ANSWERING REVIEWERS

May 12, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 10253-edit.docx).

Title: The prevalence of *Helicobacter pylori* infection and atrophic gastritis in patients with dyspeptic symptoms in Myanmar

Authors: Thein Myint, Seiji Shiota, Ratha-korn Vilaichone, New Ni, Than Than Aye, Miyuki Matsuda, Trang Thi Huyen Tran, Tomohisa Uchida, Varocha Mahachai, Yoshio Yamaoka

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript No: 10253

The manuscript has been improved according to the suggestion of reviewers

1. Format has been updated.

2. Revision has been made according to the suggestions of the reviewers as follow and change in yellow color in the manuscript.

Responses to comments raised by Reviewer 1

(1) Since the cutoff values for PG I and the PG I/II ratio seem to be different from each other country, the authors had better demonstrate the value of PGI and PGII concentration in the text or the table and discuss based upon each value instead of PG method criteria.

According to your comments, we showed the real value of PGs according to the status of *H. pylori* in Table 2. Furthermore, we re-evaluated the relationship between gastric mucosal atrophy and PGs in the revised version. As PG was not good indicator for atrophy in our original version, we calculated better cut-off value of PG to predict mucosal atrophy in the revised version.

(2) There are several repetitions in the Results section, especially the same wordings as those in Tables. The authors should shorten the description in the text.

According to your comment, we shorten the description, especially for the prevalence of *H. pylori* infection in the revised version.

(3) The authors had better discuss more about H. pylori, gastritis, and gastric cancer by citing the follow review article and about the molecular dynamics of by citing the following manuscripts. 1. Helicobacter pylori infection in functional dyspepsia. Nat Rev Gastroenterol Hepatol. 2013 10(3):168-74. 2. Helicobacter pylori and gastric cancer. Gastric Cancer. 2009;12(2):79-87. 3. Reactive oxygen species-induced autophagic degradation of Helicobacter pylori CagA is specifically suppressed in cancer stem-like cells. Cell Host Microbe. 2012 12(6):764-77.

According to your comments, we cited them in the revised version.

Responses to comments raised by Reviewer 2

(1) The recruitment of patients is not clear. The study is retrospective? The patients are consecutively enrolled? Why the female sex is prevalent?

According to your comments, we added the details in Method section as follows "We consecutively recruited a total of 252 volunteers with dyspeptic symptoms (155 female and 97 male; mean age of 43.6 ± 14.2 years, range 13 to 85 years old) in our prospective study in 2012." in the revised version. In addition, the number of subject was female dominant although total population in Yangon and Mandalay was not clear in this study. In general, the prevalence of dyspepsia was higher in female than male (Flier S, Rose S. Is functional dyspepsia of particular concern in women? A review of gender differences in epidemiology, pathophysiologic mechanisms, clinical presentation, and management. *Am J Gastroenterol* 2006; 101(12 Suppl): S644-653). Nevertheless, our study includes selection bias although there was no difference of H. pylori infection rate between male and female. We described the limitation in Discussion section.

(2) The study reports high prevalence of atrophic gastritis in gastric antrum (55%). This result is surprising. In fact the absence of oxintic cells in gastric antrum makes the assessment of atrophic gastritis in this site very difficult. A regard a surrogate marker-facilitating the diagnosis, can be considered the presence/absence of intestinal metaplasia. Therefore it is my opinion that the diagnosis of antral atrophic be re-calculated and restricted to patients with this marker.

We really appreciate your comments. As you mentioned, the prevalence of atrophy in the antrum was 55% in this study when samples of grade 1 or more were considered atrophy-positive according to a previous report (Bornschein J, Selgrad M, Wex T, Kuester D, Malfertheiner P. Serological assessment of gastric mucosal atrophy in gastric cancer. BMC Gastroenterol 2012; 12: 10). In addition, on the basis of the topographic locations (antrum and corpus), the gastritis stage (the severity and topography of atrophy) was assessed according to the Operative Link on Gastritis Assessment (OLGA) system in this study. It revealed that most cases with gastritis were mild but not severe in Myanmar. Furthermore, according to your comment, we calculated the prevalence of intestinal metaplasia in the antrum. Intestinal metaplasia in the antrum was found in 11.5% (14/121) in *H. pylori*-positive and 3.8% (5/131) in –negative patients. The prevalence of intestinal metaplasia in the antrum was significantly higher in *H. pylori*-positive subjects than that of negative subjects (P = 0.01). Nevertheless, the score of intestinal metaplasia in the antrum was lower in Myanmar than that of Japan even in *H. pylori*-positive subjects (0.19 ± 0.59 in Myanmar, 0.50 ± 0.07 in Japan). Therefore, milder gastritis might be related with a low incidence of gastric cancer in Myanmar in spite of high *H. pylori* infection rate. We described them in the revised version.

