Format for ANSWERING REVIEWERS

October 28, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 13938-review.doc).

Title: Association between *Helicobacter spp* infections and hepatobiliary malignancies: a review

Author: Fany Karina Segura López, Alfredo Güitrón Cantú, Javier Torres.

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 13938

The manuscript has been improved according to the suggestions of reviewers:
1 Format of manuscript: no changed
2 Revision has been made according to the suggestions of the reviewer

(1) A number of studies in the literature have evaluated the association between Helicobacter pylori infection and hepatobiliary malignancies, especially for pancreatic cancer. The study covers very well the aspects of this association. Regarding the sentence that "It has been suggested that enterohepatic Helicobacter infection may be a factor in the development of cholesterol gallstones and intrahepatic cholelithiasis, which may further lead to carcinogenesis" i believe that for the aformentioned association between h. Pylori, bile stone formation and gall bladder cancer there is not strong evidence. Despite that it is a very well written and interesting review article.

In response at this comment, we agree that there is not strong evidence with the association between *H.* pylori, bile stone formation and gallbladder cancer. Although Helicobacter pylori DNA has been found in human bile, whether this organism rarely colonizes the bile duct epithelium was not clearly documented [105]. We make specific reference at infection with enterohepatic Helicobacter species such as *H. bilis* and *H. hepaticus*. Cholangiocarcinoma has the histological characteristics of an adenocarcinoma of the bile-duct epithelial cells, and it is suggested that the progression to cancer is similar to what is observed in the intestinal type of gastric cancer, from hyperplasia, to metaplasia, to dysplasia, and to the development of adenocarcinoma [9-11, 28-30]. In other hand, there are multiple human clinical studies relating infection with Helicobacter spp. and biliary diseases. *H. pylori* has also been suggested to infect the biliary tract and cause cancer. In this infection, carcinogenesis includes the activity of virulence factor CagA, an oncoprotein that interferences with signal transduction pathway, and host response to Helicobacter antigens in form of cytokines, and other inflammatory mediators. [101, 104]

(2) 1-The authors reviewed the role of Helicobacter species in the development of hepatobiliary malignancies. As it was stated in the manuscript, culturing the Helicobacter species is difficult and molecular methods have been used to show the presence of Helicobacter species in malign tissues (Yang J, et al. Helicobacter hepaticus infection in primary hepatocellular carcinoma tissue. Singapore Med J 2013;54(8):451-7.). additionally, most studies have been carried out on animals.

2-Abbreviations (HCC, ICCA, ECCA, etc) must also be written in the manuscript, not only in abstract.

In response at the comment of 2o reviewer, we have included abbreviations in the manuscript (HCC, ICCA, ECCA, etc).

(3) We have carefully evaluated this new review article. This is a review article that describes Helicobacter species pathogenicity and their potential association with hepatobiliary malignancies. However, most studies have been performed in animals. Because this is a translational review article, the authors need to clearly demonstrate the potential linkage between concepts that have been developed from animal models and their attempts to reference human studies. Multiple clinical studies have shown a significantly higher pooled infection rate of Helicobacter species in the biliary tract of the cancer group as well as in the benign biliary disease patients compared with the asymptomatic group. What are the potential reasons for the increased prevalence of Helicobacter species in individuals with benign biliary diseases? The authors also touch upon the difficulty in culturing these Helicobacter species in humans, and why molecular methods like PCR or immunologic assays have become the standard methods for diagnosis. The authors need to provide a clear vision about how they foresee diagnostic methods being utilized by clinicians in the future, when caring for individuals with benign and maligant biliary tract diseases.

According to questions of 3rd reviewer:

What are the potential reasons for the increased prevalence of Helicobacter species in individuals with benign biliary diseases?

Enterohepatic Helicobacter infection has been suggested to participate in the development of cholesterol gallstones and intrahepatic cholelithiasis, which may subsequently lead to carcinogenesis [54, 95]. Its promotion of stone formation has been suggested to be due to its serving as a foreign body nest around which the stone develops, likely by producing hydrolyzing enzymes or nucleating proteins, such as immunoglobulins [54,96]. The risk for lithogenicity might also be associated with the modulation of enterohepatic cycling of conjugated bile acid, including the transit time through the gut [54, 82,87, 97-101].

