review Board of Hamamatsu University School of Medicine (Number: 23-185) and written informed consent was obtained from patients enrolled in this study. The registration number of this clinical research is UMIN 000023336.

### Subjects

One hundred eight patients receiving maintenance hemodialysis at 8 Dialysis center in Shizuoka, Japan (Sanaru Sun-Clinic, Sano Clinic, Yamashita Clinic, Tadokoro Clinic, Hiryu Clinic, Sanarudai Asahi-Clinic, Satsuki no Mori Clinic and Hamana clinic), who had received regular hemodialysis for 4 h three times per week and 13 non-hemodialysis *H. pylori*-negative control subjects (eGFR > 50) in Hamamatsu University Hospital were enrolled in this study. After written informed consent was obtained, all hemodialysis patients and control subjects underwent gastroduodenoscopy and blood samples were taken for measurement of plasma ghrelin level on a non-dialysis day. Of 108 hemodialysis patients, 78 patients who received regular hemodialysis at Hamana clinic (Hamamatsu, Japan) was used in previous report which investigated associations with plasma ghrelin level and gastric mucosal atrophy as single center study<sup>[19]</sup>. In this study, because previous our study could not show significant association with ghrelin level and present *H. pylori* infection (present infection and present non-infection including of non-infection and past infection), we focused in associations with plasma ghrelin level and infection status of *H. pylori* (non-infection, past infection and present infection) and we did as multicenter trial.

Exclusion criteria were a history of *H. pylori* era-

**Table 1** The criteria of the classification of *Helicobacter pylori* infection

	(1) Anti- <i>H. pylori</i> IgG (2) RUT (3) Culture test	Endoscopic gastric mucosal atrophy
Present infection	Positive at least one detection systems	
Past infection	Negative with all of three detection systems	Positive for atrophy
Non-infection	Negative with all of three detection systems	No atrophy

*H. pylori*: *Helicobacter pylori*; RUT: Rapid urease test.

dication, history of gastrectomy, significant clinical illness (e.g., cancer) and lack of informed consent.

### Assay of plasma ghrelin levels

Blood samples were taken from all patients while fasting on the morning of a non-dialysis day. Samples were collected in an aprotinin/EDTA-containing tube and immediately centrifuged at 1500 × *g* for 15 min at 4 °C. The resultant plasma was agitated with the addition of 1 mol/L hydrochloric acid equal to one-tenth of the plasma volume and stored at -80 °C until assay. We used a two-site sandwich enzyme-linked immunosorbent assay specific for acyl ghrelin and des-acyl ghrelin (Active Ghrelin ELISA Kit and Des-acyl Ghrelin ELISA Kit, SCETI, Tokyo, Japan).

### Diagnosis of *H. pylori* infection and assessment of gastric mucosal atrophy

The diagnosis of *H. pylori* infection was achieved with an anti-*H. pylori* IgG serological test (E plate Eiken *H. pylori* antibody<sup>®</sup>; Eiken Chemical Co. Ltd., Tochigi, Japan), a rapid urease test (RUT) (Helicocheck<sup>®</sup>; Otsuka Co., Tokyo, Japan) and culture test (E-MR82; Eiken Chemical Co, Ltd., Tochigi, Japan). When positive results were observed with at least one of the detection systems, we diagnosed as positive for *H. pylori*. We classified *H. pylori* infection status into three groups: present infection (positive results were observed with at least one of the detection systems), past infection (negative with all three detection systems and positive for endoscopic gastric mucosal atrophy) and non-infection (negative with all three detection systems and no endoscopic gastric mucosal atrophy) (Table 1). Past infection group had not been received previously *H. pylori* eradication therapy with proton pump inhibitor and two kinds of antimicrobial agents. Hemodialysis patients in this group were considered to be naturally eradicated *H. pylori* infection without receiving eradication therapy.

