Author's response to reviews

Title: Pandemic influenza A/H1N1 virus infection and TNF, LTA, IL1B, IL6, IL8, and CCL polymorphisms in Mexican population: a case-control study

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Version: 4 Date: 26 September 2012

Author's response to reviews: see over
Dear Dr. Marshall,

We submit our manuscript, entitled "Pandemic influenza A/H1N1 virus infection and TNF, LTA, IL1B, IL6, IL8, and CCL polymorphisms in Mexican population: a case-control study", for consideration for publication in BMC Infectious Diseases by Morales-García et al.

We review our manuscript

1. The authors presented a group of patients as “Asymptomatic Healthy Controls (AHC)” or Healthy Controls in table 3 which the authors described as persons who had personal contact with A(H1N1)pdm09 patients and confirmed exposure with the presence of anti-influenza A(H1N1)pdm09 antibodies. However, the presence of antibody does not confirm infection with A(H1N1)pdm09 as there is the likelihood of cross-reactivity (Hancock et al. NEJM 2009) especially in an older age group which seems to be the case in this patient group. Therefore several issues have to be addressed as this group consists of a large sample size of 176 patients and seems to play a major role in subsequent analysis. The result of which is featured in Table 4, 5, and maybe 6 (as non-fatal group? See below) where the authors appear to include this group of patients as being infected with A(H1N1)pdm09:
   a. the authors should describe the methods used in determining the presence of antibody (micro-neutralization assays?) and the data should be shown,

   To evaluate the presence of antibody, we use haemagglutination inhibition technique (HAI); contacts exhibited significant titers of specific anti-A/H1N1 antibodies, supporting the fact that they were in contact with the A/H1N1 virus. By serially diluted aliquots of serum samples; those individuals with titers greater than 1:16 were considered positive for A/H1N1 infection/exposure. Additionally is necessary clear that is not a patients group, we just include unrelated contacts in this study (e.g. family in law persons, home workers, etc.). They were in close contact with patients when the latter exhibited acute respiratory illness. None of these household contacts developed respiratory illness.

   b. can sero-conversion or rising titer for these patients be demonstrated?

   It wasn’t evaluated.

   c. were antibodies for A(H1N1)pdm09 tested for in the ILI patients (they could been previously infected as well).

   Not assessed, only asked directly.
d. Was the AHC group included into the non-lethal cases of A(H1N1)pdm09 infection for analysis to give the results in Table 6 i.e. what are the inclusion criteria to compare with mortality group?

Table 6 compares the risk of influenza A/H1N1 infection between genotypes in the A/H1N1 patients, not versus others groups.

We reality appreciate the comment of the author. With respect to point 1 and 1.d, the article by Hancock et al NEJM 2009 included population that had not contact with patients infected with A(H1N1)pdm09, where the argument for positivity is the cross-reactivity. However when a person with positivity of antibodies that had contact with an A/H1N1 virus infected patient, the infection cannot be excluded (Reference 34). The gold standard for identifying the infection to A(H1N1)pdm09 is real time PCR test, however to identify the presence of the virus in symptomatic patients is necessary that the patients be assessed during the first days from the beginning of the disease, the probability of identifying the virus by molecular test in asymptomatic patients is very low and not practical (Reference 35). Therefore we use an immunoassay to identify the antecedent of infection just in patients with contact with corroborated infection by A(H1N1)pdm09.

2. The timing and dosage of anti-viral drug administration could impact outcome significantly, although the authors stated that all patients were treated “upon admittance to at hospital” (please note grammatical mistake!), further information is needed:
   a. the days after symptom onset in which the patients were admitted into the hospital (ie. sample collection) should be shown, for without this data it is difficult to determine whether the timing of treatment will confound the results?
   b. Did all the patients receive the same dosage of anti-viral drug?

We correct the grammatical mistake, thank you for the point. We include in methods the moment when the patients received the antiviral drug. It is real that the moment where the antiviral drug is administered is important for the evolution of the patients. However, the antiviral drug is not a variable related with the polymorphisms therefore it is not a variable that would confound the results of our study.

3. One of the most common complications of influenza infection is secondary bacterial infection. In the serious cases, did the authors investigated whether these patients have bacterial infection as this may have confounded the analysis?

This is an important point. However our control groups were ILI and negative to real time PCR to A(H1N1)pdm09 and another group was asymptomatic patients with contact and seropisitivity to A(H1N1)pdm09, where this relation can not be studied.

4. in background (line 6): “In Mexico, the global lethal rate was estimated to be 1.2% .......).” Does the authors mean globally, in Mexico or both?
The correction was done.

5. On page 7 line 3 (Method): “These samples were analyzed for direct antigen detection of influenza specific RNA by using real-time reverse-transcriptase (rtRT)-PCR (RespiFinder)” does not make sense. Maybe the direct antigen detection bit should not be there.

The correction was done.

We appreciate the comments of the reviewer. Many thanks in advance for the observation and the opportunity of reviewing our manuscript.

Sincerely

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