Author's response to reviews

Title: Anemia, diet and therapeutic iron among children living with HIV: a prospective cohort study

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Author's response to reviews: see over
30 September 2015

Dear Editor-in-Chief,

BMC Pediatrics

Ref: Resubmission of revised manuscript: MS: 7114348291747486

Title: Anemia, diet and therapeutic iron among children living with HIV: a prospective cohort study

Many thanks for your reply and the comments from the reviewers. We would like to re-submit our manuscript titled “Anemia, diet and therapeutic iron among children living with HIV: a prospective cohort study” to BMC Pediatrics.

We appreciate the comments from the reviewers and have made all the modifications according to the reviewers’ suggestions. Point-by-point responses to the reviewers’ comments are attached below. The changes in the main manuscript corresponding to the reviewers’ comments are shown in red text.

We are grateful for this opportunity to resubmit the modified manuscript, and feel that these changes have contributed towards improving the readability of this manuscript.

Sincerely,

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Reviewer 1: Ilaria Mariotti

1. in Table 2 Legend the conjunction "and" could be substituted by "are" in the first line.
Response: This is done.

2. The legend of Figure 1 appears distant from the image which is referring to
Response: The legend for Figure 1 is appended to the end of the manuscript after the tables and the table legends. This appears the best place for the figure legend, as the figure is in .tiff format as per the journal’s submission instructions.

Discretionary Revisions:
1. since South India population presents an high incidence of hemoglobin disorders, it would have been interesting to include in laboratory examination hemoglobin electrophoresis to distinguish this kind of anemia from others
Response: We agree with the reviewer that it is always a good idea to look for other causes of anemia. Prevalence of hemoglobinopathies in this geographic area has been studied extensively and is very low. One study I have been involved in, was a community-based anemia study in our own area among healthy young children, where hemoglobinopathy (alpha and beta-thalassemia) was also assessed; this study concluded that the hemoglobinopathy prevalence was 1% or less\(^1\). Hence we believe that laboratory examination for hemoglobinopathies would not have changed the results presented in this manuscript.

2. it would have been also interesting to have a population of control of South India not affected by HIV to evaluate anemia and chronic inflammation which seems, in my view, a parameter quite opinable

Response: The aim of our present study was to examine anemia prevalence and etiology, and effect of iron supplementation on HIV disease progression among children living with HIV. While it would be interesting to compare a similar phenomenon in HIV-non-infected children, this would have been beyond the scope of this particular project. National data from India indicates that anemia prevalence varies in children between 40-80% based on age, socio-economic conditions and geographic area \(^2\). Our community based study\(^1\) also showed that anemia was prevalent in 75%, and iron-deficiency anemia was present among 62%. Chronic inflammation measured by CRP was prevalent in less than 10%.


Reviewer 2: Elizabeth Kibaru

A) Major compulsory revisions

1. The title suggests that iron was given as a supplement but in the methodology it was given for treatment to only the children with anemia. FDA defines a dietary supplement as a product intended for ingestion that contains a "dietary ingredient" intended to add further nutritional value to (supplement) the diet.

What is the true position?

Response: We agree with the reviewer wholeheartedly; although the term ‘supplementation’ has been used in several settings to indicate treatment, this is not entirely accurate. Hence we have changed the title to reflect ‘therapeutic’ iron that has been dispensed in treatment doses. We have also changed this term in several places in the main manuscript in order to maintain accuracy.

2. On line 85 and 86 the authors mentioned that perinatally acquired infected was indicated by history or documentation of one or both parents being infected. Does having infected parents translated to a confirmed source of infection considering the age of children in the study?

Response: All the children included in the analysis were those who were diagnosed with HIV before they attained 10 years of age, and all had evidence of their mothers being infected with HIV. Some had evidence that their fathers also had HIV infection. There were no cases where only the father was known to have HIV infection. These children were also young, with over half of them being 6 years of age or below. Although strictly speaking, only molecular phylogenetic analysis of the virus obtained from both the child and the mother can prove direct vertical transmission, this was beyond the scope of this project, and for all practical purposes we assessed perinatal transmission through careful history and documentation combined. Children whose maternal status was unknown were not included in the analysis (explained in the Results section).
• **If only father was infected what is the likelihood that the child will be infected?**
  
  **Response:** There were no cases where only the father was known to have HIV infection.

• **Was early infant diagnosis to confirm diagnosis in early childhood? Was there any evidence of in utero exposure to antiretrovirals having an influence on baseline hematological parameters? J Infect Dis. 2006 Oct 15; 194(8):1089-97**

  **Response:** All of these children were diagnosed during childhood and none were exposed to in utero maternal antiretroviral drugs. None of these children in this study was diagnosed during infancy within the early infant program.

