

Replies to dear Reviewers and Editors:

First of all, we thank both reviewers and editors for their positive and constructive comments and suggestions for our manuscript (NO: 53656), titled "*Helicobacter pylori* and gastric cardia cancer: what do we know about the risks?". We have revised the manuscript, according to the comments and suggestions, point by point to, and responded as listed below. And we have marked the changes in the manuscript in red.

Response to Reviewers

Reviewer's code: 02446061

Comment 1: Please check the meaning of some sentences like "we 'believed' that..." and be concise.

Answer: We are so sorry for the inappropriate words in the manuscript. We have now reviewed and edited our manuscript based on your suggestions.

Comment 2: Abstract should include clear conclusions.

Answer: Thank you very much for your suggestions, changes have been made as suggested. We agree with you that it is very important to show clear conclusions in the abstract.

Comment 3: The following recent articles should be considered. Their content is relevant to the content of your manuscript: Liou JM, Lee YC, El-Omar EM, Wu MS. Efficacy and Long-Term Safety of *H. pylori* Eradication for Gastric Cancer Prevention. *Cancers* (Basel). 2019 Apr 28;11(5). pii: E593. Rawla P, Barsouk A. Epidemiology of gastric cancer: global trends, risk factors and prevention. *Prz Gastroenterol*. 2019;14(1):26-38. doi: 10.5114/pg.2018.80001. Venerito M, Link A, Rokkas T, Malfertheiner P. Review: Gastric Cancer-Clinical aspects. *Helicobacter*. 2019 Sep;24 Suppl 1: e12643. doi: 10.1111/hel.12643.

Answer: Thank you very much for your valuable suggestions, these articles have made us a lot of gains. In particular, the explanation of the cause of gastric cardia cancer in these articles gives us a lot of inspiration. Actually, this aspect of the relationship between *H. pylori* eradication and gastric cardia

cancer was not covered in the manuscript. We regret to point out this problem in the shortcomings of the manuscript. Because we feel that the relationship between these two cannot be fully shown through a simple description, so we hope that we can explore this aspect in the future, which will be a good direction. We are very grateful to you for recommending these documents to us for reference and bringing us such a great inspiration. We also hope not to simply mention them in this manuscript, but to show the relationship between these two in more detail, because this is indeed a very exciting idea.

Reviewer's code: 03769068

Comment 1: The abstract needs revision. The text presents problematization. However, this session text needs more content.

Answer: Thank you very much for your suggestions, changes have been made as suggested. We have now reviewed and edited our abstract. The conclusions of this study are explained in more detail in the abstract.

Comment 2: There is a lot of text in the introduction without bibliographic reference. In addition, the numbers referring to these references need to be standardized in the text.

Answer: Thank you very much for your suggestions, we have now reviewed and edited our manuscript based on your suggestion about "There is a lot of text in the introduction without bibliographic reference". Regarding the question of standardization, you mentioned, we also agree with you. It would be better if they can be standardized uniformly, but the comparison between the data cited in our article was made in the data of the same literatures. The comparison was comparable, so the citation data of the manuscript as a whole was not standardized, and we hope to get your forgiveness and understanding.

Comment 3: It is not necessary to cite table 1 twice in the text on epidemiology.

Answer: Thank you very much for your suggestions, changes have been made as suggested. We are so sorry that this part was not clear in the original

manuscript. We wanted to explain that the age-standardized incidence of gastric cardia cancer (per 100,000 cases) and the *H. pylori* infection rates were originally described separately according to the structure of the manuscript, before. It appeared in the prevalence of gastric cardia cancer and the prevalence of *H. pylori*, but now we have reworked this part to make the manuscript look better.

Comment 4: It would be important to discuss the prevalence of *H. pylori* in Latin America.

