Dear Editor,

Thank you for giving us the opportunity to revise our manuscript. We would like to submit our revised manuscript for consideration of publication in the World Journal of Gastroenterology. Enclosed please find our revised manuscript entitled "Prevalence and risk factors for Barrett's Esophagus in Taiwan". We highly appreciate the valuable comments from the Editor/Editorial board and reviewers and have made an itemized, point-by-point response to the comments, referring to page numbers where possible.

This paper may be of particular interest to your readers because this study showed that the prevalence of Barrett's esophagus in subjects undergoing routine health check-up in Taiwan was 2.6%. The finding indicates that the prevalence of Barrett's esophagus among the general population in Taiwan is comparable with that in the western countries, ranging from 0.5% to 2%. In addition, the study also demonstrated that old age, male gender, ingestion of tea, and hiatal hernia were the independent risk factors predicting the presence of Barrett's esophagus.

We appreciate your consideration of this manuscript for publication in the World Journal of Gastroenterology. We hope it will now meet with your approval.

Sincerely Yours,

Dr. Ping-I Hsu
Professor
Division of Gastroenterology and Hepatology
Department of Internal Medicine
Kaohsiung Veterans General Hospital
386 Ta-Chung 1st Road, Kaohsiung, Taiwan

Name of journal: World Journal of Gastroenterology

Manuscript NO: 45468

Manuscript Type: RETROSPECTIVE STUDY

Prevalence and risk factors for Barrett's Esophagus in Taiwan

Yan-Hua Chen, Hsien-Chung Yu, Kung-Hung Lin, Huey-Shyan Lin, Ping-I Hsu

Reply to reviewer's comments

First reviewer's comments

The authors did a thorough review of a fairly discrete topic and covered their goals well. There are relatively few studies on this topic. This data refutes the previously held concept that BE is rare in Asia. The 2015 CGH systematic review was a good background and gave similar results. Since the predominance of BE in Taiwan is short segment, I do not know why one cm interval Seattle protocol biopsies are not employed. The tea ingestion issue should be further elaborated on in the discussion. Overall, I enjoyed the manuscript.

Comment 1: Since the predominance of BE in Taiwan is short segment, I do not know why one cm interval Seattle protocol biopsies are not employed.

Reply to comment: We thank the reviewer for this valuable comment. In this study, we adopted the Seattle protocol with four quadrant biopsies, 2 cm-apart, throughout the columnar-lined esophagus. Additionally, target biopsy was used for individuals with small tongues of columnar mucosa and for all patients with any suspicious IM and dysplastic lesions under NBI evaluation. Although obtaining 4-quadrant biopsy specimens at interval of every 1 cm throughout the columnar-lined esophagus might increase the yield rate of IM, the procedure time, the dose of anesthetic agents and biopsy-related bleeding rate would increase. Our Health Evaluation Center therefore

used the Seattle protocol with 4-quadrant biopsies at interval of every 2 cm for ESEM. We have discussed this important issue in the revised manuscript (P19, lines 9~18: *In this study, we adopted the Seattle protocol with four quadrant biopsies, 2 cm-apart, throughout the columnar-lined esophagus. Additionally, target biopsy was used for individuals with small tongues of columnar mucosa and for all patients with any suspicious IM and dysplastic lesions under NBI evaluation. Although obtaining 4-quadrant* biopsy *specimens at interval of every 1 cm throughout the columnar-lined esophagus might increase the yield rate of IM, the procedure time, the dose of anesthetic agents and biopsy-related bleeding rate would increase. Our Health Evaluation Center therefore used the Seattle protocol with 4-quadrant* biopsies *at interval of every 2 cm for ESEM.*).

Comment 2: The tea ingestion issue should be further elaborated on in the discussion.

