Author's response to reviews

Title: Seroprevalence of Cytomegalovirus among pregnant women and hospitalized children in Palestine

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Version: 2 Date: 2 October 2013

Author's response to reviews: see over
October, 2\textsuperscript{nd}, 2013

Re: MS: 2125152393953475
Seroprevalence of Cytomegalovirus among pregnant women and hospitalized children in Palestine
Tahani Neirukh, Ayda Qaisi, Niveen Saleh, Areej Abu Rmaileh, Eman Abu Zahriyeh, Lina Qurei, Firas Dajani, Taghreed Nusseibeh, Hatem Khamash, Sabri Baraghithi and Maysa Azzeh

Dear Dr. Meylan,

Thank you for the opportunity to submit a revised manuscript.

We answered the comments and questions to the best of our knowledge. We believe that we were able to address all points raised by the reviewers.

Our detailed responses to the comments of the review follow in bold.

Furthermore, we made few corrections (figure 3 and the real time PCR section adding the reference for the method used). Finally, we sent the manuscript for review by two different English native speakers, Prof. Robert Stern and Kristen Reynolds for editing.

Best Regards
Maysa Azzeh, PhD

Reviewer's report#2
Title: Seroprevalence of Cytomegalovirus among pregnant women and hospitalized children in Palestine
Version: 1 Date: 2 July 2013
Reviewer: Kamran Yusuf
Reviewer's report:
A descriptive study of CMV prevalence from a part of the world where data on this is lacking.
Minor revisions
1. The first sentence of the results section needs to be rewritten (Lines 53 and 54)
First, we would like to thank Dr. Yusuf for his valuable comments
Lines 52-54 (now 53-56, conclusion section) were re-written:
"A comprehensive study with a long term follow-up examination of offspring born to HCMV IgM-positive mothers would be required to provide estimates of an accurate percentage of symptomatic congenital HCMV infection in Palestine"

2. On page 9, line 248, the word among is repeated twice
The repeated "among" was deleted

3. On page 10, lines 280-282, please rewrite the sentence to make the meaning more clear.
The sentence was re-written for clarification: now lines 281-284
"Since vertical transmission of HCMV is 15-20% higher in primary infected women compared to women with recurrent infection [9, 11, 12, 51], Palestinian infants may be considered at lower risk of perinatal HCMV infection as most Palestinian pregnant women seem to suffer from recurrent infection"

4. In all the figures, the y-axis needs to be labelled as percentage.
Y-axis was labeled as "percentage"

5. On page 8, second para, third sentence needs to be rewritten (lines 224-226)
The sentence was re-written, now lines 219-223
"Sixteen newborns were subjected to HCMV viral load testing in urine; thirteen were born to mothers with recurrent HCMV infection. The remaining three newborns were admitted to MICH following birth (aged 1, 10 and 19 days) and tested for HCMV because of symptomatic congenital infection"

Major Revisions
1. The authors did not include 160 tests (8.6%) where the IgG and IgM were not tested simultaneously. How many of these were IgM positive?
First we would like to clarify that these 160 tests were single (IgG or IgM) due to the following reasons:
   A. Either test kit was not available at the MICH laboratory
   B. Sample was insufficient
   C. Sample came from other clinics and the clinician is not aware of the importance of testing both simultaneously
   D. Patient was tested outside and sample was re-tested at MICH for conformation, especially IgM!
For these reasons, we did not include these results, as we considered them biased! See below the results of these 160 single tested cases (please compare with table 2):

<table>
<thead>
<tr>
<th></th>
<th>Pregnant Women</th>
<th>Children</th>
<th>Newborn</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG+</td>
<td>91.7%</td>
<td>88.4%</td>
<td>88.5%</td>
</tr>
<tr>
<td>IgG-</td>
<td>8.3%</td>
<td>11.6%</td>
<td>11.5</td>
</tr>
<tr>
<td>IgM+</td>
<td>22.6%</td>
<td>24%</td>
<td>38.9%</td>
</tr>
<tr>
<td>IgM-</td>
<td>70.9%</td>
<td>74%</td>
<td>61.1%</td>
</tr>
<tr>
<td>IgM grey</td>
<td>6.5%</td>
<td>2%</td>
<td>0</td>
</tr>
</tbody>
</table>

2. All the women tested were in the first trimester. Were any women tested later in pregnancy?

No, all women were tested in the first trimester. If women were not booked and came to the hospital for delivery, the hospital performs HBsAg test only.

