Author's response to reviews

Title: An association between Helicobacter pylori infection and cognitive function in children at early school age: a community-based study

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Author's response to reviews: see over
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Rachel Neilan, MSc
Executive Editor
BMC-series Journals

re: Manuscript 1191253392448967

Dear Editor

Enclosed is a revised version of the manuscript entitled: "An association between Helicobacter pylori infection and cognitive function in children at early school age: a community-based study" by Muhsen Kh et al.

We have revised the manuscript in accordance with the comments and suggestions raised by the reviewers. See please our point-by-point reply.

We believe that the revised manuscript incorporates the reviewers' suggestions and hope that it is now suitable for publication. Thank you for considering our manuscript.

Sincerely,

Dani Cohen
Response to the reviewers' comments
We thank the reviewers for their important and valuable comments and suggestions.

Reviewer 1: Prof. Pelayo Correa
This interesting work appears to have been well conducted. It needs to be tested in other populations before being fully accepted. Hopefully its publication will stimulate other investigators.
Quality of written English: Acceptable

Reply:
We added a sentence on the need of additional studies in other populations to replicate and confirm our findings. See please the abstract and the discussion section page 14, lines 15-16.

Reviewer 2: Prof. Marianne M Hillemeier
This paper examines the association between H. pylori infection and Stanford-Binet IQ score. The introduction discusses evidence that H. pylori infection is associated with iron deficiency, which in turn is associated with lower cognitive development and school performance. It is then hypothesized that H. pylori itself might negatively affect cognitive development independently of socioeconomic and nutritional factors. No rationale or conceptual framework is provided at the outset to explain why this independent effect might be expected.

Reply: In the past few years there has been a growing evidence in the literature showing that gastrointestinal infections acquired in childhood may be associated with diminished cognitive function, school performance and psychomotor development (Berkman et al. 2002, Niehaus et al. 2002, Lortz et al. 2006, Simsek et al. 2004), independent of nutritional status and socioeconomic variables (Berkman et al. 2002, Niehaus et al. 2002, Lortz et al. 2006). The impact of H. pylori infection on cognitive function in children was not assessed in any of these studies, although hypotheses were raised on potential negative effect of H. pylori infection on developmental outcomes (Lee 2007, Windle et al. 2007). This was the rational and conceptual framework of conducting the current study. We added this information to the introduction section. See please page 3, lines 2-7, lines 19-22.

More information about the target population and study sample is also needed. The methods section states that the 200 children who were included in the present analyses were part of a previous project three to five years prior to the current data collection. It is not clear how the sample in the original project was chosen and recruited, and the degree to which they are representative of the identified target population, Israeli Arabs.

Why is the study focused on this target population? Also, how were the three villages included in the study selected? Are they typical? Without this information it is difficult to determine whether the findings are at all generalizable beyond the study sample.

Reply: Following the reviewer's comment, we added more information about the study target population, the study sample, and the sampling and recruitment procedures in the original study. See please page 4, lines 4-13, 20-22, page 5, lines 8-20.
The characteristics of the villages that were included in the study are similar to those of other Arab villages and towns in Israel. For example, the median age in the Israeli Arab population is 20 years, and in three villages it is 18-21 years. In the Israeli Arab population 34% of the families have ≥ 6 persons, 21% of the women hold a job, as compared with 33% and 24%, respectively in the study sample. The mean number of rooms per a household is 3.7, and the median year of schooling is 11.3 in the Israeli Arab population, as compared with 3.8 and 10 years, respectively in the study sample. The three villages were selected to represent different socioeconomic background within the Arab population. We added this information to the methods sample. See please page 5, lines 8-16.

The reason for which we focused on the Israeli Arab population relied on the unique characteristics of this population. The Arab population in Israel is in transition, it has developed countries features in terms of infrastructure, educational and medical systems, while the rates of H. pylori infection and anemia are comparable to those in developing countries. In addition, the previous studies that addressed the association between gastrointestinal infections and cognitive development were conducted mainly in populations from developing countries. We added this information to the methods section (See please page 4, lines 4-13).

