Author’s response to reviews

Title: Helicobacter pylori vacA, cagA and iceA genotypes in dyspeptic patients from the southwestern region of Saudi Arabia: Distribution and association with clinical outcomes and histopathological changes

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Author’s response to reviews:

Dear respected Editor

BMC Gastroenterology

We would like to resubmit our revised manuscript (# BMGE-D-18-00409R1 edited). We would like to thank you and the reviewers for the insightful and helpful comments. We believe these suggested changes significantly enhanced the quality of our manuscript.

The study team

Reply to the editor and reviewers’ comments (point-to-point response). All changes to the manuscript are indicated in the text by red font and yellow highlighting.
Response to editor’s comments

Comment 1:
Please move the list of abbreviations to after the Conclusions section. In addition, please move the Declarations section to after the list of abbreviations.

Response:
The list of abbreviations was moved to after the Conclusions section, and the Declarations section was moved to after the list of abbreviations.

(See List of abbreviations and Declarations sections in the revised manuscript, lines 331-349, 351-379, pages 13 and 14, revised manuscript).

Comment 2:
Please reorder the subsections of the Declarations as outlined in Submission Guidelines (https://bmcgastroenterol.biomedcentral.com/submission-guidelines/preparing-your-manuscript/research-article#declarations).

Response:
Subsections of declarations were reordered as outlined in Submission Guidelines. (See declarations subsections, pages 13 and 14, revised manuscript).

Comment 3:
Under the heading, “Ethics approval and consent to participate” in the Declarations, please remove the sentence starting “Dr Ibrahim Elhassan is the head…”.

Response:
Under the heading, “Ethics approval and consent to participate” in the Declarations, the sentence starting “Dr Ibrahim Elhassan is the head…” was removed

(See “Ethics approval and consent to participate” section in the revised manuscript, lines 352-355, page 13, revised manuscript).
Response on reviewer 1

Comment 1:
The title is too long and descriptive. I would prefer to see more informative one. Generally, it is not acceptable to say everything in the title for a cross sectional paper.

Response:
The title was shortened to be more informative.

(See title in the revised manuscript, lines 1-3, page 1, revised manuscript).

Comment 2:
In background section of abstract: the actual aims stated by the authors are in a line, why they divided these same goals into two different ones? Please correct it.

Response:
The aim of the study was corrected as recommended.

(See the background section of abstract, lines 35-37, page 2, revised manuscript).

Comment 3:
The result section for abstract should be reduced in word count. I see some unnecessary parts in it, so please delete them all.

Response:
The result section for abstract was reduced in word count as recommended.

(See the result section of abstract, lines 44-51, page 2, revised manuscript).

Comment 4:
The word H. pylori in many of sentences are not italic, please address this correction all over the manuscript.

Response:
The word H. pylori were written italic all over the manuscript.
(See the word H. pylori all over the revised manuscript).

Comment 5:

Basically, if the authors want to talk about the virulence genes, they should have think about many of proposed virulence genes for HP. For example, dupA, babA and homB are the minimum candidates which deserve more investigation by the researchers before they can conclude about virulence determinants of HP. So I would see the rationale for such selection by the authors or performing this survey as I mentioned in revised manuscript afterwards.

Response:

We completely agree on this and convinced with it as dupA, babA and homB are too important to screen in studying H. pylori virulence, but because our current study is the first largest and sophisticated one which studied H. pylori virulence in our region in KSA (Southwestern region) and our aim in this study was to study virulence by determining the genotype patterns and relating them to clinical outcomes and histopathological findings in dyspeptic patients in the region, we began with screening for the most basic virulence genes (cagA, vacA, and icA1, 2 in full details as we screened cagA and cagA empty site, all subtypes of s and m regions of vacA including: s1a, s1b, s1c, m1,m2 and icaA1 and iceA2) to give us a reasonable view on status of H. pylori virulence, to take it as the baseline (1st phase) study, as we intend to extend our work in future in another study searching for more important and diverse virulence genes including dupA, babA and homB, sabA and oipA to study in depth more associations with the clinical outcomes to gain a benefit for effective eradication of infection caused by these bacteria in our region.

