

ANSWERING REVIEWERS



November 13, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 6810-review.doc).

Title: Can *Helicobacter pylori* infection influence human reproduction?

Authors: Moretti E, Figura N, Collodel G, Ponzetto A

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 6810

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

1 Revision has been made according to the suggestions of the reviewer

Reviewed by 00503963

It is really interesting article described the relation between H. pylori infection and reproduction. There are some suggestions raised by me, as followed: 1. How about the epidemiology in health population?

Answer. It is impossible to answer to this question in a few lines. The prevalence of infection is declining starting from the early '60ies and cagA + strain infections are declining even more rapidly: Perez-Perez GI, Salomaa A, Kosunen TU, Daverman B, Rautelin H, Aromaa A, Knekt P, Blaser MJ. Evidence that cagA(+) *Helicobacter pylori* strains are disappearing more rapidly than cagA(-) strains. *Gut*. 2002 Mar;50(3):295-8.

The ethnicity is important as shown by the results of the following study: Katsanos KH, Tatsioni A, Tsakiris V, Christodoulou D, Tsianos EV. *Helicobacter pylori* is a major public health priority in western Balkans: an endoscopy referral center experience. *Eur J Intern Med*. 2010 Aug;21(4):306-9.

These are only two examples. It is impossible to forecast the future situation, because it mostly depends on how intense will be the exodus of population of underdeveloped world (where are almost all infected) towards to the technologically developed world (where the infection is declining and concerns a minority of inhabitants).

The author described that almost everyone infected H. pylori with in Africa, but no epidemiological information in so called" Western world"?

Answer. This query is not very clear. We understood that the referee possibly wonders which are the prevalence of H. pylori infection in Africa and the outcome of disease. The prevalence of CagA positive H. pylori infection in Africa is high and resembles that one in certain Western countries; however, the consequences are less drastic, due to the concomitant infestation (that concerns most people), which does modify the cell response to the infection from predominantly Th1 to Th2 lymphocytes (Campbell DI, Warren BF, Thomas JE, Figura N, Telford JL, Sullivan PB. The African enigma: low prevalence of gastric atrophy, high prevalence of chronic inflammation in West African adults and children. *Helicobacter*. 2001 Dec;6(4):263-7.)

This point can help us to explain/understand the infertility difference between Western country and Africa.

2. The author mentioned that the anti-H. pylori (anti-CagA) is a possible mechanism to explain the H. pylori-mediated infertility in women and in men. Because of there are some antigenic mimicry, please described.

Answer: Franceschi et al. demonstrated the existence of an immune cross reactivity between CagA and beta-actin of trophoblast: anti-CagA antibodies recognized β -actin of cytotrophoblast cells, showing a dose-dependent binding. Incubation of cytotrophoblast cells with increasing doses of anti-CagA antibodies significantly reduced their invasiveness and determined a significant decrease in phosphorylated ERK expression and a reduced NF- κ B translocation activity. In addition, we showed a linear homology between CagA and beta-tubulin, the main constituent of sperm flagella (vide infra).

1. how much sequence (nucleotide or amino acid) homology in each case?

Answer. In order to find regions of similarity between amino acid sequences, we used the BLAST (Basic Local Alignment Search Tool) program in the protein databases at the National Center for Biotechnology Information (NCBI; Bethesda, MD, USA; www.ncbi.nlm.nih.gov). The program compares protein sequences to sequence databases and calculates the statistical significance of matches. Although the minimum number of amino acids able to form an antigen is five, we have included sequences longer than five aminoacids. The program also takes into consideration different, though antigenically equivalent ("similar"), aminoacids. Results include percentages of identical and similar aminoacids and the number of intervals lacking linear homology between two homologue sequences ("gaps"). The higher the percentage of identical or similar aminoacids and the lower the number of gaps, the greater is the structural homology between two proteins. In case of linear homology of CagA with tubulin, there are two different segments of CagA that align; for the first one, the identity is 31% and the percentage of matching amino acids is 50, as shown in the figure.

(2) How anti-Cag A can penetrate cell membrane, then react with intracellular antigen, such as tubulin, enzymes of Krebs cycle or actin? I can not understand.

Answer. We suppose they do at the same manner in which anti-nuclear antibodies react with nucleus antigens in lupus and other autoimmune diseases.