Endoscopic diagnosis of gastric mucosal atrophy was shown significant correlations with morphological and histological diagnosis<sup>[20,21]</sup>. In addition, because 30% of hemodialysis patients enrolled in this study were taking anti-coagulant agents, we diagnosed

**Table 2** Characteristics of patients who received hemodialysis

Age (yr)	68.8 ± 11.2
Male/female (n/n)	68/40
Duration of hemodialysis (yr)	8.7 ± 8.6
Body weight (kg)	51.4 ± 11.4
Body mass index (kg/m <sup>2</sup> )	20.2 ± 3.5
Albumin (g/dL)	3.4 ± 0.4
Total cholesterol (mg/dL)	159.5 ± 36.4
Cholinesterase (U/L)	230.3 ± 87.2
Infection of <i>H. pylori</i> : non/past/present	29/30/49
Acyl-ghrelin (fmol/mL)	25.9 ± 19.8
Desacyl-ghrelin (fmol/mL)	256.2 ± 158.4
Pepsinogen I (ng/mL)	349.9 ± 294.5
Pepsinogen II (ng/mL)	43.5 ± 32.3
Pepsinogen I / II ratio	8.3 ± 4.7

Values are presented as mean ± standard deviation. Non, negative with all three detection systems (anti-*H. pylori* IgG serological test, rapid urease test and culture test) and endoscopic gastric mucosal atrophy; past, negative with all detection systems for *H. pylori* and positive for endoscopic gastric mucosal atrophy; present, positive results were observed with at least one of the detection systems. *H. pylori*: *Helicobacter pylori*.

gastric mucosal atrophic by endoscopic findings, not pathological evaluation using gastric biopsy specimens, to prevent hemorrhage events. The endoscopic gastric mucosal atrophic pattern was evaluated according to the Kimura-Takemoto classification and expressed in six grades: Close (C)- I, C- II, C- III, Open (O)- I, O- II and O- III<sup>[22]</sup>. We categorized the severity of gastric mucosal atrophy into four groups as follows: non-atrophy; mild, C- I and C- II; moderate, C- III and O- I; severe, O- II and O- III.

#### Assay of serum pepsinogen levels

Serum levels of PG I and PG II were measured using a commercially available kit (Pepsinogen CLEIA®; Fuji Rebio. Ltd, Tokyo, Japan) by chemiluminescence enzyme immunoassay (EIA), and PG I/PG II ratio was calculated as a serological marker of gastric mucosal atrophy<sup>[23]</sup>.

#### Statistical analysis

Normally numerical values were expressed as mean ± standard deviation (SD). Statistical differences in plasma ghrelin levels and serum pepsinogen levels among subgroups were assessed using one-way ANOVA (analysis of variance) with Scheffe's multiple comparison test. Continuous variables were compared using *t*-test or Mann-Whitney *U*-test and categorical variables were compared using the  $\chi^2$  test. All *P*-values were two-sided. *P* < 0.05 was considered statistically significant. Calculations were carried out using StatView 5.0 statistical software (SAS Institute, Cary, NC, United States).

## RESULTS

#### Patient characteristics

The mean age of hemodialysis patients was 68.8 ±

11.2 years and the mean duration of hemodialysis was 8.7 ± 8.6 years (Table 2). The causes of end-stage renal disease in patients receiving hemodialysis were chronic glomerulonephritis (52/108, 48.1%), diabetic nephropathy (29/108, 26.9%), nephrosclerosis (7/108, 6.5%), and others (20/108, 18.5%).

Among the 108 hemodialysis patients, 49 (45.4%) had current *H. pylori* infection (present infection group) (Table 2) while 30 (27.8%) were in the past infection group, irrespective of eradication history (Table 2). Seventy-six patients (70.4%) had endoscopic gastric mucosal atrophy (46 patients in the present infection group and 30 patients in the past infection group). Although plasma ghrelin levels affected by body weights, sex and BMI, there were no significant differences in these parameters among three different groups related with *H. pylori* infection (non-infection, past infection and present infection groups) and among different groups of severity of gastric mucosal atrophy (Tables 3 and 4). Total cholesterol and cholinesterase in the *H. pylori* present infection group or the severe gastric mucosal atrophy group were lower than those in the *H. pylori* non-infection group or the non-atrophy group, but not significant (Tables 3 and 4).

