January 18th, 2016.

Jing Yu
Science Editor
World Journal of Gastroenterology

Dear Dr. Jing Yu,

We are pleased that our study has been considered for a resubmission. We are sincerely thankful to the reviewers, whose remarks helped strengthen our manuscript.

Reviewers comments.

Reviewer's code: 03086186

Herrera-Goepfert R et al. explored gene methylation in esophageal columnar metaplasia, and correlated these findings with the status of *H. pylori cagA*+. It is well written and contains information which readers may be interested. Because it is suggested that intestinal metaplasia in the esophagus arises from gastric-type metaplasia, I suggest authors to include intestinal metaplasia in the study.

Answer. We did not include cases of intestinal metaplasia, because the aim of the present study was to search for the potential association between *Helicobacter pylori* and the methylation of some genes in gastric-type columnar metaplasia of the esophagus, which in fact had been previously reported as methylated in intestinal metaplasia of the esophagus (Barrett esophagus) (Ref. 21). Considering that gastric-type comes before intestinal-type metaplasia of the esophagus and that *Helicobacter pylori* do not colonize intestinal-type epithelium, the rationale was to demonstrate that *Helicobacter pylori* could play a substantial role in the epigenetic changes seen during the early stages of columnar non-intestinal metaplasia of the esophagus. These issues are contended within the second and third paragraphs of the Discussion Section (page 10) of the manuscript.

Reviewer's code: 00057996

Herrera-Goepfert and colleagues presented in their manuscript "Methylation of DAPK and THBS1 genes in esophageal gastric-type columnar metaplasia" data on a potential influence of HP infection on methylation of relevant tumor suppressor genes in gastric-type columnar metaplasia. In summary, the study presents some interesting findings, and the manuscript is well written. I have only a few comments for the authors:

1) The result section in abstract does not provide any data on methylation status of investigated genes.

Answer. Information regarding methylation status of investigated genes has been incorporated into the Results Section in the Abstract: "*DAP kinase*, *THSB1*, *CDH1*, and *p14* gene promoters were methylated by MS-PCR in 40 (58.8%), 33 (48.5%), 46 (67.6%), and 23 (33.8%) cases of the 68 esophageal samples."

2) The numbering of tables seems incorrect. The results state for example "Histopathological variables according to the Updated Sydney System for the classification and grading of gastritis were significantly associated with H. pylori cagA+ status (Table 5).", but table 5 shows "Association of Helicobacter pylori cagA+ infection as detected by PCR with DNA methylation of the promoter regions of target genes in 33 esophageal and gastric biopsies."

Answer. The order and number of Tables have been corrected in the Results Section. In addition, the information of the age variable has been filled-out in the Table 5.

3) The authors should add clinical data on severity or symptoms of reflux, extent of metaplasia etc. In addition, I would ask the authors to provide more detailed information on the indication of endoscopy and biopsy as this might bias results.

Answer. We did not include information regarding the symptoms of patients because the primary objective of the study was to seek for the association of *Helicobacter pylori* with epigenetic changes in the esophageal metaplastic mucosa, regardless of clinical symptoms. In addition, several studies have shown that there is no correlation between the severity of reflux symptoms and morphological changes observed in the distal esophagus. Nevertheless, we added brief clinical information: "*All biopsies were obtained by means of the panendoscopy procedure at the Endoscopy Service outpatient clinic, in patients with upper gastrointestinal complaints and with endoscopic suspicion of columnar metaplasia.*" (Material and Methods Section; pages 6 and 7; last and first lines, respectively). Finally, in the Results Section, page 8, first paragraph, lines 5 and 6, endoscopic findings are commented: "*Endoscopic findings at the distal esophagus included variable degrees of mucosal erosion, salmon-colored mucosal tongues and irregular Z line*".

4) The low number and especially missing samples of the gastric samples (there is no reason given why some patients have gastric biopsies and why others have not) makes interpretation of these data extremely difficult. The authors should acknowledge this in more detail.

