Response to the reviewers,

Please find enclosed the edited manuscript in Word format (file name: **29627**-revised version.doc).

Title: Role of *dupA* in virulence of *Helicobacter pylori*

Authors: Amin Talebi Bezmin Abadi*, Guillermo Perez-Perez

Name of Journal: *World Journal of Gastroenterology*
ESPS Manuscript NO: 29627

The manuscript has been improved according to the suggestions of reviewers.

Reviewer *03010025*:

1- This review by Abadi and his colleague summarized the role of dupA, an H pylori virulence factor, in disease process. This paper showed the importance of having more research investigating the role of H pylori virulence factor and their roles in disease pathogenesis. Because it is a review paper, there are some missing references that might help increasing the strength of the discussion. Therefore, I have the following comments Virulence of H pylori
2- The authors stated that the definition of a virulence factors is referring to the ability of a bacterium on the severity of disease The definition needs more clarifications; I would reword it with a clearer definition. Also, as long as virulence factors are mentioned, cagA and vacA must be mentioned, only one paragraph for each. There is a good review article by JC Atherton (Annu Rev Pathol. 2006; 1; 63-96) that might be helpful.

3- dupA The authors stated that Therefore, among most of Asian countries, a significant association between disease development and dupA status can be observed Some studies are missing and the author should include them in his review including Prevalence of Helicobacter pylori cagA, babA2, and dupA genotypes and correlation with clinical outcome in Malaysian patients with dyspepsia Osman et al published in Turkish journal of Med sci A study by Hussein et al comparing Iraqi samples and Iranian samples is missing here (Journal of clinical microbiology 46 (5), 1774-1779). Helicobacter pylori virulence genes in the five largest islands of Indonesia by Miftahussurur et al published in Gut pathogen

4- The authors stated that Antral induction of IL-8 production is a main character of dupA pathogenesis causing predominant gastritis The author should elaborate more about the role of dupA in IL8-secretion. One helpful paper is published by Hussein et al Infection and immunity 80 (8), 2971-2972

5- Conclusion remarks The authors stated that Interestingly, the presence of dupA was significantly associated with H. pylori eradication failure with no biologic explanation

The association of dupA and clarithromycin resistance was studied before by Hussein et al (New microbes and new infections 6, 5-10). This should be involved and commented on.

Authors' response:

Author response #1:

In agreement with reviewer s suggestion, we have now added text about definition of virulence factor to make it clear in the revised manuscript.
Strains possessing these virulence factors are isolated more frequently from patients with the more serious clinical manifestations. It is logic to observe the increase in the chance of survival within harsh gastric condition, furthermore, it induce more cell damage which infiltrate immune cells to the location and thus inflammation will be the high priority event in epithelial cells. Due to the chronic aspects of the *H. pylori* infection, scientists can expect to observe particular definition of virulence factor in this bacterium.

Author response #2:

In agreement with reviewer s comment; we added two paragraphs (vacA and cagA) in the paper as follow.

CagA

cagA is located at the end of the cag pathogenicity island (PAI), which is a 39kbp region transferred horizontally from an unknown bacterial source. The "pathogenicity islands" include cytotoxin-associated gene A (*cagA*) encode proteins contributing in signal transduction cascades that result in cytoskeletal rearrangement via actin polymerization and host cell protein phosphorylation. Virulent strains of *H. pylori* possess the cagPAI. Many of *H. pylori* strains from patients with peptic ulcer or gastric cancer carry *cagA*, whereas many of those strains from asymptomatically infected persons lack this gene. Currently, we know two major types of *H. pylori* isolates: cagA gene-negative and cagA gene- positive strains. Counting a virulence factor for *cagA* needs another classification which is based on polymorphism in EPIYA motifs. In *cagA* positive strains, there is a region contains the Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs, which contains a tyrosine phosphorylation site. Briefly, two major types (Western and Eastern *cagA*) were determined according to this polymorphism. Though, we need more biologic rationale in consistent with clinical evidences to present better information on how to interoperate this classic virulence factor in *H. pylori*.

VacA

To now, VacA is the second most extensively investigated virulence factor of *H. pylori*. Virtually all *H. pylori* strains have a functional vacA gene which codes for the secreted pore-forming protein VacA. The main difference in carrying bacteria is in expression levels and disease severity which are associated with sequence variation in different domains of secreted protein. There are a big gap on our knowledge regarding biologic function of this protein since still many contradictory findings are exist. So we need more investigation to determine how to count on *vacA* as useful *H. pylori* virulence factor.

Author response #3:

Because of the reviewer's request, we have now added a reference as mentioned by the reviewer. However, other requested references were inserted in revised manuscript.