#### Relationship between *H. pylori* status and plasma ghrelin level in hemodialysis patients

Mean plasma acyl- and desacyl-ghrelin levels of the 108 patients were 25.9 ± 19.8 fmol/mL and 256.2 ± 158.4 fmol/mL, respectively (Table 2). Acyl-ghrelin level in the non-infection group (39.4 ± 23.0 fmol/mL) was significantly higher than in the past (23.4 ± 19.9 fmol/mL, *P* = 0.005) and present infection groups (19.5 ± 14.0 fmol/mL, *P* < 0.001) (Figure 1A and Table 3). Furthermore, desacyl-ghrelin level in the non-infection group (353.2 ± 190.2 fmol/mL) was significantly higher than those in the past (234.9 ± 137.5 fmol/mL, *P* = 0.008) and present infection groups (211.8 ± 124.2 fmol/mL, *P* < 0.001) (Figure 1B and Table 3). However, there were no significant differences in acyl- or desacyl-ghrelin levels between the present and past infection groups (Figure 1A and B).

The acyl- or desacyl-ghrelin levels in the non-infection group of hemodialysis patients were significantly higher than those in *H. pylori* infection-negative non-hemodialysis control subjects (*P* = 0.010 and 0.004, respectively) (Figure 1A and B).

#### Relationship between endoscopic gastric mucosal atrophy and plasma ghrelin level

Acyl-ghrelin and desacyl-ghrelin levels were significantly decreased in accordance with the progress of endoscopic gastric mucosal atrophy (both *P* < 0.001) (Table 4). Acyl-ghrelin levels were significantly lower in patients with mild (C- I and C- II), moderate (C- III and O- I) and severe atrophy (O- II and O- III) (24.5 ± 23.1 fmol/mL, 20.2 ± 14.9 fmol/mL and



**Table 3** Relationship between *Helicobacter pylori* status and plasma ghrelin level

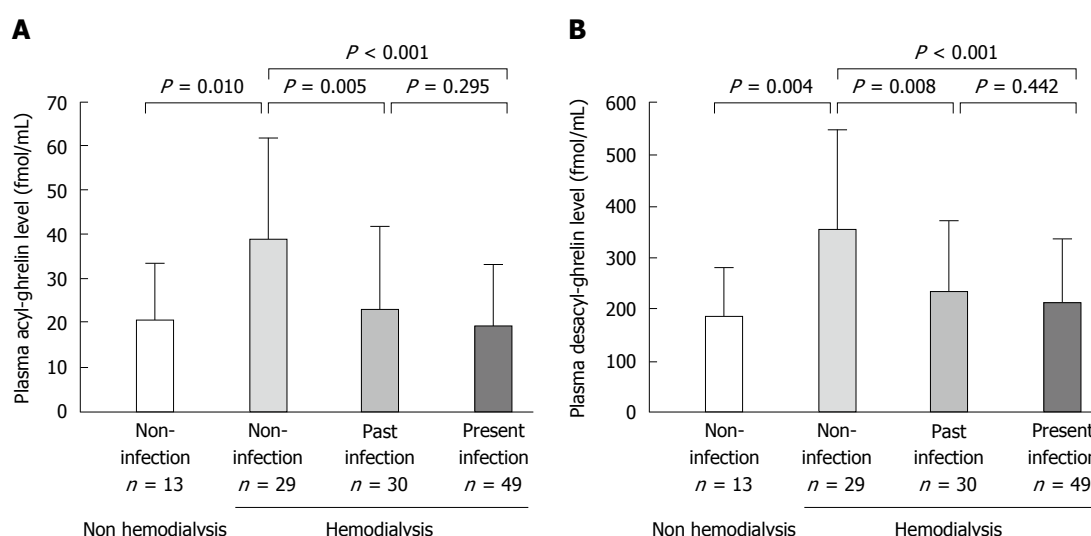
<i>H. pylori</i> status	Non-infection	Past infection	Present infection	<i>P</i> value
Number	29	30	49	
Male/female ( <i>n/n</i> )	19/10	22/8	27/22	0.275
Age (yr)	67.9 ± 10.7	71.7 ± 11.3	67.6 ± 11.3	0.119
Body weight (kg)	51.2 ± 12.1	49.0 ± 8.3	51.9 ± 12.4	0.468
BMI (kg/m <sup>2</sup> )	19.9 ± 3.4	19.3 ± 2.3	21.0 ± 4.0	0.160
Albumin (g/dL)	3.5 ± 0.3	3.5 ± 0.4	3.3 ± 0.4	0.156
Total cholesterol (mg/dL)	167.5 ± 36.1	149.5 ± 29.0	161.5 ± 44.2	0.175
Cholinesterase (U/L)	247.0 ± 109.3	228.5 ± 64.3	207.5 ± 75.3	0.310
Acyl-ghrelin (fmol/mL)	39.4 ± 23.0	23.4 ± 19.9 <sup>a</sup>	19.5 ± 14.0 <sup>a</sup>	< 0.001
Desacyl-ghrelin (fmol/mL)	353.2 ± 190.2	234.9 ± 137.5 <sup>a</sup>	211.8 ± 124.2 <sup>a</sup>	0.001
Pepsinogen I (ng/mL)	551.8 ± 319.2	286.5 ± 268.2 <sup>a</sup>	269.3 ± 238.0 <sup>a</sup>	< 0.001
Pepsinogen II (ng/mL)	46.4 ± 24.5	28.5 ± 17.4 <sup>a</sup>	51.0 ± 39.9	0.002
Pepsinogen I / II	12.2 ± 4.5	9.3 ± 4.1 <sup>a</sup>	5.3 ± 2.9 <sup>a</sup>	< 0.001

