ANSWERING REVIEWERS

December 11, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: **manuscript WJG-5822-edited_ 11122013.docx)**.

Title: Carcinogenic H. pylori in gastric pre-cancer and cancer lesions: association with tobacco-chewing

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We really appreciate your critical review, valuable comments and favorable decision to give us a chance to improve our manuscript. We carefully read your suggestions and the reviewers' comments. We have now revised the manuscript as per suggestions of the reviewers and tried to respond to each of their comments as indicated below in the revised manuscript text and highlighted by yellow background.

Response to Reviewer 1 (Code: 00068357)
General comments: This manuscript is a well-written article with some clinic-epidemiological significance in the Indian population.

Author's response: We are thankful for reviewer's appreciation and understanding the clinic-epidemiological significance of the study.

1. What is the threshold set up for the HP infection?

Author's response: We are thankful to the reviewer's comment on our assay description. We have now incorporated the description of PCR sensitivity and assay threshold in Methods section at page 6, para 3rd, line 6 as follows:

"PCR assays based on *ureC* and *cagA* pre-standardized primer could detect at least 3.6 fg of bacterial DNA which correspond to approximately two *H. pylori* genomes [28].

2. What is the exact definition of the tobacco chewing habit, once a day or some other?

Author's response: We are thankful to the reviewer's valuable comment. We have now included the description of tobacco chewing habits daily (at least one cigarette or bidi/tobacco chew/alcohol shot daily) and regularly (at least 3 days/week) and the manuscript text have been revised accordingly at page 5, para 2nd, line 17

"Any person having these habits at the time of examination or used tobacco or alcohol daily (at least one cigarette or bidi/tobacco chew/alcohol shot daily) and regularly (at least 3 days/week) for 6 months or more in the past were grouped as tobacco user or alcoholic."

3. When doing the statistical analysis, were the age and sex bias balanced?

Author's response: We are thankful to the reviewer for raising this issue.

We used two-way crosstab analysis (for Fischer's Exact Test, χ2 test) to analyze the data by SPSS Statistics Software version 20 and the age and sex bias were monitored. We also performed Multivariate linear regression analysis with age and gender stratification and and these factors were adjusted while doing the statistics. The manuscript has been revised accordingly to include these details both in Methods and Results section.

Methods (page 7, para 2, line 4)

"Multivariate linear regression analysis was performed with age and gender stratification and the age gender bias was adjusted while doing the statistics."

Results (page 8, para 2, line 4)

"To understand the effect of confounding factors on the final outcome, the age and gender stratification was performed and adjusted while doing the statistics. We also performed the multivariate linear regression analysis, which again showed that these two factors did not play any significant role in the final outcome (data not shown)."

4. I observed that in the normal tissues, Hp antigen expressions were particularly higher in female than in male, but in the tumor tissues the difference are not that obvious, how do you explain that phenomenon? What is the condition that women are more likely to chew tobacco in India?

Author's response: We are thankful to the reviewer for the observation. However, we would like to clarify that the present study is Hp DNA-based, which evaluate presence of Hp DNA only but not the antigen expression. Hp DNA positivity was predominantly high in tissues of histopathologically normal females but not in case of tumor tissues. As further explained in the revised manuscript, this phenomenon could be linked with tobacco chewing. Recent epidemiological survey demonstrated that women significantly (p<0.00001) preferred smokeless/chewable tobacco as

compared to smoking. The information has been incorporated in the revised text at Page 12, para 1, line 3 as follows:

"The high prevalence of *H. pylori* infection may be due to poor household hygiene in rural areas and addiction to tobacco habit, ==particularly the tobacco chewing in females which is quite frequent in north India [11,46]. According to population-based epidemiological study, women significantly (p<0.00001) preferred smokeless/chewable tobacco as compared to smoking [11]. Moreover, it has been generally observed that females from rural areas usually do not come to referral hospitals till they have significant symptoms whereas males report even if symptoms are milder. It is quite likely that this phenomenon might have resulted in higher percentage of females who came for endoscopy had *H. pylori* infection than males in control and pre-cancerous groups whereas in cancer cases where both genders had equally severe symptoms such a bias was not there.=="

Response to Reviewer 2 (Code: 0004678)
General comments: Well written study. Suitable for priority publication
Author's response: We are thankful for reviewer's appreciation and understanding the value of the present investigation.

