OPEN PEER REVIEW

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Peer Review Report for "Evaluation of *miR-21* Expression Level in *Helicobacter pylori*-Infected Gastric Mucosa"

Author(s): Mona Noohi, Mojdeh Hakemi-Vala, Jamileh Nowroozi, Seyed Reza Fatemi , Mehrouz Dezfulian

Review Timeline:

Submit Date: 5 May 2020
Revised Date: 9 Aug 2020
Accepted Date: 30 Aug 2020

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

Farhad Safarpoor Dehkordi: Reviewer | Revision (0) 28 Jun 2020

Dear AE,
Well, this is an original research work about the Evaluation of miR-21 expression level during Helicobacter pylori-infected gastric mucosa. The findings of this paper revealed that miR-21 expression was significantly up-regulated in infected samples with H. pylori compared to healthy samples. Therefore, our results confirmed that H. pylori infection can alter the expression of various miR-21 in gastric epithelial cells and in gastric mucosal tissues. The exact role of the miRNA changes in H. pylori infection will require further experiments.
However, this is an interesting work, but authors should address the following comments before its full acceptance.
1- Text is full of grammatical and typographical mistakes. Please use from a native English speaker person who is an expert in microbiology to revise your text.
2- First of all, the abstract contained mostly the introduction of the topic. Please mention the introduction, methods, results, and discussion in the same way and equal to each other.
3- Keywords used by authors didn't have a relation with the topic and purpose of the study. Please use it from more related keywords.
4- Authors mentioned: Endoscopy was performed by Gastroenterologists and five biopsy

samples (4 from gastritis part and 1 from adjacent healthy part)… In my opinion, the adjacent healthy parts of patients who were H. pylori-positive is not a suitable sample as a control. In my opinion, authors should use the biopsy samples of a negative patient as a control of their study. Please add your explanation about this matter.

5- Please add the figures for the histopathological examination.

6- Please add the properties of the media and devices used in the study. I mean brands, city, and country of manufacture.

7- Please add the concentrations of antibiotics used for the specific growth of H. pylori by their related references.

8- Why the names of media and antibiotics written in Italic?!!

9- oxidase, catalase, and Gram staining tests are not sufficient for the identification of H. pylori. Please add the full biochemical tests used for the identification of bacteria with related references.

10- There were some concerns in the contents of the paper:

- Authors used from culture for isolation and identification of bacteria from gastric biopsies. Then, the authors used the PCR-based identification of the cagA gene to detect the invasive H. pylori in the gastric samples. So, what was the basic method for their study? Which of the method used for data analysis?
- CagA positive strains cannot be surely considered as invasive strains.
- Authors mentioned: H. pylori-positive samples were amplified with specific primers of cagA gene (Interactive, England) in order to determine the invasive form of H.pylori….. How did the author detect the invasive strains by PCR as they didn't extract DNA samples from the bacteria?! In fact, authors should extract DNA from culture-positive bacteria and then detect the cagA gene.
- Why the authors didn't consider antibiotic therapy in the inclusion or exclusion criteria?
- I cannot find any statistical analysis of data in the result section.
- Please add the full history of patients.
- Please add the incidence of bacteria according to sex, age, and even type of burn wounds of patients. This is because of the data of this study are low and authors should increase the scientific base of their article using more data.
- How the quality and quantity of DNA were measured?
- How the purity of extracted DNA was measured?
- Some parts of the text need additional gaps and some parts of the text need additional space between letters and words of the text.
- Please add the figures for PCR gel electrophoresis.
- How the quality and quantity of RNA were measured?
- How the quality and quantity of cDNA were measured?

11- Authors should read all parts of the text another time and revise the text and their main goals. Some parts of the text are inconsistent with others.

12- In my opinion, cagA is not a good candidate for the detection of invasive H. pylori as there were so many related genes such as vacA. What is the reference of authors for this statement?

13- All text should check another time by me after the revision.

Thanks.

Farhad Safarpoor Dehkordi: Associate Editor | **Revision (0)** 28 Jun 2020

Dear EIC,

Well, this is an original research work about the Evaluation of miR-21 expression level during Helicobacter pylori-infected Gastric mucosa. The findings of this paper revealed that miR-21 expression was significantly up-regulated in infected samples with H. pylori compared to healthy samples. Therefore, our results confirmed that H. pylori infection can alter the expression of various miR-21 in gastric epithelial cells and in gastric mucosal tissues. The exact role of the miRNA changes in H. pylori infection will require further experiments.

However, this is an interesting work, but authors should address the following comments before its full acceptance.