<sup>a</sup>*P* < 0.05 vs the non-infection group. Values are presented as mean ± SD. BMI: Body mass index; *H. pylori*: *Helicobacter pylori*.

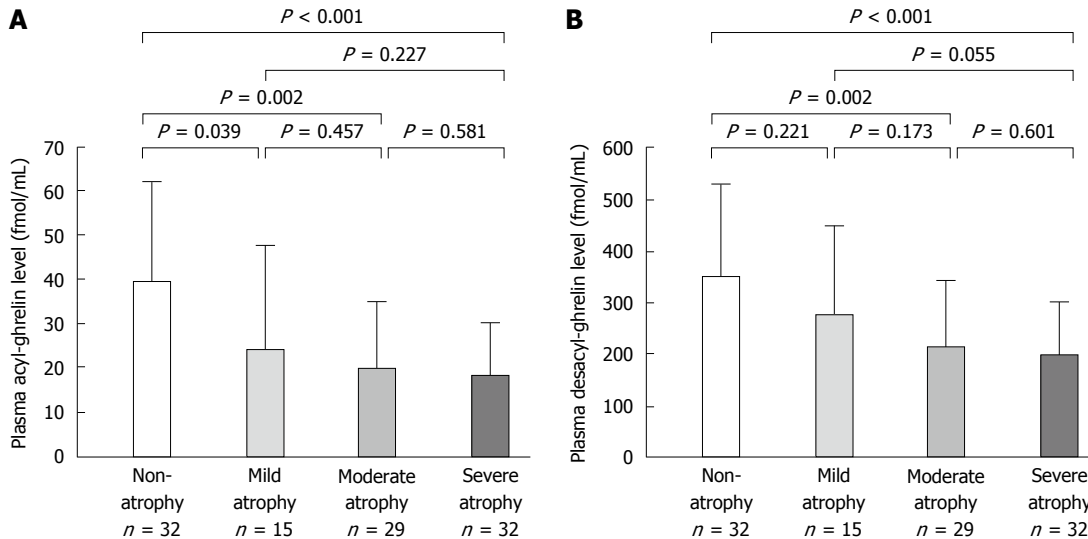
**Table 4** Relationship between endoscopic gastric mucosal atrophy and plasma ghrelin level