The authors also touch upon the difficulty in culturing these Helicobacter species in humans, and why molecular methods like PCR or immunologic assays have become the standard methods for diagnosis. The authors need to provide a clear vision about how they foresee diagnostic methods being utilized by clinicians in the future, when caring for individuals with benign and maligant biliary tract diseases.

Because the biliary tract is only accessible via invasive procedures or surgery, it is necessary to develop PCR assay protocols, more suitable antigens for immunohistochemistry, and easy and effective serological methods for the early detection of Helicobacter spp. to help reduce the incidence of biliary diseases, as well as the morbidity and mortality of this group of patients.

3 References and typesetting were corrected.

Before: 1.- Guitron-Cantu A. Endoscopic treatment in malignant obstructive jaundice: In Endoscopic procedures in Gastroenterology. In: Cordova VJA, De la Torre BA. 2a ed. Endoscopic Procedures in Gastroenterology. Mexico. Ed Panamericana, 2009: 489-504.

After: 1. Guitron-Cantu A. Endoscopic treatment in malignant obstructive jaundice: In Endoscopic procedures in Gastroenterology. In: Cordova VJA, De la Torre BA. 2a ed. Endoscopic Procedures in Gastroenterology. Mexico. Ed Panamericana, 2009: 489-504. **ISBN: 978-607-7743-05-7**

We added a new reference:

104. Peek RM Jr, Crabtree JE. Helicobacter infection and gastric neoplasia. J Pathol. 2006;**208**(2): 233-248. [PMID: 16362989] DOI:10.1002/path.1868

By the new reference, the other one had changed:

Before: 104. Myung SJ, Kim MH, Shim KN, Kim MH, Kim EO, Kim HJ, Park ET, Yoo KS, Limb BC, Seo DW, Lee SK, Min YI, Kim JY. Detection of Helicobacter pylori DNA in human biliary

tree and its association with hepatolithiasis. *Dig Dis Sci.* 2000; **45** (7): 1405-12. [PMID: 10961722]
After: 105. Myung SJ, Kim MH, Shim KN, Kim MH, Kim EO, Kim HJ, Park ET, Yoo KS, Limb BC, Seo DW, Lee SK, Min YI, Kim JY. Detection of Helicobacter pylori DNA in human biliary tree and its association with hepatolithiasis. *Dig Dis Sci.* 2000; **45** (7): 1405-12. [PMID: 10961722]

Before: 105. Ananieva O, Nilsson I, Vorobjova T, Uibo R, Wadström T. Immune responses to bile-tolerant Helicobacter species in patients with chronic liver diseases, a randomized population group, and healthy blood donors. *Clin Diagn Lab Immunol.* 2002; **9**(6): 1160–1164. PMID: 12414744. PMCID: PMC130091]
After: 106. Ananieva O, Nilsson I, Vorobjova T, Uibo R, Wadström T. Immune responses to bile-tolerant Helicobacter species in patients with chronic liver diseases, a randomized population group, and healthy blood donors. *Clin Diagn Lab Immunol.* 2002; **9**(6): 1160–1164. PMID: 12414744. PMCID: PMC130091]
Before: 106. Zhou D, Wang JD, Weng MZ, Zhang Y, Wang XF, Gong W, Quan Zw. Infections of Helicobacter spp. in the biliary system are associated with biliary tract cancer: a meta-analysis. *Eur J Gastroenterol Hepatol.* 2013; **25**(4): 447- 54. [PIMD: 23470268. DOI: 10.1097/MEG.0b013e32835c0362]
After: 107. Zhou D, Wang JD, Weng MZ, Zhang Y, Wang XF, Gong W, Quan Zw. Infections of Helicobacter spp. in the biliary system are associated with biliary tract cancer: a meta-analysis. *Eur J Gastroenterol Hepatol.* 2013; **25**(4): 447- 54. [PIMD: 23470268. DOI: 10.1097/MEG.0b013e32835c0362]
Before: 107. Xuan SY, Li N, Qiang X, Zhou RR, Shi YX, Jiang WJ. Helicobacter infection in hepatocellular carcinoma tissue. *World J Gastroenterol.* 2006; **12**(15): 2335 – 340. [PMID: 16688821. PMCID: PMC4088066]
After: 108. Xuan SY, Li N, Qiang X, Zhou RR, Shi YX, Jiang WJ. Helicobacter infection in hepatocellular carcinoma tissue. *World J Gastroenterol.* 2006; **12**(15): 2335 – 340. [PMID: 16688821. PMCID: PMC4088066]

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

Sincerely yours,

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