1. Transmission of Helicobacter infection by contaminated tobacco: it is known that onset of Helicobacter infection starts in early childhood…. Small children don't chew tobacco. So please explain your conclusion bit more in detail or delete.
    Author's response: We are thankful to the reviewer for the interesting and important and highly relevant comment. As already indicated in discussion page 9, para 2, line 9-14 "the high prevalence of *H. pylori*………in adult population", In addition, we have tried to address this issue in more detail and accordingly reframe the manuscript text at page 13, para 1 which now reads as:

    ==**"Although the onset of *H. pylori* infection starts in early childhood, our study group comprised subject above 17 years (except two cases of 7 and 13 years with no tobacco use in any form). Therefore, *H. pylori* positive subjects in the present study might have got prior exposure of *H. pylori* infection in early childhood, which could either be by tobacco use or by other means. A significant association between tobacco chewing and *H. pylori* in cancer group but not in control and pre-cancer group further shows that *H. pylori* and tobacco might act as a co-carcinogen. It is likely that toxic effect of tobacco may further promote cagA+ *H. pylori* -mediated carcinogenic transformation in gastric epithelial cells over a long time period. A large cross-sectional epidemiological study in school children demonstrated that 11.2% of schools going children were addicted to tobacco in any form. Interestingly, 2.5% of the study subjects were "exclusive tobacco chewers" and the mean age of initiation of these habits in school children was found to be around 12.4 years [52]. Moreover, the chances of concurrent *H. pylori* infection with contaminated tobacco preparations usually prepared on palm by rubbing the tobacco cannot be ruled out."**==

2. I would like to know about the frequencies of antrum-predominant, pan-gastritis and corpus-predominant gastritis as well as post eradication Helicobacter gastritis, frequency of autoimmune gastritis and chemical reactive gastritis and as well % of active Helicobacter gastritis?

Author's response: We sincerely appreciate reviewer's concern regarding prevalence of *H. pylori* infection in gastritis. However, inclusion of gastritis data would have lead to loss of focus from our main aim of the study. Moreover, frequency of general *H. pylori* prevalence in different types of gastric ailments like gastritis, duodenal ulcers and gastric ulcers [18-21] in Indian context is already well documented. Present study was planned and executed primarily to understand prevalence of *cagA* gene positive *H. pylori* infection particularly in pre-malignant and malignant tissues and its correlation with tobacco consumption is currently lacking. As already mentioned in Discussion at page 9, para2, line 8-9, however, to emphasize on this point and for clarity of the readers on this point, we have modified the text in Introduction at page 4, para 2, line 13 as indicated below.

"Despite disparity in high rates of *H. pylori* infection and low incidence of gastric carcinogenesis in India[2, 18] and reports of *H. pylori* prevalence in different types of gastric ailments primarily focus on non-malignant conditions like gastritis, duodenal ulcers and gastric ulcers [19-22] in Indian context, there is a strong need of a comprehensive analysis of the prevalence of *H. pylori* infection and carcinogenic *cagA* positivity in gastric pre-cancer and cancer lesions along with its correlation with the habits of tobacco and alcohol use that are considered potential risk factors.

Response to editor for manuscript formatting (Editorial/ Administrator comment on manuscript formatting)

Admin comment 1: I'm sorry "Supplementary Data" could not be attached as an appendix to the back of the body text, it does not meet our publishing requirements, I hope you can understand.

Authors Response: Supplementary table has been removed from appendix and added to main body text as Table 2.

Admin comment 2: Please provide language certificate letter by professional English language editing companies (Classification of manuscript language quality evaluation is B).

Authors Response: English language is primary official language of our institute, which is governed by the Department of Health Research, Government of India. Therefor submission of language editing certificate by professional English language editing companies may be waived/exempted in present case. To improve the linguistic quality of the manuscript, we have once again reviewed the text and corrected the errors and typos.

Admin comment 3: Please provide the author contributions. Authors must indicate their specific

contributions to the published work. This information will be published as a footnote to the paper. See the format in the attachment file-revision policies.

Authors Response: Author's contribution has been added at page 1 last para to manuscript as suggested.

Admin comment 4: An informative, structured abstracts of no less than 200 words should accompany each manuscript. Abstracts for original contributions should be structured into the following sections. AIM (no more than 20 words): Only the purpose should be included. Please write the aim as the form of "To investigate/study/…; METHODS (no less than 80 words); RESULTS (no less than 120 words): You should present P value where necessary and must provide relevant data to illustrate how it is obtained, e.g. 6.92 ± 3.86 vs 3.61 ± 1.67, P < 0.001; CONCLUSION (no more than 26 words, in a definite, conclusive, and short statement, not indefinite, vague, or suggestive sentences).

Authors Response: Structured Abstract has been added at page 2 to manuscript as suggested.

Admin comment 4: Core tip: Please write a summary of less than 100 words to outline the most innovative and important arguments and core contents in your paper to attract readers.

Authors Response: Core tip has been added at page 2 to manuscript as suggested by editor.

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

Sincerely yours,

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