Mucosal atrophy	Non-atrophy	Mild atrophy	Moderate atrophy	Severe atrophy	<i>P</i> value
Number	32	15	29	32	
Male/female ( <i>n/n</i> )	19/13	10/5	19/10	20/12	0.969
Age (yr)	67.5 ± 11.8	64.5 ± 12.3	71.9 ± 10.9	69.3 ± 10.0	0.293
Body weight (kg)	50.7 ± 12.0	53.6 ± 10.5	50.6 ± 11.4	51.5 ± 11.4	0.708
BMI (kg/m <sup>2</sup> )	19.9 ± 3.4	20.3 ± 2.8	20.1 ± 3.6	20.6 ± 3.9	0.896
Albumin (g/dL)	3.5 ± 0.3	3.5 ± 0.3	3.3 ± 0.4	3.4 ± 0.5	0.351
Total cholesterol (mg/dL)	166.4 ± 34.8	159.9 ± 34.9	153.6 ± 37.5	151.4 ± 40.4	0.510
Cholinesterase (U/L)	246.0 ± 104.3	250.5 ± 77.7	201.9 ± 59.3	218.6 ± 77.6	0.283
Acyl-ghrelin (fmol/mL)	39.4 ± 22.2	24.5 ± 23.1 <sup>a</sup>	20.2 ± 14.9 <sup>a</sup>	18.3 ± 11.8 <sup>a</sup>	< 0.001
Desacyl-ghrelin (fmol/mL)	345.7 ± 183.7	276.1 ± 169.4	212.6 ± 129.4 <sup>a</sup>	196.8 ± 104.4 <sup>a</sup>	< 0.001
Pepsinogen I (ng/mL)	525.9 ± 316.6	461.9 ± 330.3	284.2 ± 207.3 <sup>a</sup>	181.0 ± 198.8 <sup>a</sup>	< 0.001
Pepsinogen II (ng/mL)	45.9 ± 23.8	49.3 ± 32.0	45.8 ± 40.8	36.3 ± 31.6	0.120
Pepsinogen I / II	11.6 ± 4.7	10.0 ± 4.6	6.9 ± 3.4 <sup>a</sup>	5.3 ± 3.3 <sup>a</sup>	< 0.001

<sup>a</sup>*P* < 0.05 vs the non-atrophy group. Values are presented as mean ± SD. BMI: Body mass index.



**Figure 1** Plasma acyl-ghrelin (A) and desacyl-ghrelin (B) levels according to *Helicobacter pylori* status in hemodialysis patients (*n* = 108) and non-hemodialysis patients with non-*Helicobacter pylori* infection (*n* = 13). Acyl-ghrelin and desacyl-ghrelin levels in the non-infection group were significantly higher than those in the present and past infection groups in hemodialysis patients. Both levels in the non-infection group of hemodialysis patients were significantly higher than those in the non-hemodialysis control group.



**Figure 2** Plasma acyl-ghrelin (A) and desacyl-ghrelin (B) levels between hemodialysis patients with different severities of gastric mucosal atrophy. Acyl-ghrelin and desacyl-ghrelin levels were decreased in accordance with the progress of endoscopic gastric mucosal atrophy.

18.3 ± 11.8 fmol/mL) than in those with non-atrophy (39.4 ± 22.2 fmol/mL,  $P = 0.039$ ,  $P = 0.002$  and  $P < 0.001$ , respectively) but not in those with mild atrophy (Figure 2A and Table 4). Furthermore, desacyl-ghrelin levels were also significantly lower in patients with moderate and severe atrophy (212.6 ± 129.4 fmol/mL and 196.8 ± 104.4 fmol/mL) than in those with non-atrophy (345.7 ± 183.7 fmol/mL,  $P = 0.002$  and  $P < 0.001$ , respectively) (Figure 2B and Table 4).

#### Serum pepsinogen levels in hemodialysis patients and association with pepsinogen and plasma ghrelin levels

Serum PG I, PG II levels and PG I/II ratio were 349.9 ± 294.5 ng/mL, 43.5 ± 32.3 ng/mL and 8.3 ± 4.7, respectively (Table 2). These values were higher than those generally reported in subjects with normal renal function<sup>[24-27]</sup>. Serum PG I level and PG I/II ratio were significantly decreased in accordance with the progress of endoscopic atrophy (both  $P < 0.001$ ) (Table 4).