3. **In line 145 on follow-ups, no interventions was offered apart from iron for treatment, was there any food supplements or multivitamins provided? And if so what was the impact on Hb level? These have been shown to have a positive influence on the hematological parameters.**

  **Ref:** Multivitamin supplementation improves hematologic status in HIV-infected women and their children in Tanzania by Wafaie W Fawzi, Gernard I Msamanga, Roland Kupka, Donna Spiegelman, Eduardo Villamor, Ferdinand Mugusi, Ruilan Wei, and David Hunter

  **Response:** We agree completely that food supplements and multivitamins were known to have a beneficial effect on hemoglobin. However, food supplements and multivitamins were not part of the national guidelines[^3] and hence were not included in the routine management or assessment. The aim of the study was to assess the impact of therapeutic iron as prescribed by the WHO and followed within the National Anemia Control program[^3] on children with HIV, who also have anemia, and hence no intervention other than iron was offered to the children.

4. In line 208 on the etiology of anemia and changes after iron 43.4% were already on HAART at baseline

• What was the impact of this at base line ?

Response: We did not find any impact of HAART at baseline on the etiology of anemia. Although there was a slight trend in decreased prevalence of anemia of inflammation among those on HAART at baseline, there was no statistical significance obtained; this could be due to the small numbers included in each of the etiology groups. Hence we have not reported this data. This information has been added to the manuscript (line 215).

• Were there other drugs used in this children that could have have affected the Hemoglobin levels e.g. sulphamethoxazole trimethoprim?

Response: Yes, some children were taking co-trimoxazole, but we found no impact of this drug on anemia prevalence or hemoglobin levels. This information has been added to the manuscript (line 204 - 205).

• Line 210; Vitamin A was noted to cause anaemia in 26.6% which criteria was used to conclude this? Note that the Attachment on etiology is not clear as most of the etiologies were combined 2 or more.

Response: Criteria used to define Vitamin A deficiency were as follows: Retinol binding protein (RBP) measured as < 0.7 µmoles/L. This marker is a known sensitive and specific marker for vitamin A deficiency in the context of HIV infection and malnutrition. There were several overlapping micronutrient deficiencies as well as evidence of inflammation that was associated with anemia, and this is depicted in Figure 1. The box in Figure 1 also consolidates the main causes of anemia in this population, and should help with clarifying the overlapping etiologies. This is explained in the manuscript (lines 209 - 213).

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At least the prevalence of major hemoglobinopathies in the population studied should be discussed since this is likely to have an effect on the hemoglobin levels seen. This includes HbS and thalassemia. (See Curr HIV Res. 2013 Apr;11(3):187-92.)

Response: Thalassemia and other hemoglobinopathies can definitely have an impact on anemia. However the prevalence of hemoglobinopathies in this geographic area in southern India has been studied extensively and is very low. One study we have been involved in, was a community-based anemia study in our own area among healthy young children, where hemoglobinopathy (alpha and beta-thalassemia) was also assessed, concluded that the hemoglobinopathy prevalence was 1% or less. Hence we believe that this would not have changed the results presented in this manuscript.

This has been added to the manuscript (lines 373 – 377).

5. In 25I on effect of iron supplementation there was reported positive changes on hemoglobin levels but were all these children on HAART and what was the difference in changes between those on HAART and iron versus HAART and no iron. HAART alone has been shown to markedly increase the hemoglobin levels ref

Response: Yes, we agree that ART alone can markedly increase the hemoglobin levels. In our study, we found that children who were on ART plus iron had higher hemoglobin increase compared to children who were on ART alone, without iron (Hb change 1.3 gm/dl versus 0.4 gm/dl, respectively. p=0.009). This data has been added in the manuscript (lines 254 – 256). We have also added the reference shown above to

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our manuscript in the Discussion section (lines 352 – 353).

6. In the discussion from line 273 there is minimal reference of the study findings as the authors are discussing. There is need to refer more and more to your findings

Response: In the Discussion, we state the main findings of our study, namely the significant prevalence of anemia among children with HIV, the main causes of anemia which includes iron deficiency and chronic inflammation, and the associations of anemia which includes stunting and poor disease control (detectable viral load and low CD4 counts). We also summarize the impact of using therapeutic iron among these children. We have structured the rest of the discussion, to reflect literature from other parts of the world that have similar findings, and discuss our findings within the context of findings from other researchers. We highlight our findings in contrast with the studies where iron supplementation was used in the context of malaria, which resulted in many safety concerns. We have now referred to our findings as well as other similar findings and hope that there is more clarity.