More fundamentally, however, the present analyses are not sufficient to support the conclusion as stated that H. pylori infection is associated with lower cognitive function, independent of socioeconomic and nutritional status. Although the authors do note that conclusions regarding a causal association cannot be drawn, the analyses are limited by small sample size and other considerations such that the claim of association is also in doubt. In multivariate models, H. pylori was significantly associated with IQ score in only the highest SES village out of the 3 villages included in the study, which appears to be a non-robust finding since that village seems quite similar to the “intermediate SES” village as measured by educational level (Table 1) in which the H. pylori/IQ association was not seen.

More fundamentally, the inclusion of only maternal education, maternal age, and hemoglobin level in the high SES village analysis leaves open the strong possibility that other factors affecting cognitive functioning have been omitted from the model, allowing their effects to load on the H. pylori variable. It is likely, for example, that other aspects of social position not captured by maternal education alone predispose children to both H. pylori infection and lower cognitive development. It is also not clear why other available variables including the crowding index were not included in the high SES village model.

Reply:
All along the manuscript we were cautious in our conclusions, and emphasized that the negative association between H. pylori infection and IQ scores was found only in children from the higher socioeconomic community, in which we assume that the negative effect of H. pylori infection is not overwhelmed/ masked by other factors affecting cognitive development.

The high socioeconomic status village and the intermediate socioeconomic status village differ in various characteristics. Following the reviewer's comment, we highlighted the difference among the villages (see please the revised table 1). We also added an appendix with data that the Israel Central Bureau of Statistics published on these villages. See please additional file 1.
In the current study, the variables socioeconomic level and nutritional status are covariates that we sought to control for their impact on cognitive function. Socioeconomic status was assessed by several parameters: (1) community socioeconomic rank as classified by the Israel Central Bureau of Statistics, (2) household socioeconomic characteristics: (a) maternal education, (b) paternal education, (c) crowding index: number of people living in the household divided by the number of room in the household, and (d) reported family income. Nutritional status was also assessed by several parameters: (1) current hemoglobin level, (2) hemoglobin level in early childhood (3) current BMI Z score, (3) Height for age Z score (HAZ) in early childhood, and (4) Weight for Height Z scores (WHZ) in early childhood.

We used methods accepted in the literature to control for covariates that can affect IQ scores/cognitive development:

1. Restriction: the study was restricted to children born after week 34 of gestation and at a birth weight of $\geq 2$ kg (See please page 5, lines 21-22). We also excluded children with medical conditions that might affect cognitive development (see please page 6, lines 1-6). By this way we limited/controlled the impact of these variables on the study outcome.
2. Stratified analyses by village of residence (Tables 2-4): assures comparisons within homogenous groups in terms of socioeconomic characteristics and other unmeasured environmental variables.
3. Multivariate analysis stratified by village of residence (Table 5), while including into the models individual level/household socioeconomic characteristics and nutritional variables that were found to be significantly associated with IQ score in univariate analysis.

In the high socioeconomic status village, the variables that were significantly associated with IQ (except of *H. pylori* infection) in the univariate analysis were maternal education and current hemoglobin level, so these factors were included in the multivariate analysis, in which the negative association between *H. pylori* infection and IQ scores remained statistically significant. This means that after controlling for community socioeconomic level (by stratification), individual level maternal education, and current hemoglobin level (as a marker of nutritional status), this significant association persisted, despite the small sample size (the chance of type one error was less than 0.05). In the intermediate and low socioeconomic villages, household socioeconomic characteristics (maternal education, crowding index), and nutritional status (current hemoglobin levels, hemoglobin levels in early childhood, and HAZ score in early childhood) were the main determinants of IQ scores. Actually, a main finding in our study is that the association between *H. pylori* infection and IQ scores was evident only among children from the high socioeconomic community. We discussed the potential explanations for such a difference among the three villages. See please page tables 2-5 and the results section.