Responses to comments raised by Reviewer 3

(1) The study population was the 252 volunteers with dyspeptic symptoms. The statistical estimator of this population drew a conclusion of the prevalence of H. pylori in Myanmar. However, there could be selection bias in this population. I wonder about H. pylori status of patients with dyspeptic symptom could represent the true prevalence of population of Myanmar. There should be effort to overcome selection bias or authors have to state this as the limitation of this study.

We agree with your comments. Our study included selection bias. Therefore we added limitations in Discussion as follows "Our study includes limitations. We obtained the samples from patients living in Yangon and Mandalay which are the largest and the second largest cities in Myanmar. In general, the prevalence of H. pylori infection is higher in country sides than that of cities due to the difference of environmental factors including sanitary condition. Therefore, our results cannot be generalized in Myanmar. In addition, we included only patients with dyspeptic symptoms but not general population. " in the revised version.

Minor comments

(1) Is there any reason of no relationship between H. pylori infection rate and age in Myanmar?

In general, the prevalence of H. pylori infection will be decreased according to the improvement of sanitary condition. However, the percentage of improved sanitation facilities in 2011 was still 77% in Myanmar (UNICEF, http://www.unicef.org/), which might be the reason for constant infection rate in every age group. We stated them in the revised version.

(2) Could you explain the reason why the prevalence of H. pylori infection is different in Yangon and Mandaly? (besides unidentified genetic or host factors)

According to your comment, we obtained the information for drinking water and sanitary condition in Myanmar. The percentage of usage of pit latrine is higher in Mandalay than in Yangon (Myanmar Multiple Indicator Cluster Survey 2009 – 2010, UNICEF, http://www.unicef.org/myanmar). In addition, drinking water sources is improved in Yangon than in Mandalay (http://www.unicef.org/myanmar). Therefore, it is difficult to explain the difference of H. pylori infection rate by the differences of sanitary condition. Therefore, we think unidentified genetic or host factors might be contributed to this difference. We mentioned them in the revised version.

(3) In page 10, line number 16: Table 4->Table 2. In page 14, line number 8: in in ->in

We really appreciate your pointed out. We corrected them.

Responses to comments raised by Reviewer 4

(1) The paper, as written, is nearly a copy template of other reports from this same lab with many sections (including figures and tables) of the paper copied word for word with numbers changed-out to represent the new data. This same data are discussed redundantly in numerous sections, which should be changed.

According to your comments, we modified some sentences. In addition, as the cut-off value as used in Japan was not better indicator for gastric mucosal atrophy, we calculated the best cut-off value of PG I/II for the OLGA score in the revised version.

(2) It is not clear why Myanmar was chosen for the study—and then why two large cities were chosen. It might have been more interesting to evaluate the city and countryside or both cities if novel information was available to test an important hypothesis that could only be done in these two cities. Thus, it would be helpful to clearly delineate the justification for Myanmar as a location for the study—other than it hasn't been studied already.

Myanmar is located in Southeast Asia bordered by China, Thailand, India, Laos and Bangladesh. The age-standardized incidence rate (ASR) of gastric cancer in Myanmar was reported to be 11.2/100,000 per year (http://globocan.iarc.fr/), which is higher than that of India and Thailand, and lower than that of China (6.1, 3.1 and 22.7/100,000, respectively). To our knowledge, there is no previous study published focusing on the *H. pylori* infection in Myanmar. To understand the reason for higher incidence of gastric cancer in Myanmar than India or Thailand, it is important to elucidate of *H. pylori* infection rate in Myanmar. In addition, phylogeographic analyses with genomic difference of *H. pylori* strains can assume the migration of human populations. Therefore, analyses of *H. pylori* strains isolated from Myanmar might be contributed to the exploration of human migration pattern in south Asian countries.