Author response #4:

In agreement with reviewer, we used this paper and now more elaborated text are available in revised manuscript as in below:

It has been extensively reported that there is an association between increased expression levels of IL-8 and *dupA* in the gastric mucosa of *H. pylori*-colonized individuals. As expected, many reports are indicating on gastric mucosal inflammatory cell infiltration was significantly higher in patients with *dupA*-positive *H. pylori* than in patients with *dupA*-negative strain.

Author response #5:

With thanks to reviewer, we inserted mentioned reference in the text.

Reviewer ***00039368***:

Authors' response:

With thanks to the reviewer, we asked English native speaker to reread the paper once again before re-submission.

Reviewer *00053556*:

Comments to the Editor: Thanks for inviting me to review the review article entitled" Role of dupA in virulence of Helicobacter pylori ". Minor Comment:

1- Minor editing revision is required. ? Language level: B. Revision is needed in term of grammar and structure.

2- TITLE Reflect the major content of the article. 2. ABSTRACT partially fulfill the journal requirements. Conclusion is missing and is better to be added. 3. TEXT: The section is almost well organized and the overall theoretical analysis concerning the provided topic is nearly fulfilled, as, following points are better to be considered: o When describing H. pylori and in order to satisfy the reader, more details are better to be added regarding plasticity region.
3- The same also goes for H. pylori different virulence factors. o “Lu et al” was mentioned within the text with different reference number (19, 10, and 43), actually, it was reference number (43) in reference section and this has to be revised and corrected to be in its proper site.
4- Role of DupA in IL-8 production is not clearly identified.
5- Final remark: ? No: 1 has to be stated at the end of the article as a recommendation, while No: 2&3 have to be included within the text under the subheading: “dupA”. Conclusion within final remark No: 3 has to be added to the subheading “Conclusion remarks”. ? Final remark No: 2: Needs more clarification to identify dupA cluster. Also, T4SS: has to be fully written when mentioned for the first time (type IV secretory system). ?
6- Final remark No: 3: The work of the cited reference has to be clearly identified. Its resuls were missing and have to be mentioned. o
7- Conclusion remarks: This section is better to be revised; no new data has to be mentioned. The part of this paragraph with cited references (34-36, 42, 49, 50- 52)and even the last sentence, are better to be put within the text under the subheading: “dupA”. 3. References: Finally relevant adequate references, especially the most current literatures are cited, however, this section needs major revision as the following points have to be considered: ?
8- Numbering of references was not comparable to that mentioned within the text. This has to be carefully revised. e.g.: Ref. No:43:Lu et al, it was ref. No: 19, 10 & 43 within the text. ?
9- Reference No: 1 has to be completed. It is missing the volume and page numbers. ? Ref. No:46 is not Yamaoka et al as it was mentioned in final remarks No: 3 ? Some cited references were repeated: e.g.: Ref (20) is the same as Ref. (49) and ref. 26 is the same as ref. (41). ? PMID is not maintained for all references.

Authors' response:

Comment #1:

In agreement with the reviewer, we asked an English native speaker to reread the paper once again before resubmission.

Comment #2:

Thanks for comment by the reviewer, done.

Comment #3:

In agreement with the reviewer, we inserted new text for all topics. Thanks for this comments as well. Regarding the references, we adjust the reference list once again and corrected the mistakes.

Comment #4:

With thanks to reviewer; we described the case in detailed in revised manuscript.

Comment #5:

Due to the reviewer s comment, we corrected all mistakes and revised manuscript improved a lot.

Comment #6:

With thanks to reviewer; we corrected all references in revised manuscript.

Comment #7:

In agreement with reviewer, we already rewrite the text.

Comment #8:

In agreement with reviewer, we already corrected the text in revised manuscript.

Comment #9:

In agreement with reviewer, we checked all reference numbers and position in the manuscript in the body text.

Reviewer #: 03474938

The report is a review of gene dupA which belongs to the zone of plasticity of the genome of Helicobacter pylori.

1- Although it described about of this gene and its role in virulence, the review does not address epidemiological aspects of their frequency in child population compared with adult population, I dont know if whether there is enough information or simply mentioning that no information exist.
2- So too the fact belong to the set of genes that make up the area of plasticity is not mentioned if there is information about the association DupA with other virulence genes or with other genes of the plasticity zone itself. So I suggest to include more information in relation to: Prevalence in children and adults, association DupA presence of other virulence genes or with genes plasticity zone. For example Romo-Gonzalez et al.,2015. They conducted a study on the presence of genes of the plasticity zone H. pylori strains from children and also show the prevalence of dup A in this population.

Authors' response:

Comment #1:

In agreement with reviewer, we would have this comparison but there is no data on it. However, investigating this interesting population will be interesting.

Comment #2:

In revised manuscript, we incorporated many text as reviewer requested to describe the dupA story more in detailed.

Sincerely yours,
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