Our responses to comments raised by the Reviewer (No 2816646)

1. As the authors described in the discussion section, plasma ghrelin levels were affected by body weights, sex, and hormonal parameter. Data of body weights, sex and hormonal parameter such as serum insulin levels in each group should be presented and it should be at least confirmed that there are no differences in these parameters between the groups.

   Response:
   As your comment, in general, plasma ghrelin levels are reported to be affected by body weights, sex, and hormonal parameter. Although unfortunately, we have no data of hormonal parameter including insulin and leptin, we add data of body weights, sex and BMI in each group (different severity of endoscopic gastric mucosal atrophy and different three *H. pylori* status groups of non, past and present infection**)** in revised version.

   With agreement to your comments, data of body weights, sex and BMI in each group were presented in the “Result” section of revised version and we showed that there were no significant differences in these parameters among groups, as shown in Table 3 and 4.

2. Please examine the relationship between observed plasma ghrelin levels and PEW markers (serum albumin, transthyretin, cholesterol, or body composition).

   Response:
   We also think that it is important to show the relationship between plasma ghrelin levels and metabolic or nutritional markers in hemodialysis patients. According to your comments, we added data of serum albumin, total cholesterol, and cholinesterase levels in hemodialysis patients in revised version. In this study, although there were no significant differences in three parameters among three groups related with *H. pylori*

infection status and among different severity groups of gastric mucosal atrophy, 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.

Because this study is preliminary, we think that it is required to clarify the relationship between observed plasma ghrelin levels and PEW markers by enrolling many hemodialysis patients in multicenter study and the long-term observation study.

3. I am confused with the criteria of the classification of *H. pylori* infection. Is there any possibility that high titer of anti-H pylori IgG antibody reflect the past infection? In that case, I think the titer cannot distinguish the present and past infection.

Response:

Thank you for your comments. In this study, we diagnosed *H. pylori* infection by using three detection systems: an anti-*H. pylori* IgG serological test, a rapid urease test and culture test. When positive results were observed with at least one of three detection systems, we diagnosed as positive for *H. pylori*.

In addition, we classified three groups including of the present infection, past infection and non-infection of *H. pylori*, as below (please see Table). We defined that the past infection was negative with all three detection systems including of anti-*H. pylori* IgG antibody titers <10 and positive for endoscopic gastric mucosal atrophy.

As your comments, it might be difficult to understand our classification of *H. pylori* infection in the first version. Therefore, we modified explanations of our classifications and added as newly made Table 1 in the revised version.

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

4. Page8, Line 25. Page14, Line 2. Did this cohort include patients with the history of *H. pylori* eradication? The history of *H. pylori* eradication seems to be included in the exclusion criteria.

Response:

As your comments, the history of *H. pylori* eradication was included in the exclusion criteria in our study. Therefore, this study did not include hemodialysis patients with the history of *H. pylori* eradication therapy with proton pump inhibitor and two kinds of antimicrobial agents.

Previously, we did the follow-up survey of *H. pylori* infection in hemodialysis patients [1]. 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. In addition, the mean dialysis duration in *H. pylori* positive patients was significantly shorter than in uninfected patients. Therefore, *H. pylori* infection might be actually eradicated after beginning hemodialysis treatment, whereas patients did not receive eradication therapy.

In this study, we used the “Past infection” group as patients group who had been naturally cured *H. pylori* infection without receiving eradication

therapy. To avoid confusion, we added explanations criteria in revised version.

5. Page 13, Line5. Did the author use the same kits for measuring serum PG levels as those used in the study of Araki et al?

Response:

To evaluate serum pepsinogen levels, we used a commercially available kit (Pepsinogen CLEIA®; Fuji Rebio. Ltd, Tokyo, Japan) by chemiluminescence enzyme immunoassay. On the other hand, Araki, et al. measured serum pepsinogen levels by radioimmunoassay (RIA). Although a method of assay in our study differed from Araki's method, strong correlation was proved between assays of EIA and RIA [2-4].

To avoid misunderstandings, we added explanations of these points in revised version: (1) methods of pepsinogen assay, and (2) strong correlation of EIA and RIA.

Our responses to comments raised by the Reviewer (No. 39368)

1. The main problem of this study is the luck of control group. Notwithstanding description of background of the study in Introduction it is not sufficiently clear the role of the level of ghrelin in patients with chronic kidney disease.

   Response:
   In the first version, as your comments, the main problem of this study may be the luck of control group using non-hemodialysis subjects. Therefore, although sample number is limited (n=13), we added data of *H. pylori* infection-negative patients without hemodialysis (eGFR>50) as control group in revised version. The acyl- or desacyl-ghrelin levels in *H. pylori*-negative hemodialysis patients were significantly higher than those in *H. pylori* infection-negative non-hemodialysis control subjects (p = 0.010 and 0.004, respectively) (Figure 1).

   As your comments, it might not be clear the role of serum ghrelin level in hemodialysis patients in the first version. Therefore, we modified the "Introduction" section to clarify understanding of the role of the ghrelin level in hemodialysis patients in revised version.

2. In Introduction by description of the role of *H. pylori* infection as the main cause of atrophic gastritis, peptic ulcer and gastric cancer the authors used predominantly self-citation instead of reference to classical *H. pylori* studies.

   Response:
   As your comments, we revised in appropriate references for description of the role of *H. pylori* infection as the main cause of atrophic gastritis, peptic ulcer and gastric cancer in "Introduction" section of revised version.

3. In Material and Methods: why diagnosis of atrophy of gastric mucosa has

not been done by morphological examination of gastric biopsy specimens? The authors' diagnosis of gastric mucosa atrophy is based on endoscopic gastric mucosal atrophic pattern and on the level of serum pepsinogen.

Response:

Thank you for your comments. As your comments, we diagnosed gastric mucosal atrophic by endoscopic and serological findings, not pathological evaluation using gastric biopsy specimens. Pathological findings of gastric mucosal atrophy are suitable to evaluate severity of gastric mucosal atrophy according to the up-fate Sydney system and the OLGA classification. However, pathological evaluation system has any disadvantage, as below: (1) increased risk of gastric bleeding by taking biopsy, especially in patients taking anti-coagulant agents. (2) cost for evaluation, and (3) diagnostic error due to patchy formation of gastric mucosal atrophy.

Recently, endoscopic diagnosis of gastric mucosal atrophy was demonstarted significant correlation with morphological, histological diagnosis [5, 6]. In Japan, most of endoscopists diagnose gastric mucosal atrophy only by endoscopic findings, not pathological evaluations, at routine endoscopy. In addition, the recently developed Kyoto classification of gastritis which are based endoscopic characteristics of *H. pylori* infection-associated gastritis allows grading of endoscopically-visible risk factors for the development of gastric cancer [7, 8].

In this study, 30% of hemodialysis patients enrolled in this study were taking anti-coagulant agents. Therefore, we evaluated gastric mucosal atrophy by endoscopic finding, not pathological evaluations using gastric biopsy specimens, to prevent hemorrhage events. We added comments why diagnosis of atrophy of gastric mucosa has not been done by morphological examination of gastric biopsy specimens in this revised version.

4. “Conclusion” part begin with statement which does not based on actual results of present study.

Response:

As your comment, the first sentence of “Conclusion” part in the first version did not be reflect actual results which plasma ghrelin level was associated with the endoscopic and serological severity of atrophy related to *H. pylori* infection in hemodialysis patients. Therefore, according to your suggestions, we modified this part of the “Conclusion” section into appropriate form in revised version.

References

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