Reply to comment: We thank the reviewer for this constructive comment. Several studies have shown that caffeine from tea induced or aggravated acid reflux by decreasing lower esophageal sphincter pressure (LESP). Gudjonsson et al. conducted a blinded crossover study of 12 healthy subjects to evaluate the effect of coffee and tea upon LES function. LESP was significantly lower after intra-gastric instillation of regular coffee and tea. The data for lower esophageal pH paralleled those for LESP. Another single-blinded experimental study performed by Lohsiriwat et al. evaluated the effect of caffeine on LES and esophageal peristaltic contractions in healthy Thai adults. The result indicated that caffeine affected esophageal function, resulting in a decrease in basal LESP and distal esophageal contraction, which is known to promote esophageal reflux. Additionally, tea consumption has been shown to increase gastric acid secretion. Theophylline existing in black tea and green tea was also reported to induce esophageal acid reflux through inhibition of LESP. It is therefore reasonable to expect that tea ingestion might be a risk factor for BE. We have discussed this important issue in the revised manuscript (P17, lines 5~22: *However, it undoubtedly poses a great impact on our daily clinical practice and care of the patient with BE, especially in Asian countries where the prevalence of tea ingestion is high. Several studies have shown that caffeine from coffee and tea induced or aggravated acid reflux by decreasing lower esophageal sphincter pressure (LESP)*[28, 29]. *Gudjonsson et al. conducted a blinded crossover study of 12 healthy subjects to evaluate the effect of coffee and tea upon LES function. LESP was significantly lower after intra-gastric instillation of regular coffee and tea. The data for lower esophageal pH paralleled those for LESP*[28]. *Another single-blinded experimental study performed by Lohsiriwat et al. evaluated the effect of caffeine on LES and esophageal peristaltic*

contractions in healthy Thai adults. The result indicated that caffeine affected esophageal function, resulting in a decrease in basal LESP and distal esophageal contraction, which is known to promote esophageal reflux[29]. Additionally, tea consumption has been shown to increase gastric acid secretion[30]. Theophylline existing in black tea and green tea was also reported to induce esophageal acid reflux through inhibition of LESP[31]. It is therefore reasonable to expect that tea ingestion might be a risk factor for BE.).

Thanks for the reviewer's valuable and constructive comments!

Second reviewer's comments

The study is focused on prevalence and risk factor for BE and it demonstrates an higher prevalence of BE in Taiwan than previously reported. The study is well conducted and improve general knowledge on BE prevalence and on risk factor. Some points need to be addressed in my opinion: Authors state: Periodic endoscopic surveillance for dysplastic or cancerous lesions is suggested for patients diagnosed with BE, although disagreement exists regarding the long-term survival benefit of such surveillance [4]. This is an old reference of more than 10 years ago; I suggest to use a more recent reference. Authors state: The majority of these individuals were physically robust without medical illness and underwent their health check-up to rule out physical disorders, particularly malignancy. The remaining individuals were either employees who were undergoing a regular physical check-up arranged by their employers or those suffering from physical discomforts. I think that this sentence needs to be clarified with data showing at last the percentage of the three categories that has been recognized. In particular the percentage of participants 'suffering from physical disconforts' seems to be important. ESEM was found in 423 (12.5%) individuals, and 89 among them were confirmed to have IM and presence of goblets cells via biopsy examination. Table 3 compare different characteristics in group of ESEM with or without specialized IM. Unfortunately, among the endoscopic findings, it is not reported if any difference was found in ESEM length between these two groups. As reported by authors different papers has demonstrated that specialized IM is more easily histologically recognized dependently on several factors, and among them also length of Barrett's esophagus. I think that an explanation on the reasons for a value as high as 12.5 of ESEM with an histological confirmation in only 21% of them is necessary. Furthermore, while information are provided on the number of endoscopists participating in this work was provided, we have no information about pathologists. The overall agreement (K) in the histological diagnosis of Barrrett's

esophagus is 0.599 (Virchows Arch 2016 Feb;468(2):159-67. doi: 10.1007/s00428-015-1878-5. PMID: 26563401). This could also be an important factor to explain the difference between ESEM and histological BE.