3. In the results section, the authors use a lot of percentages. They should try to summarize their results better with less use of percentages.

The percentage part was modified, shortened and simplified as much as possible, in order not to lose important information.

4. The authors make a statement that on page 9, that congenital CMV infections in Palestine can be attributed to recurrent infection. How do they reach this conclusion as only 40 women were tested with the IgG avidity test?

We deleted this sentence, as we agree with the reviewer that we do not have enough data regarding congenital infections to make this assumption.

We made the assumption based on the fact that most Palestinian pregnant women (96.6%) were HCMV IgG positive. This by itself is an initial indication for unlikely primary infection to happen. Out of the 556 pregnant women tested, 63 were HCMV IgM positive. 38 women showed high HCMV avidity and these are already 60% of the 63 HCMV IgM positive women.

Nevertheless, we hope this publication will enable us to garner additional funding for better and more complete studies to verify HCMV infection in pregnant women and the congenital infections in offspring.
5. It might be useful to provide some demographic data from Palestine and also some data from the hospital where the study was done i.e. number of deliveries and how many were inborn babies in the study.

**Basic necessary demographic data is provided in the study population, line 98-101. Similarly basic necessary information regarding MICH is also provided in the study population, line 97-98. Data of deliveries were added.**

6. Do the authors have any data on the hearing test results of the newborns with congenital CMV?

**Unfortunately, we were unable to recruit these data, as most babies/children continue follow up on other day care clinics**

Reviewer's report#1
Title: Seroprevalence of Cytomegalovirus among pregnant women and hospitalized children in Palestine
Version: 1 Date: 27 June 2013
Reviewer: Maria Grazia Capretti
Reviewer's report:
The paper by Neirukh et al. investigate the seroprevalence of HCMV infection in an heterogeneous population of 1480 patients in Palestine. Data about the seroprevalence of HCMV infection among pregnant women are interesting, However the paper suffers of major weak points.

**Major Compulsory Revisions:**
1. The study population includes newborns, children and pregnant women; as the impact of HCMV infection is different in these categories, it is advisable to better focus the paper. The Authors state that the aim of the study is to “shed light on the incidence of HCMV infection in Palestine pregnant women and children including newborns” (page 4 lines 89-90). However, when referring to newborns, it is necessary to clearly define if it is aim of the Authors to provide data about the incidence of congenital disease, or if they are talking about post-natal infection. This is a key point when evaluating newborns. The method used to investigate
newborns and younger infants (IgG and IgM assay) seems to be inappropriate. CMV serology is not useful when studying newborns: they often did not produce IgM, and neonatal IgG reflect the placental transfer of maternal antibodies (as Authors themselves state in the Discussion section – pag. 9 line 250). In the Discussion section Authors state “During the study period, four (1.6%) HCMV congenital infection out of 249 tested newborns were symptomatic and treated with Gancyclovir” (page 9 lines 288-290): were all 249 infants considered to have HCMV congenital infection based on positive anti-HCMV IgG? If the aim of the study is to evaluate the incidence of HCMV congenital infection Authors should provide the diagnostic criteria of both symptomatic and asymptomatic HCMV congenital infection. Authors should clarify these points, or analysis and considerations about newborns should be removed.