Answer: Thank you very much for your suggestions, changes have been made as suggested. We have supplemented this part in the seventh paragraph in the article. The content is as follows: “The prevalence of *H. pylori* in Latin America cannot be underestimated. A meta-analysis in a study by Curado et al. suggested that *H. pylori* infection rates are high in all age groups in Latin America”. (Curado MP, de Oliveira MM, de Araujo Fagundes M. Prevalence of *Helicobacter pylori* infection in Latin America and the Caribbean populations: A systematic review and meta-analysis. *Cancer Epidemiol* 2019; 60: 141-148). And the data in table 1 and figure 1 of the manuscript also show the prevalence of *H. pylori* in Latin America.

Comment 5: Severe diseases resulting from *H. pylori* infection depend on the host, bacteria and environment. It has to be in the text.

Answer: Thank you very much for your suggestions, changes have been made as suggested. Through your suggestions, we realized that the causes of the problem that may cause the inconsistent relationship between *H. pylori* and gastric cardia cancer need to be considered from the perspective of the host, bacteria and environment of *H. pylori*. This is indeed a very important point, and we have supplemented this point in the manuscript.

Comment 6, 7: The relationship between *cagA* EPIYA sites and carcinogenesis is not mentioned in the text. This topic is extremely relevant. The authors should discuss *cagA* protein as an oncoprotein.

Answer: Thank you very much for your suggestions, this is really important

and will make our manuscript more complete. We have supplemented content, on these two points, in the thirteenth paragraph in the text. The content is as follows: *H. pylori* cagA protein appears as a bacterial oncoprotein. Lee et al. showed that people infected with *H. pylori*, which contains the cagA protein, produce more reactive oxygen species and have an increased risk of gastric cancer. CagA protein is the only bacterial oncoprotein identified to date. cagA contains two repeatable protein-binding motifs, the Glu-Pro-Ile-Tyr-Ala (EPIYA) motif and the cagA multimerization (CM) motif. There are two major pathological and biochemical processes that contribute to *H. pylori* cagA-induced gastric cancer: abnormal cancer-promoting signals caused by SHP2 imbalance via the EPIYA motif, and gastric epithelial destruction caused by CM-mediated PAR1 inhibition. EPIYA motifs are divided into four categories (EPIYA-A, -B, -C, -D), depending on the amino acid sequence surrounding each EPIYA motif, and they have different characteristics.

1. Hayashi T, Senda M, Morohashi H, Higashi H, Horio M, Kashiba Y, Nagase L, Sasaya D, Shimizu T, Venugopalan N, Kumeta H, Noda NN, Inagaki F, Senda T, Hatakeyama M. Tertiary structure-function analysis reveals the pathogenic signaling potentiation mechanism of *Helicobacter pylori* oncogenic effector CagA. *Cell Host Microbe* 2012; 12: 20-33 [PMID: 22817985 DOI: 10.1016/j.chom.2012.05.010]
2. Lee DY, Jung DE, Yu SS, Lee YS, Choi BK, Lee YC. Regulation of SIRT3 signal related metabolic reprogramming in gastric cancer by *Helicobacter pylori* oncoprotein CagA. *Oncotarget* 2017; 8: 78365-78378 [PMID: 29108235 PMCID: 5667968 DOI: 10.18632/oncotarget.18695]
3. Nishikawa H, Hatakeyama M. Sequence Polymorphism and Intrinsic Structural Disorder as Related to Pathobiological Performance of the *Helicobacter pylori* CagA Oncoprotein. *Toxins* 2017; 9: 136 [PMID: 28406453 DOI: 10.3390/toxins9040136]
4. Chen SY, Zhang RG, Duan GC. Pathogenic mechanisms of the oncoprotein CagA in *H. pylori*-induced gastric cancer (Review). *Oncol Rep* 2016; 36: 3087-3094 [PMID: 27748858 DOI: 10.3892/or.2016.6111]

10.3892/or.2016.5145])

Reviewer's code: 03538269

Comment 1: Most of the studies are from the western countries which have already established no link between the gastric cardia cancer and *H. pylori*. The outcome was obvious.