Comment 1: Periodic endoscopic surveillance for dysplastic or cancerous lesions is suggested for patients diagnosed with BE, although disagreement exists regarding the long-term survival benefit of such surveillance [4]. This is an old reference of more than 10 years ago; I suggest to use a more recent reference

Reply to comment: We thank the reviewer for this constructive comment and have replaced the original reference with a guideline from American Society for Gastrointestinal Endoscopy in 2012 (P25, reference number *4* ***Evans JA****, Early DS, Fukami N, Ben-Menachem T, Chandrasekhara V, Chathadi KV, Decker GA, Fanelli RD, Fisher DA, Foley KQ, Hwang JH, Jain R, Jue TL, Khan KM, Lightdale J, Malpas PM, Maple JT, Pasha SF, Saltzman JR, Sharaf RN, Shergill A, Dominitz JA, Cash BD. The role of endoscopy in Barrett's esophagus and other premalignant conditions of the esophagus. Gastrointest Endosc 2012;* ***76****: 1087-1094 [PMID: 23164510 DOI: 10.1016/j.gie.2012.08.004]*).

Comment 2: The majority of these individuals were physically robust without medical illness and underwent their health check-up to rule out physical disorders, particularly malignancy. The remaining individuals were either employees who were undergoing a regular physical check-up arranged by their employers or those suffering from physical discomforts. I think that this sentence needs to be clarified with data showing at last the percentage of the three categories that has been recognized. In particular the percentage of participants 'suffering from physical disconforts' seems to be important.

Reply to comment: We thank the reviewer for this constructive comment. In this study, the majority of these individuals (68.5%, n=2321) were physically robust and underwent their health check-up to rule out physical disorders, particularly malignancy. The remaining individuals were either employees (21.9%, n=741) who were undergoing a regular physical check-up arranged by their employers or those suffering from physical discomforts (9.6%, n=325). We have described the percentage and number of subjects in each relevant category in the revised manuscript. (P13, lines 11~16: *The majority of these individuals (68.5%, n=2321) were physically robust and underwent their health check-up to rule out physical disorders, particularly malignancy. The remaining individuals were either employees (21.9%, n=741) who were undergoing a regular physical check-up arranged by their employers or those suffering from physical discomforts (9.6%, n=325)*.).

Comment 3: Table 3 compare different characteristics in group of ESEM with or without specialized IM. Unfortunately, among the endoscopic findings, it is not reported if any difference was found in ESEM length between these two groups. As reported by authors different papers has demonstrated that specialized IM is more easily histologically recognized dependently on several factors, and among them also length of Barrett's esophagus

Reply to comment: We thank the reviewer for this valuable comment and have compared the length of metaplastic epithelium between ESEM with and without IM in revised Table 3. There was no significant difference in the length of ESEM between the two groups (1.42±0.84cm *vs* 1.31±0.48cm, *P*= 0.243).

Comment 4: I think that an explanation on the reasons for a value as high as 12.5 of ESEM with an histological confirmation in only 21% of them is necessary. Furthermore, while information are provided on the number of endoscopists participating in this work was provided, we have no information about pathologists. The overall agreement (K) in the histological diagnosis of Barrrett's esophagus is 0.599 (Virchows Arch 2016 Feb;468(2):159-67. doi: 10.1007/s00428-015-1878-5. PMID: 26563401). This could also be an important factor to explain the difference between ESEM and histological BE.

Reply to comment: We thank the reviewer for the valuable and constructive comments. In this study, the esophageal biopsy specimens were interpreted by eight experienced general pathologists. We have mentioned this point in the revised manuscript (P12, lines 7~9: *All specimens acquired were embedded in paraffin, stained with hematoxylin and eosin and then reviewed by eight experienced general pathologists)*.