First, we would like to thank Dr. Capretti for the valuable comments and questions, please see our response below:

The aim of this manuscript was not to evaluate the incidence of congenital HCMV infection. The data regarding congenital HCMV infection presented in this manuscript was a part of the data we recruited on HCMV infection cases at MICH. We have clearly concluded that another study would be necessary to estimate an accurate percentage of congenital HCMV infection (line 53-56). Furthermore, we mentioned that the 1.6% percentage of symptomatic congenital HCMV infection may not reflect a true incidence rate (discussion, page 10, line 292-295). We actually changed "may not" to "does not" in the revised manuscript to be clear.

We consider it important and relevant to shed light on the limited data and number of congenital HCMV infection cases presented here, as we want to draw attention to the largely unrecognized congenital infections in Palestine.

We intentionally divided the pediatric cases in children and newborns to differentiate between “possible” postnatal infection/sequelae of perinatal infection and “possible” perinatal infection. IgG and IgM are "not appropriate" for congenital CMV detection, but they were ordered as a part of TORCH surveillance as indicated in the methods, line 107-111. A part of the clinical manifestations for TORCH order were added in line 109.

Most of these newborns were admitted to MICH as a referral hospital, when other hospitals failed to diagnose them. The medical team at MICH exhausts all testing possibilities to diagnose and treat these babies. TORCH is on the top list
of diagnostic tests ordered in such cases. MICH laboratory is the only to provide TORCH testing in the entire Palestinian territories (West Bank, Gaza strip and East Jerusalem district). These circumstances created the large data of newborn and children tested for TORCH. Therefore, we did not make any attempt to estimate the incidence of congenital CMV infection in Palestine based on these current records.

So, most of the 249 were not diagnosed with congenital HCMV infection, but were tested for HCMV IgG and IgM as part of TORCH surveillance. Newborn with “suspected” congenital HCMV infections or those even asymptomatic ones born to IgM-positive mothers were subjected to both TORCH surveillance and HCMV DNA testing in urine. These were the 16 newborns mentioned in line 219-220.

2. It is unclear how the study population was selected: was TORCH test ordered in children and independently from the age and from the maternal serological status? (page 4 line 101, 104). Which were considered to be clinical signs of infection?

First, we did not select the study population, rather than we analyzed the HCMV serology records and then arranged the tested patients based on their age and the wards, which ordered the tests. Please see page 2, line 32-35.

Yes, TORCH test was ordered independently from age and maternal serological status for children as the case for most newborns. According to the policy at MICH pediatric unit, TORCH is ordered for children if they presented with jaundice and/or prolonged fever of unknown origin (line 101-102). Many of these children suffer from intractable medical conditions and were submitted to MICH as a referral hospital, when other hospitals failed to diagnose them (line 272-274).

3. In the HCMV Avidity section (page 7 line 185): how Authors determined IgG avidity in a IgG negative woman?

The serum was tested as one of the 40 IgM+ cases, even though it was clear that the avidity will be low or negative. The sentence line 186-187 was changed, so that it does not sound like a surprising finding.

4. Clinical observations section (page 8 line 207-219): the clinical findings in infants aged 23 days-14 years with positive anti-CMV IgM seem to be suggestive of congenital HCMV infection, especially as regards the visual and hearing
impairment. However, given the age range and the positive IgM, it is unclear what Authors mean. The mild bilateral conductive hearing loss (page 8 line 216) is probably unrelated to HCMV.

Clinical finding were summarized to show the clinical status of a subgroup of the children subjected to TORCH testing. Many of these clinical finding could be attributed to sequelae of HCMV congenital infection, proposed in line 276-280 in the discussion.

We removed the part with mild bilateral conductive hearing loss agreeing that it may be caused by other factors.

Minor Essential Revisions:
1. Page 9 line 248: remove "among"
   Among was removed

2. If data on prenatal findings in pregnant women undergoing a HCMV infection are available, they should be added.

   There were no critical prenatal findings in the 63 IgG+, IgM+ cases; we have no records for the only IgG-/IgM+ case, as this woman did not return for further consultation to the outpatient clinic, although her physician was immediately informed.