Answer: First of all, thank you very much for your valuable comments. We are so sorry our manuscript has brought you this feeling. In our manuscript, there are also examples of negative correlations between *H. pylori* and cardia cancer in western countries. For example, the eleventh paragraph in the manuscript: Data from some western countries showed that *H. pylori* was a protective factor for gastric cardia cancer, or there was no pathogenic relationship between these two. A nested case-control study of Norwegian population by Hansen and others found that gastric cardia cancer was negatively associated with *H. pylori* (OR=0.27, 95%CI: 0.12-0.59). Ye et al. found no correlation between gastric cardia cancer and *H. pylori* infection based on the native Swedish population who were younger than 80 years. (1. Hansen S, Vollset SE, Derakhshan MH, Fyfe V, Melby KK, Aase S, Jellum E, McColl KEL. Two distinct aetiologies of cardia cancer; evidence from premorbid serological markers of gastric atrophy and *Helicobacter pylori* status. *Gut* 2007; 56: 918-925 [PMID: 17317788 DOI: 10.1136/gut.2006.114504] 2. Ye WM, Held M, Lagergren J, Engstrand L, Blot WJ, McLaughlin JK, Nyren O. *Helicobacter pylori* infection and gastric atrophy: Risk of adenocarcinoma and squamous-cell carcinoma of the esophagus and adenocarcinoma of the gastric cardia. *J Natl Cancer Inst* 2004; 96: 388-396 [PMID: 14996860 DOI: 10.1093/jnci/djh057])

As for the meta-analysis of the relationship between *Helicobacter pylori* *cagA* and gastric cardia cancer in the manuscript, all the selected articles were included under the conditions of retrieval and following the inclusion criteria, as far as we could. We also carried out bias analysis through funnel plot, Egger's and Begg's test.

Comment 2: More of Asian studies should be included

Answer: Thank you very much for your suggestions, changes have been made as suggested. We have supplemented more of Asian studies in the article. Our article referenced some research on Asians, such as: 1. China has a higher incidence of gastric cardia cancer in the world. Epidemiological data showed that the incidence of esophageal and gastric cardia cancer was consistent. China was a high incidence area of esophageal cancer, and many studies suggested that the incidence of gastric cardia cancer was also high in the area, which has a high incidence of esophageal cancer in China. This phenomenon had been observed in China's Linxian (Henan province), Cixian (Hebei province), Chaoshan (Guangdong province) and other areas with high incidence of esophageal cancer. 2. However, researches in China, Japan, and other Asian countries had shown that *H. pylori* were the pathogenic factor of gastric cardia cancer. A cohort study by Kamangar et al. on 29,584 residents in Linxian (Henan province in China) suggested that *H. pylori* infection was a risk factor for gastric cardia cancer (HR=1.64; 95%CI: 1.26-2.14). Yasuo et al. also found that 75% of Japanese patients with gastric cardia cancer had *H. pylori* infection, and *H. pylori* infection was closely associated with gastric cardia cancer. On this basis, we have added some relevant studies on Asian. (Such as: 1. Kim JY, Lee HS, Kim N, Shin CM, Lee SH, Park YS, Hwang JH, Kim JW, Jeong SH, Lee DH, Park DJ, Kim HH, Jung HC. Prevalence and clinicopathologic characteristics of gastric cardia cancer in South Korea. *Helicobacter* 2012; 17: 358-368 [PMID: 22967119 DOI: 10.1111/j.1523-5378.2012.00958.x] 2. Wang LD, Zheng S, Zheng ZY, Casson AG. Primary adenocarcinomas of lower esophagus, esophagogastric junction and gastric cardia: in special reference to China. *World J Gastroenterol* 2003; 9: 1156-1164 [PMID: 12800215 DOI: 10.3748/wjg.v9.i6.1156])

We look forward to hearing from you regarding our submission. We would be glad to respond to any further questions and comments that you may have. We hope that this research paper can be accepted in this journal.

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Thanks very much for your attention to our paper. I am looking forward to your reply.

Sincerely yours,

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