The acyl-ghrelin level was positively correlated with serum PG I level and PG I/II ratio ( $|R| = 0.484$ ,  $P < 0.001$  and  $|R| = 0.403$ ,  $P < 0.001$ , respectively). In addition, desacyl-ghrelin level was also positively correlated with serum PG I level and PG I/II ratio ( $|R| = 0.538$ ,  $P < 0.001$  and  $|R| = 0.426$ ,  $P < 0.001$ , respectively).

Serum PG I level in the non-infection group (551.8 ± 319.2 ng/mL) was significantly higher than those in the past (286.5 ± 268.2 ng/mL,  $P = 0.001$ ) and present infection groups (269.3 ± 238.0 ng/mL,  $P < 0.001$ ) (Table 3). Furthermore, PG I/II ratio in the non-infection group (12.2 ± 4.5) was significantly higher in the past (9.3 ± 4.1,  $P = 0.014$ ) and present infection groups (5.3 ± 2.9,  $P < 0.001$ ) (Table 3).

## DISCUSSION

In this study, we showed that plasma acyl and des-

acyl ghrelin levels were significantly associated with the endoscopic and serological severity of gastric mucosal atrophy in patients who were hemodialyzed, and that both ghrelin levels gradually decreased according to the advanced endoscopic and serological severity of atrophy. In general, although *H. pylori* infection down-regulated plasma and gastric ghrelin levels in subjects with normal renal function<sup>[28-30]</sup>, these results also demonstrate the possibility that not only long-standing enhanced gastric mucosal inflammation induced by *H. pylori* infection but also gastric mucosal atrophy influenced plasma and gastric mucosal acyl- and desacyl-ghrelin levels<sup>[18]</sup>. In fact, in subjects with normal renal function, plasma ghrelin level was lower in *H. pylori*-positive patients with severe mucosal atrophy than in those without<sup>[18]</sup>. Here, our observation might suggest that the interaction with plasma ghrelin levels, gastric mucosal atrophy and *H. pylori* infection in patients who were hemodialyzed was similar to that in subjects with normal renal function. From the observation that improvement of PEW in hemodialysis patients was important for improving prognosis, measures for *H. pylori* infection in hemodialysis patients will be required not only for prevention of gastric cancer but also for improvement of nutritional disturbance and prognosis.

#### Association with *H. pylori* infection, gastric atrophy and ghrelin levels

Although ghrelin is released from tissues including the small and large intestines, lung, kidney, the nucleus of the hypothalamus and A cells of the pancreatic islet, ghrelin-producing endocrine cells had been found mainly in the oxyntic mucosa of the stomach<sup>[11]</sup>. Ghrelin plays central and peripheral biological roles in food intake, gastric motility and acid secretion, and major factors that influenced plasma ghrelin levels were sex, body weight, hormonal parameter and *H.*