Comment 6:

The page 3- line 65, add the word "bacterial" before the carcinogen.

Response:

The word "bacterial" was added before the carcinogen. (See line 66, page 3, revised manuscript)

Comment 7:

The page 3, line 73, whole line needs a reference, and without the reference this section should be removed. Mainly from the region authors work. For example, these references are suggestive:

a- High correlation of babA 2-positive strains of Helicobacter pylori with the presence of gastric cancer
b-Prevalence of Helicobacter pylori vacA, cagA, cagE, iceA, babA2, and oipA genotypes in patients with upper gastrointestinal diseases

c-High frequency of vacA s1m2 genotypes among Helicobacter pylori isolates from patients with gastroduodenal disorders in Kermanshah, Iran

d-Clinical relevance of the cagA, tnpA and tnpB genes in Helicobacter pylori

Response:
2 References were added from those recommended above. They are numbered 8 and 9 in the text and in reference section in revised manuscript.

(See the background section (lines 76-77, page 3) and reference section (lines 414-419, page 15), revised manuscript).

Comment 8:
Whole page-4 needs rewrite in English.
Response:
The whole manuscript had undergone English Language editing. "English Language Editing certificate is enclosed".

Comment 9:
All genes should be italic, it is large number of genes which wrote not-italic through the paper.
Response:
All genes were written in italic (see all genes through the revised manuscript).

Comment 10:
Of how many biopsies, the authors could take 128 positive biopsy specimens which hare all positive for HP? This should be clarified in the M&M. This is very important since I think this paper designed as cross sectional study but something are lacking in my opinion. However, the
other criteria for a cross-sectional study is not met and I think that they selected manually those 128 samples! This is a major query should be replied in detailed by the authors before final decision.

Response:

Gastric biopsies were collected from 404 Saudi dyspeptic patients attending gastroenterology clinics in Jazan region during the study period. There were non-sufficient biopsy materials in two cases, and from all the remaining 402 cases DNA was extracted and submitted to real-time PCR (RT-PCR) shown in M&Ms to detect H. pylori. Overall, RT-PCR was positive in 187 specimens. All of these 187 DNA positive samples were surveyed for cagA, vacA and iceA virulence genes by using genotyping PCRs shown in M&M section. The genotyping PCR reactions were positive only in 128 specimens.

This part was clarified in M&M section in the revised manuscript (See lines 103-104, 109-110, 114-115, 126-127, page 5, revised manuscript). Also, see results section: lines 158-159, page 7, revised manuscript.

Also, according to this, we modified the sections of methods and results in the abstract. (See lines 40-42, 44, page 2, revised manuscript).

Comment 11:

Page 7 is a bit elongated text, please reduce it.

Response:

The text in page 7 was reduced as we removed the following parts:

Cases that were positive for both genes were considered as mixed or multiple infections by different H. pylori strains.

Presence of both iceA1 and iceA2 was considered as a mixed infection with H. pylori of different genotypes and 21.1% (27/128) were negative for iceA gene s2m2 subtype was found in 14.8% (19/128) of tested H. pylori. The least genotypes were s1am1, s1bm1, s1a1bm1 and m1 (0.8% (1/128) for each). Mixed genotypes were found in 5/128 (3.9%) of tested specimens while in cagA negative subtypes the most prevalent vacA alleles were s2m2 and m2 (29% for each), and two of the cagA mixed three cases had were s1as1bm2 and the remaining one was mixed vacA genotype.
(See the modified page 7, revised manuscript).

Comment 12:

PAGE 4 Line 108-109, should be deleted! Since it is obvious statement and not necessary for a scientific paper

Response:

Lines 108 -109 were deleted.