We agree with the reviewer's comments that an explanation on the reasons for a value as high as 12.5 of ESEM with a histological confirmation in only 21% of them is necessary. Many factors may lead to false negative detection of IM in daily practice. For example, the number of endoscopic biopsies taken may directly affect the yield rate of IM. Harrison et al. found that the diagnostic yield of IM was 34.7% when four biopsies were taken, which increased to 67.9% with eight biopsies, and would have reached 100% if more than 16 biopsies were taken. Moreover, the distribution of IM over the columnar-lined esophagus is markedly heterogeneous, which could cause sampling error. Chandrasoma et al. demonstrated that the prevalence and density of goblet cells between the most proximal and most distal levels were markedly different, and the probability of finding IM was highest when the biopsies were focused in the most proximal area of the columnar-lined esophagus. In this study, we adopted the

Seattle protocol with four quadrant biopsies, 2 cm-apart, throughout the columnar-lined esophagus. Additionally, target biopsy was used for individuals with small tongues of columnar mucosa and for all patients with any suspicious IM and dysplastic lesions under NBI evaluation. Although obtaining 4-quadrant biopsy specimens at interval of every 1 cm throughout the columnar-lined esophagus might increase the yield rate of IM, the procedure time, the dose of anesthetic agents and biopsy-related bleeding rate would increase. Our Health Evaluation Center therefore used the Seattle protocol with 4-quadrant biopsies at interval of every 2 cm for ESEM. In this study, the esophageal biopsy specimens were interpreted by eight pathologists. It was also a possible confounding factor responsible for the different detection rates between ESEM and BE since the overall agreement rate of the diagnostic category of "BE with IM" between pathologists is moderate. We have addressed the important issue in the Discussion section (P18, line 20 ~ P19, line 23: *Of the 423 subjects labeled as ESEM in this study, IM was detected in 89 individuals. The detection rate of IM in metaplastic epithelium was 21% only. Many factors may lead to false negative detection of IM in daily practice. For example, the number of endoscopic biopsies taken may directly affect the yield rate of IM. Harrison et al. found that the diagnostic yield of IM was 34.7% when four biopsies were taken, which increased to 67.9% with eight biopsies, and would have reached 100% if more than 16 biopsies were taken*[40]*. Moreover, the distribution of IM over the columnar-lined esophagus is markedly heterogeneous, which could cause sampling error. Chandrasoma et al. demonstrated that the prevalence and density of goblet cells between the most proximal and most distal levels were markedly different, and the probability of finding IM was highest when the biopsies were focused in the most proximal area of the columnar-lined esophagus*[41]*. In this study, we adopted the Seattle protocol with four quadrant biopsies, 2 cm-apart, throughout the columnar-lined esophagus. Additionally, target biopsy was used for individuals with small tongues of columnar mucosa and for all patients with any suspicious IM and dysplastic lesions under NBI evaluation. Although obtaining 4-quadrant biopsy specimens at interval of every 1 cm throughout the columnar-lined esophagus might increase the yield rate of IM, the procedure time, the dose of anesthetic agents and biopsy-related bleeding rate would increase. Our Health Evaluation Center therefore used the Seattle protocol with 4-quadrant biopsies at interval of every 2 cm for ESEM. Furthermore, the esophageal biopsy specimens were interpreted by eight pathologists. Mastracci et al. revealed that the overall agreement rate of the diagnostic category of "BE with IM" between pathologists is moderate, with a K value of 0.599*[42]*. This phenomenon might also be one of the confounding factors responsible for the different detection rates between ESEM and BE*.).

Thanks for the reviewer's valuable and constructive comments!

Third reviewer's comments

This is a well-designed retrospective study. Authors clearly demonstrated that prevalence of Barrett's esophagus (BE) among the general population in Taiwan is 2.6% and old age, male gender, ingestion of tea and hiatal hernia are significant risk factors for BE. Furthermore, they demonstrated that he independent risk factor for the presence of intestinal metaplasia in endoscopically suspected esophageal metaplasia lesions was old age alone. It is well known that BE is a pre-malignant condition associated with the development of esophageal adenocarcinoma. Therefore, it is important to know the risk factors predicting the presence of BE. The present study provided such data from Taiwan district and enriched the database in this field. It is relevant for the readership in WJG.

Comment 1: The present study provided such data from Taiwan district and enriched the database in this field. It is relevant for the readership in WJG.

Reply to comment: We are grateful for the reviewer's appreciation.