In addition, we brought all reagents for *H. pylori* cultures (e.g., disposable forceps, transport mediums) from Thailand and Japan. As you mentioned, it is interesting to examine the status of H. pylori infection in other cities and country side; however, unfortunately, it is impossible to do the additional survey at this point. We described limitations in the revised version.

(3) Unless there is a reason that is not elucidated in the paper as it is written, it seems to be incorrect to conclude that the prevalence of H. pylori infection is 48% in Myanmar. Unless justified, it is imperative that this statement be changed to indicate that the prevalence of H. pylori infection is 48% in patients with dyspeptic symptoms, which is not representative of the population as a whole—but of patients with pre-existing gastric disease. This is written numerous places in the paper and as such, needs to be revised in numerous places unless the statement is better justified/clarified.

We agree with your comments. Our study included selection bias. Therefore we added limitations in Discussion as follows "Our study includes limitations. We obtained the samples from patients living in Yangon and Mandalay which are the largest and the second largest cities in Myanmar. In general, the prevalence of *H. pylori* infection is higher in country sides than that of cities due to the difference of environmental factors including sanitary condition. Therefore, our results cannot be generalized in Myanmar. In addition, we included only the patients with dyspeptic symptoms but not general population. The percentage of female was also higher than that of male although there was no difference of *H. pylori* infection rate between male and female. In general, the dyspeptic symptom is more common in female than in male. In further study, it is necessary to investigate the subjects from all Myanmar to elucidate the prevalence of *H. pylori* infection in Myanmar." in the revised version.

(4) The work is solely descriptive and because such papers have been published by this group already, it is disappointing that mechanistic issues were not drilled-down further. First, rather than having anything to do with sanitation, it is more likely that H. pylori positive patients with gastric cancer

have a regionally-specific H. pylori isoform, have major dietary differences, the lack of adequate healthcare, co-infections making the ASR lower than other Asian nations, or other novel issues--none of which were investigated.

As you mentioned, this study was the descriptive paper. However, to our knowledge, this is the first collaborative study for H. pylori infection in Myanmar. In this study, we brought all reagents for H. pylori culture. This collaborative study can help to investigate the infectious diseases in Myanmar in the future study.

(5) If the authors intend to conclude that significant differences in H. pylori infection rates occur with the stated differences in improved sanitation rates, which were 77% in Myanmar versus 100% in Japan, this needs to be studied in detail. Most sanitation improvements occur in large cities first—so was there any difference in improved sanitation in the two cities in Myanmar versus Japan. The authors indicate being unable to obtain regional information on this issue and as such, the entire paragraph should be deleted unless justified scientifically.

As you mentioned, information of sanitary condition in these cities are not sufficient to conclude in this study. Therefore, we cannot discuss the difference of the incidence of gastric cancer between Japan and Myanmar. We deleted these statements from Discussion section in the revised version.

(6) Please indicate from where biopsy samples were taken from the corpus. It would be important that they are all taken from the same location.

According to your comments, we added the detail as follows "During each endoscopy session, 4 gastric biopsy specimens were obtained (three from the lesser curvature of the antrum approximately three cm from the pyloric ring and one from the greater curvature of the corpus)." in the revised version.

(7) With so many patients studied, why don't the data in Figure 1 have standard errors or standard deviations shown on the graph? Error bars must be included. Additionally, is there any statistical difference in the positive rate with age?

According to your comment, we added the standard errors in the revised figure. In addition, there was no statistical difference in the positive rate with age.

3. References and typesetting were corrected.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

Yoshio Yamaoka, MD, PhD

Department of Environmental and Preventive Medicine,

Oita University Faculty of Medicine

1-1 Idaigaoka, Hasama-machi, Yufu-City,

Oita 879-5593, Japan

Fax: +81-97-586-5749

E-mail: yyamaoka@oita-u.ac.jp