3. About Table 1, each description should included specific reference. Please add one more column.

Answer. Following the ref.'s suggestion, we have reported references in an additional column.

4. The inflammatory response might be a mechanism involved in PE and in sperm damage (page 9 and 12). In my opinion, there are many different kinds of inducers could elicit (systemic) inflammation, thus, is there specific factor or specific situation happened upon H. pylori infection?

Answer. Each person has a proper systemic inflammatory status. In individuals infected by H. pylori, such a level is increased and if the strains express CagA, it is augmented even more. If they have stigmata of infection (i.e., specific IgG) by Chlamydia pneumoniae, cytomegalovirus, herpes simplex virus, Epstein-Barr virus etc., such level is maximum (the concept of pathogen burden). Secretion of inflammatory cytokines largely depends also by the apotype of inflammatory cytokines of each person; for instance, those with a mutation in the cytosine in position -31 of the gene encoding interleukin-1 β (if I remember well) produce far higher levels of cytokine. We mean that variables are many; what matters is the systemic concentrations of proinflammatory cytokines, which have deleterious consequences upon pregnant women (facilitating the development of PE) and men (contributing to damage spermatozoa).

5. Page 12, line 16, spelling error, tumor necrosis factor-"alfa" should be tumor necrosis factor-alpha,

please correct it.

Answer. We have corrected the misprint.

6. One more interesting question, why people in Africa elicit different immune response from people lived in developed country?

Answer. We have answered this question in the manuscript; if it is not clear, we formulate the answer in a more concise manner: in the absence of parasitic infestation (that is the common situation in people living in Western world), the cell immune response to *H. pylori* infection is predominantly Th1, which does not cure the infection and contribute to damage the gastric mucosa. In the presence of co-infestation, the cell immune response to *H. pylori* infection switches from Th1 to Th2 lymphocytes that secrete anti-inflammatory cytokines, such as IL-4, IL-10 etc., thus contributing to hinder the development of the most severe consequences of such bacterial infection. This is demonstrated for severe chronic gastritis, peptic ulceration and gastric carcinoma. Our hypothesis is that, in underdeveloped countries, the presence of parasitic infestation reduces the systemic inflammatory status of patients infected by *H. pylori*, thus protecting pregnant women and diminishing the sperm damage exerted by high concentrations of pro-inflammatory cytokines.

Reviewed by 01445931

This review summarized the evidences to support an interesting hypothesis that *H. pylori* might affect the human reproduction. Although the literatures collected are up to date and this topic is thought-provoking, the major limitation is that all the studies addressing this issue were constrained by small case numbers. For example, case number was 167 and 21 for the association between *H. pylori* infection and infertility (reference 11 and 12, respectively) and 35 for the association between *H. pylori* infection and PCOS (reference 17). Reverse association can be seen not only in African but also in Asia, where both the prevalence of *H. pylori* infection and the birth rate are declining. Also, there is no clinical trial to confirm the causal relationship. This indicates that the cause of infertility is in fact a multifactorial, complicated issue. I would like to suggest the authors to describe this association in a more conservative way.

Answer: We agree that the infertility is multifactorial disorder and that the number of the studies on this subject is not very high, however their conclusions are concordant; one of the tasks of our review is to prompt other groups to investigate in this direction. Even though the patient groups in the opinion of the referee is not high, we would like to underline that, in the reference 11 the control group was composed by more than 800 individuals, and that in reference 12, 21 patients were those in whom the cause of infertility was unknown, while the whole number of examined patients was 204. As suggested by the referee we tried to describe the association in a more conservative way.

Reviewed by 02536349

Congratulations for exploring a new extragastric manifestation of Hp. The manuscript have to be edited by native English editors and must be certified by them before publication.

Answer: An English mother tongue person revised the manuscript

Reviewed by 02536325

The authors present a very interesting review about the possible mechanisms of H. pylori infection in reproductive disorders. As the authors mention, there is important evidence that CagA positive strains are related to these disorders and antigenic mimicry seems to play a role in the process. Furthermore, I found interesting to approach the female and male problems separately. I have general and specific comments addressed to authors.

Answer: Thank you for the positive comment and look forward to receiving the general and specific comments that we did not find enclosed.

2 References and typesetting were corrected

Thank you for consideration.
Sincerely yours,

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