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4- Authors mentioned: Endoscopy was performed by Gastroenterologists and five biopsy samples (4 from gastritis part and 1 from adjacent healthy part)… In my opinion, the adjacent healthy parts of patients who were H. pylori-positive is not a suitable sample as a control. In my opinion, authors should use from the biopsy samples of a negative patients as a control of their study. Please add your explanation about this matter.

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increase the scientific base of their article using more data.

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- Please add the figures of PCR gel electrophoresis.
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- How the quality and quantity of cDNA were measured?

11- Authors should read all parts of the text another time and revise the text and their main goals. Some parts of the text are inconsistent with others.

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13- All text should check another time by me after the revision.

14- Authors can complete their text using the following references:

Ranjbar R, Yadollahi Farsani F, Safarpoor Dehkordi F. Phenotypic analysis of antibiotic resistance and genotypic study of the vacA, cagA, iceA, oipAand babA genotypes of the Helicobacter pyloristrains isolated from raw milk. Antimicrobial Resistance and Infection Control. 2018;7:115.

Zohreh Mashak, Sedigheh Jafariaskari, Iman Alavi, Mohammad Hossein Sakhaei Shahreza, Farhad Safarpoor Dehkordi. Phenotypic and Genotypic Assessment of Antibiotic Resistance and Genotyping of vacA, cagA, iceA, oipA, cagE, and babA2 Alleles of Helicobacter pylori Bacteria Isolated from Raw Meat. Infection and Drug Resistance. 2020:13 257–272.

Ghorbani F, Gheisari E, Dehkordi FS. Genotyping of vacA alleles of Helicobacter pylori strains recovered from some Iranian food items. Trop J Pharm Res. 2016; 15;1631-1636.

Mousavi, S., Dehkordi, F.S., & Rahimi, E. Virulence factors and antibiotic resistance of Helicobacter pylori isolated from raw milk and unpasteurized dairy products in Iran. Journal of Venomous Animals and Toxins including Tropical Diseases, 20, 51.

Ranjbar R, Yadollahi Farsani F, Safarpoor Dehkordi F. Antimicrobial resistance and genotyping of vacA, cagA, and iceA alleles of the Helicobacter pylori strains isolated from traditional dairy products. Journal of Food Safety. 2018.

Yahaghi, E., Khamesipour, F., Mashayekhi, F., Safarpoor Dehkordi, F., Sakhaei, M.H., Masoudimanesh, M., & Khayyat Khameneie, M. (2014). Helicobacter pylori in vegetables and salads: genotyping and antimicrobial resistance properties. Biomed Research International, 2014, 1-11

Atapoor, S., Dehkordi, F.S., Rahimi, E. (2014). Detection of Helicobacter pylori in various types of vegetables and salads. Jundishapur Journal of Microbiology, 7, e10013.

Thanks.

REFEREE: EIC | **Revision (0)** 11 Jul 2020

Dear Author,

Thank you for submitting your manuscript to JJM. I have completed the review of your manuscript and a summary is appended below (Pleases find attached files). The reviewers recommend the reconsideration of your paper following major revision. I invite you to

resubmit your manuscript after addressing all reviewer comments. Please answer point by point to reviewers' comments on a separate sheet under WORD format and write your modifications in the revised version (WORD format) in colored or highlighted letters. Your answers to reviewers’ queries are not only for the Editorial Board but for the readers and should be included, when appropriate, in the manuscript for improvement and a better understanding.

When resubmitting your manuscript, please carefully consider all issues mentioned in the reviewers' comments, outline every change made point by point, and provide suitable rebuttals for any comments not addressed. In addition, JJM INSTRUCTION attached to the mail, Pleases find it.

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Thanks.

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Dear editor in chief if Jundishapur J of Microbiology

At first, on behalf of other authors and as corresponding author like to say our thanks for the review valuable comments. In the following please find our reply to them.

Paper ID: 100724

Q1.The recent paper was edited by a native editor and a certificate was attached.

Q2.The abstract is corrected and included all items introduction, methods, results and discussion.

Q3. In the key words ***quantitative real-time PCR*** is revised and the remained *H. pylori*, mir-21, gastritis are selected based on the subject of this paper from the MeSH.

Q4. However, matched case (patients) and control (healthy people) is a favorite condition, but because of invasive ness of endoscopy and the nature of this study as a pilot study, we selected an adjacent healthy part of stomach from each patient as a control. Doing such design has done in different studies before, like in the Elliot et al., study, Lancet Gastroenterol Hepatol. 2017 Jan; 2(1): 32–42, they have done similar to us and mentioned in the study design that" In patients with a diagnosis of oesophageal adenocarcinoma, tissue samples from the tumor and matched normal squamous oesophagus were collected". We have collected 4 samples from gastritis and adjacent healthy part.