*pylori* infection in the gastric mucosa<sup>[28-30]</sup>. Of these, *H. pylori* infection inhibited ghrelin production from gastric endocrine cells, leading to lower plasma and gastric mucosal ghrelin levels<sup>[18,31,32]</sup>. Moreover, expression of gastric ghrelin mRNA significantly increased after either peptic ulcer healing or *H. pylori* eradication therapy<sup>[33]</sup>. Recently, Osawa *et al.*<sup>[18]</sup> reported that lower PG I levels and PG I / II ratio which denoted advanced gastric mucosal atrophy were significantly associated with lower plasma ghrelin levels in current *H. pylori*-positive patients with normal renal function. In this study, we divided hemodialysis patients into non-, past, and present *H. pylori*-infection groups. Previously, we did the follow-up survey of *H. pylori* infection in hemodialysis patients<sup>[6]</sup>. When the natural history of *H. pylori* infection was investigated using more than 300 patients for a 4 year-follow up survey, although nobody received eradication therapy, the prevalence of *H. pylori* infection was reported to be 51.6% at first year, 42.9% two years later, and 38.3% four years later, indicating that the infection rate gradually decreased during dialysis treatment. In other words, 26.7% of dialysis patients naturally cured *H. pylori* infection over four years. Therefore, *H. pylori* infection might be actually eradicated after beginning hemodialysis treatment. We used "past *H. pylori*-infection" group as patients group who had been naturally cured *H. pylori* infection without receiving eradication therapy. The acyl-ghrelin level in the non-infection group was significantly higher than that in the present infection group, as reported in previous studies in subjects with normal renal function<sup>[18,29]</sup>. In addition, we saw no significant differences in acyl- and desacyl-ghrelin level between the present and past infection groups and both ghrelin levels in the past infection group were significantly lower than those in the non-infection group, whereas there was no current *H. pylori* infection in the past infection group. In this study, most hemodialysis patients in the past infection group had advanced gastric mucosal atrophy (*i.e.*, severe atrophy, 36.7% and moderate atrophy, 33.3%, respectively). Importantly, this observation suggested that the determination of plasma ghrelin level was influenced more by the severity of gastric mucosal atrophy than current *H. pylori* infection in hemodialysis patients. In fact, plasma ghrelin levels and gastric ghrelin mRNA levels depended on the number of ghrelin immunoreactive cells in the gastric mucosa<sup>[18,28,34]</sup>. Chronic persistent damage to the gastric mucosa by *H. pylori* infection might affect ghrelin production, leading to changes in food intake and body weight. Because ghrelin level was associated with mortality related to cardiovascular disease and PEW in hemodialysis patients, alternative management, such as *H. pylori* eradication therapy, before the progression of gastric mucosal atrophy might be necessary to prevent the decrease of ghrelin level in hemodialysis patients<sup>[12,15]</sup>.

### **Pepsinogen level in hemodialysis patients**

PG I and II used to be considered promising biomarkers for predicting the status of gastric mucosa, such as the severity of gastric mucosal atrophy and the risk of gastric cancer development<sup>[35,36]</sup>. In Japan, recently, the combination of serum PG level and *H. pylori*-IgG level had been used mainly at health check-ups as useful markers for gastric cancer screening, namely, the ABC method<sup>[37]</sup>. However, because the kidney was the key organ for eliminating serum PGs<sup>[38]</sup>, serum PG levels were elevated in chronic kidney disease (CKD) patients including those who received hemodialysis compared with control subjects with normal renal function<sup>[27,39,40]</sup>. Araki *et al.*<sup>[40]</sup> reported that serum PG I and PG II levels and PG I / II ratio measured by radioimmunoassay (RIA), which strong correlation was proved between assays of EIA and RIA<sup>[41-43]</sup>, in *H. pylori*-negative subjects with normal renal function were  $50.7 \pm 28.7$  ng/mL,  $10.2 \pm 6.1$  ng/mL and  $5.5 \pm 2.4$ , respectively, while in this study, those parameters were  $551.8 \pm 319.2$  ng/mL,  $46.4 \pm 24.5$  ng/mL and  $12.2 \pm 4.5$  in the non-infection group and  $268.3 \pm 238.0$  ng/mL,  $51.0 \pm 39.9$  ng/mL and  $5.3 \pm 2.9$  in the present infection group, respectively. Therefore, it was considered difficult to use serum PG as a promising biomarker of gastric atrophy and gastric cancer in hemodialysis patients using the same standard for subjects with normal renal function.

In this study, the serum PG I level and PG I / II ratio in the non-infection group were significantly higher than those in the past and present infection groups. In addition, the serological severity of gastric mucosal atrophy evaluated by PG levels had a significant positive correlation with endoscopic evaluations. Therefore, serum PG may be a biomarker for gastric mucosal atrophy and represent the new standard for hemodialysis patients. In addition, because there was a significant positive correlation between PG and ghrelin levels even in hemodialysis patients, the serum PG level and PG I / II ratio might be useful determinations not only for gastric mucosal atrophy related to gastric cancer development but also nutrition status. However, in this study, because the serum PG levels of each subject showed a wide distribution and the sample number was small, we could not determine the standard PG value to evaluate the severity of gastric mucosal atrophy in hemodialysis patients. Thus, further large-scale studies are needed to verify whether the serum PG level can become the parameter of serological severity of gastric mucosal atrophy and nutrition status in hemodialysis patients.