As stated by the reviewer, we considered the small sample size as one of the study limitations (see please discussion section page 14, lines 7-8). In spite of the small sample size, we had enough statistical power to detect a significant difference in the IQ scores between *H. pylori* infection and uninfected children in the high socioeconomic community. The small sample size, nonetheless limited our precision by yielding wide confidence intervals limits. (See please discussion section page 14,
As mentioned above, we assessed the role of a wide range of socioeconomic and nutritional variables on IQ scores. Despite the methods that we used to control for confounders, we are aware to the possibility of residual confounding, which is inherited in the nature of the observational study design. We added a comment on this in the discussion section. See please page 14, lines 9-10.

Crowding index was not associated with IQ scores in children from the high socioeconomic status village, neither among children from the intermediate socioeconomic status village; therefore this variable was not included in the multivariate analyses related to children from these villages (see please table 2 and table 3). However the variable crowding index was significantly associated with IQ scores in children from the low socioeconomic status village, and it was included in the multivariate analysis of this group, see please table 4.

Following the reviewer's comment we clarified the selection of variables that were included in the multivariate analyses. See please page 9, lines 8-12.

Following the reviewer's remark we created a composite variable of individual level socioeconomic status that takes into account the parameters: maternal education, paternal education, monthly family income, and crowding index. The summative scoring of this composite variable was as following:

Each child was accredited one point if maternal education level was \( \geq \) 10 years and 0 points if maternal education level was <10 years, one point if paternal education level was \( \geq \) 10 years and 0 points if paternal education level was <10 years, one point if the monthly family income was > 4000 NIS and 0 points if monthly family income was \( \leq \) 4000 NIS, one point if the crowding index was below the median level (1.61 persons/room) and 0 if the crowding index was \( \geq \) 1.61. The higher the score was the better socioeconomic status. The socioeconomic status summative score was categorized into two categories, and was classified as "low" if the score was below the median level, and it was classified as "high" if the score was \( \geq \) the median level. The difference in IQ scores according to this variable was tested using independent t test in univariate analysis (See tables 2-4). Additional multivariate linear regression analysis was performed, in which this variable was included as a dummy variable. See please methods section, page 8, lines 7-22.

When the composite variable of socioeconomic status was controlled in the multivariate analysis, the negative association between \textit{H. pylori} infection and IQ scores remained statistically significant among children from the high socioeconomic village. See please the results section, page 11, lines 9-14.

\textit{This paper would also benefit from thorough editing to eliminate grammatical errors and awkward phrasing.}

\textbf{Reply:} The manuscript was edited as suggested by the reviewer.

\textbf{Reviewer 3: Dr. Lucia Braga}

\textit{This well presented paper is an interesting report raising the possibility that \textit{H. pylori} infection is associated with cognitive development among school age children.}

\textit{This study only found association amongst children from a relatively higher socioeconomic community. A limitation of this study is the low number of children included in each group studied.}
MINOR ESSENTIAL REVISIONS
As hemoglobin levels measure current abnormality and cognitive function is a long-term measure with a cohort effect, the authors need to discuss the likely duration of anemia and the limitations of the approach used. Similarly, only current H. pylori infection was measured, as detected by HpSA. Therefore, it would be important to mention in the discussion section that the role of the duration of the infection was not evaluated in this study and can be considered a limitation.

Reply:
We assessed the role of hemoglobin levels on cognitive development as one of the covariates. Data on hemoglobin levels in early childhood were available only for 168 children, while current (school age) hemoglobin level was measured among 194 participants. The study was not powered to assess the role of the duration of anemia on cognitive function (data on both early childhood hemoglobin level and current hemoglobin level were available for only 160 children). Given the reviewer's comment, we examined the correlation between early childhood hemoglobin level and current hemoglobin level and found a week but significant correlation (r = 0.25, P=0.001), suggesting that current nutritional status is influenced by past nutritional status.

Similarly, data on both H. pylori infection status in pre-school age and current H. pylori infection (school age) were available only for 140 children (Muhsen et al. 2010). Given this small number, we were unable to address the role of duration of the infection on H. pylori infection. We found that most H. pylori infections in this cohort of children were acquired in pre-school age and mostly persisted to school age (Muhsen et al. 2010). Given the reviewer's suggestion, we added a comment on this issue in the discussion section. See please page 13, lines 3-15 and page 14, lines 8-9.
References