In addition to biopsy samples, blood collection was done before endoscopy. After, the results of the pathology and PCR, we divided patients to healthy (not infected by *H. pylori*) and non-healthy (H. *pylori* infected) too. However, these groups were not age and sex matched and only *H.pylori* infection was used as the division criteria. The comparison and the statistical analysis of miR-21 level in blood of these two groups was done and is mentioned in figure 6 too.

Q5. The histological figures were added.

Q6. The brand of media and some devices were added.

Q7. The concentration of antibiotics and antifungal which were added to the media was included and is highlighted in the article.

Q8.The name of media and antibiotics corrected from Italic to usual style.

Q9.The reference for the gram staining, oxidase and catalase tests for *H. pylori* identification were done based on the Baily and Scott's Diagnosis Microbiology and the reference is added.

Q10. It is mentioned in different parts and replied in different items too.

- I like to mention the bacteria identification was confirmed by pathology too. Based on the different references, the gold standard of *H. pylori* are culture and pathology that both were done here. Also, by molecular PCR method, it is confirmed triplicate.

- Using the molecular PCR is related to the fastidiousing nature of *H. pylori* growth and because of no microaerophilic incubator we have use gas pack which decrease the growth rate in the first culture and even the second passage. So, to not lose the infection, we have done DNA extraction on a biopsy from each patient and confirmed the bacterial existence by PCR test using special Cinnagen kit too. Also, in the paper "**Diagnosis of *Helicobacter pylori* Using Invasive and Noninvasive Approaches" by Dr. Talebi Bezminm, doi: 10.1155/2018/9064952,** the sensitivity of culture for *H. pylori* determined 50-95% and their fastidious condition is mentioned as the major problem of the low rate of culture. Also, it is mentioned cutting a 3-4 mm^2 biopsy sample is not warranted existence of *H. pylori* and also, there is restriction in amount of biopsies during endoscopy too.
- Direct identification of *H. pylori* on biopsy samples and also, determination the existence of cagA and vacA genes was done in another studies such as one published in PLos One doi; 10.1371/journal.prone.0170616 Jan 26,2017. In similar we have done the existence of cagA gene directly on the DNA extracted from a biopsy of each patient. Detection of cagA gene not only was done on the confirmed bacterial colonies but also on the DNA extractions from the biopsy *H. pylori* positive by PCR kit (doi; 10.1371/journal.prone.0170616 Jan 26, 2017).
- Based on the valuable comment of the reviewer, the bacteria DNA extraction was done by boiling method which was forgotten to mention in the previous submitted version of the paper is added and highlighted.
-
- Based on the consultation by gastrologist all the patients with dyspeptic signs and were candidate to endoscopy were selected randomly and enrolled in this study. All confirmed a questioner form and some of the important items were mentioned in the table 1.
- The distribution of the sex and age are mentioned in the results.
- The quality and the purity of the extracted DNA is measured by OD detection at 260 and 280 nm and is mentioned in the article and is highlighted.
- The figures of PCR product after gel electrophoresis are added in the article.
- The quality and purity of the extracted RNA are detected as the same of the extracted DNA by using a namedrop instrument at 260 and 280 nm.

  Q11. The paper is revised based on the goals of this study.
- Q12. As it is mentioned in the most studies and as mentioned in the introduction too, among 60% of the invasive *H. pylori* isolated cagA gene is existed. Also. vacA gene is exist in all *H. pylori* isolates and its expression determine its role in invasiveness but existence of cagA gene is only detected in 60% invasive strains. So, based on the main goal of this article which was evaluation the role of miR-21 and not determination the situation of vacA and cagA genes as biomarker in *H. pylori* identification or no aim in genotyping *Helicobacter pylori* it is not persist on evaluation all the invasive genes in *H. pylori* and the most frequent invasive gene (cagA) gene was selected.

- At the final, we will be very grateful if the replies could be right to the valuable comments of the respect reviewer.

With best regards

Corresponding author

OPEN PEER REVIEW

Revision (1)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: EIC | **Revision (1)** 30 Aug 2020

Dear Authors
According to the associated editor report, your manuscript is accepted for publishing in JJM and send to the publisher for the next process.
Note:
Please add ORCID number into you and your collages account, then automatically appear in your published papers in JJM both in HTML and pdf.
Thanks.