(See modified “Patients and clinical specimens” part under Methods section, page 5, revised manuscript).

Comment 13:

Page 4, what was the identity of the positive control applied in the study?

Response:

The positive control applied in the study is a H. pylori positive control template DNA containing the RNA polymerase beta-subunit (rpoB) gene of H. pylori. It is supplied with the kit [“genesig Quantification of Helicobacter pylori” kit (PrimerDesign Ltd. Southampton, United Kingdom) which is primer-probe based and targets the RNA polymerase beta-subunit (rpoB) gene of H. pylori].

Comment 14:

I think that the paper can be considered as short communication rather a original paper. However, it can be an original paper only after making new experiments such as determining the new virulence factors as I suggested in above.

Response:

Thanks Sir for your opinion, however we think our manuscript is more related to an original article for the following:
1. We are reporting an original research including hypothesis, background study, methods, results, interpretation of findings, and a discussion of possible implications.

2. A Short Communication may focus on a particular aspect of a problem or a new finding that is expected to have a significant impact. It may include, discovery or development of new materials, cutting-edge experiments and theory, novelty in simulation and modeling, elucidation of mechanisms., etc

3. This is the first study conducted in our region and we exerted much effort on this first study, so as to enrich the information on H. pylori in South of Saudi Arabia.

So, we think our research is more related to original research than short communication. Publication of this study results as original article will help practitioners and researchers, especially in our region.

Comment 15:

The first sentence in discussion should be removed since it is not true! If they insist on their claim, we need to see new references confirming their idea and statistics

Response:

The first sentence in discussion was removed and the whole discussion section was rewritten, modified and updated.

(See Discussion section, pages 10-12, revised manuscript).

Comment 16:

Page 10-line 232, this sentence is not clear to me! I know what they meant but I need to read it once and get the meaning rapidly not think 20 minutes!

Response:

It was corrected. (See line 232, page 10, revised manuscript).
Comment 17:

The literature review made by the authors to excuse the result in discussion part is not enough.

Response:

The literature review in discussion section was updated. Our data is compared with more recent studies. More references were added. Kindly see lines 230-309, pages 10-12, revised manuscript. Also, see references 8,9,35,36,37,38, in the text and in references section (lines 414-419, page 15, lines 512-526, page 18), revised manuscript.

Response on reviewer 2

Comment 1:

The title of the manuscript is very extensive, you can write more accurately and directly.

Response:

The title was shortened to be more informative.

(See title in the revised manuscript, lines 1-3, page 1, revised manuscript).

Comment 2. In the background section of abstract you can add a sentence about the justification or reason for your study.

Response:

A sentence about the reason for your study was added.

(See lines 37-38, page 2, revised manuscript).

Comment 3:

What was the reason for using the chi-square test in all the statistical analysis? It is important to use the exact Fisher test when the chi-square test is invalid.
Response:

Correct, we used Fisher Exact test (when appropriate), so our results were based on Chi-square and Fisher exact test, we edited the statistical analysis section accordingly.

(See line 155, page 6, revised manuscript).

Comment 4:

It is necessary to graph the results of Figure 3 in other programs such as originlab, spss, etc.

Response:

SPSS was used to graph the results of Figure 3, as you suggested.

(See Figure 3, revised manuscript)

Comment 5:

In the discussion section it is necessary to add the most recent studies of Helicobacter pylori prevalence and genotypes in Latin American countries and compare them with your results. In addition, it is necessary to provide a possible explanation about the coincidence or discrepancy of your results with those.

Response:

The discussion section was rewritten, modified and updated. Our results were compared with more studies from different countries Including Brazil, Egypt, Mexico, Iran. Please see lines 230-309, pages 10-12, revised manuscript. Also, kindly see references: 8, 9, 35, 36, 37, 38 in the text and in the bibliography (lines 414-419, page 15, lines 512-526, page 18), revised manuscript.