Answer. Esophagus and stomach biopsies were obtained in all patients; however, in 35 of the patients, the gastric mucosa sample was insufficient for the molecular study. We added an explanatory paragraph in the Results Section, page 8, first paragraph, lines 3–5: "*In 35 subjects, gastric mucosa samples were insufficient for the molecular study, and in the remaining 33 (48.5%) subjects, the gastric samples were processed for final analysis*." Additionally, in the same Results Section, page 9, first paragraph we added the following comment: "H. pylori *cagA+ status was significantly associated with methylation of DAPK (p = 0.003) and THBS1 (p = 0.019) in the 68 esophageal samples (Table 2), and bivariate analysis confirmed the significance of this association (Table 3). In the comparative analysis between the 33 gastric and 33 esophageal paired samples, the trend for the association between H. pylori cagA+ and methylation of THBS1 and DAPK genes was maintained (Table 4). Methylation of the CDH1 and p14 gene promoters did not exhibit statistically significant differences between H. pylori cagA+ and cagA- cases, in both the esophageal and the gastric biopsies. Among the esophageal biopsies*,"

5) Inclusion of intestinal metaplasia and comparison between specialized and non-specialized columnar metaplasia if possible would be very interesting. If not available, authors should comment on this.

Answer. As previously commented, we did not include cases of intestinal metaplasia, because the aim of the present study was to search for the potential association between *Helicobacter pylori* and the methylation of some genes in gastric-type columnar metaplasia of the esophagus, which in fact had been previously reported as methylated in intestinal metaplasia of the esophagus (Barrett esophagus) (Ref. 21). We comment on such issue in the Discussion Section, page 10, first and second paragraphs.

Reviewer's code: 02537190

In the manuscript Methylation of DAPK and THBS1 genes in esophageal gastric-type columnar metaplasia by Herrera-Goepfert R et al. authors are trying to analyse the influence of H pylori infection on the methylation of some genes that can influence the risk of esophageal cancer. This idea is clinically relevant, but authors should be well aware of some limitations of their retrospective observative nature of their study. The risk of sampling bias on their results should be clearly acknowledged in the manuscript. In that way conclusions of this study can serve as a good idea for a prospective study with well-defined biopsy protocol. An important message of this study is that cag A *H. pylori* infection in GERD patients is not protective and it can even have some harmful consequences to the gastric metaplastic mucosa in esophagus. All patients with GERD and metaplastic esophageal mucosa infected with H pylori should be eradicated. This could also be one of conclusions of this study.

Answer. Indeed, we agree with Reviewer in that our study has some limitations. The risk of sampling bias is acknowledged in the Discussion Section, page 10, first paragraph, lines 10–13. We also added more commentaries in the last paragraph of the Discussion Section, page 12: "*Finally, we are aware that our study has some limitations, regarding its retrospective design, the small number of patients under study, and the inevitable sampling bias due to the varied and random distribution of the histological changes among the columnar lined esophagus.* Further *prospective* studies are warranted to adequately identify patients with GERD with higher risks for developing severe disorders including BE as well as adenocarcinoma and its precursor lesions.

Conclusions (page 13) have been also modified as follows: "In this study, we showed that CpG methylation occurs in non-specialized, gastric-type columnar metaplasia of the esophagus and is closely related to *H. pylori cagA*+ infection. Given this effect on gastric-type metaplastic mucosa, a conscious search for *H. pylori* and its eradication may be essential for halting the early mechanisms potentially involved in BE development and BE-associated carcinogenesis, among subjects suffering from GERD.

Editor's suggestions

1. The running title has been place under the corresponding heading
2. The audio core tip is attached.
3. Institutional review board statement is attached.
4. Figures 4A and 4B are now separated.

We hope that these amendments make suitable our study for its publication in your prestigious journal.

Yours, most sincerely,

Roberto Herrera-Goepfert, M.D.