### **Limitation**

Our study had several limitations, as follows. First, our study had a relatively small sample size. Second, we were unable to evaluate the control subjects with normal renal function. We therefore compared the data of control subjects with normal renal function in the

previous reports. Third, we could not evaluate whether *H. pylori* eradication therapy affected plasma ghrelin levels because we did not have data on plasma ghrelin levels before and after eradication therapy in the same subject.

In conclusion, We investigated the relationships among plasma ghrelin level, *H. pylori* status and endoscopic and serological gastric mucosal atrophy in hemodialysis patients. Acyl and des-acyl ghrelin levels decreased significantly with the progress of gastric mucosal atrophy caused by *H. pylori* infection for long-term periods. Importantly, the status of gastric mucosal atrophy was the major determinant of plasma ghrelin level. Because ghrelin influences the pathogenesis of PEW, inflammation and cardiovascular risk in CKD patients, leading to a poor outcome in hemodialysis patients, it is necessary to clarify the factors which affect the ghrelin level and to consider countermeasures to improve the ghrelin level. To increase ghrelin secretion in hemodialysis patients, improvement of gastric mucosal status may be the most important approach. Eradication therapy before the progression of gastric mucosal atrophy might be necessary to prevent the decrease of ghrelin level in hemodialysis patients.

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## COMMENTS

### Background

Ghrelin enhances the orexigenic effect, protein anabolism, anti-inflammatory actions and cardiovascular protection in hemodialysis patients. Recently, it was suggested that ghrelin is important in the pathogenesis of protein-energy wasting (PEW), which is the state of metabolic and nutritional derangement, in hemodialysis patients. *Helicobacter pylori* (*H. pylori*) infection and gastric mucosal atrophy affect plasma and gastric mucosal ghrelin level in subjects with normal renal function. However, it is unclear whether ghrelin level in hemodialysis patients relates to *H. pylori* status or gastric mucosal atrophy.

### Research frontiers

PEW has a major impact on mortality and morbidity in hemodialysis patients, especially in cardiovascular events. Administration of ghrelin improves appetite

and food intake in hemodialysis patients, finally improves nutrition status in hemodialysis patients. Therefore, clarifying the factors associated with plasma and gastric mucosal ghrelin levels affected to PEW in hemodialysis patients is important in improving their prognosis.

### Innovations and breakthroughs

Acyl and des-acyl ghrelin levels in hemodialysis patients decreased significantly with the progress of gastric mucosal atrophy caused by *H. pylori* infection for long-term periods. Importantly, the status of gastric mucosal atrophy was the major determinant of plasma ghrelin level.

### Applications

These data suggested that eradication therapy of *H. pylori* before the progression of gastric mucosal atrophy might be required to prevent the decrease of ghrelin level in hemodialysis patients. In addition, eradication therapy before the progression of atrophy would be expected to improve mortality in hemodialysis patients by improving nutrition status, such as PEW, as well as to prevent increased risk for gastric carcinogenesis. However, because this study is preliminary, The authors think that it will be better to clarify the relationship between plasma and gastric mucosal ghrelin levels and PEW markers by enrolling many hemodialysis patients in multicenter study and the long-term observation study.

### Terminology

Ghrelin is mainly released from the oxyntic mucosa of the stomach and has multiple favorable functions. PEW is defined as a state of decreased body stores of protein and energy fuels and is diagnosed if three features are present: abnormal nutrition markers; reduced body mass; and reduced muscle mass. Pepsinogen (PG) I and II, the two main precursors of pepsin, are both produced by chief cells and mucous neck cells of the stomach. PG II is also produced by pyloric gland cells. PG I and II used to be considered biomarkers for predicting the status of gastric mucosa.

### Peer-review

This clinical study considers the investigation of the plasma ghrelin level in hemodialysis patients and its association with gastric mucosal atrophy and *H. pylori* status. The main finding of this study was that the status of gastric mucosal atrophy was the major determinant of plasma ghrelin level. The authors investigated 108 patients who received hemodialysis. The plasma ghrelin level was evaluated using ELISA. The results are presented and discussed sufficiently well; the 2 figures and 3 tables give good overview about the results and are presented correctly.

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