Author's response to reviews

Title: Human immunodeficiency virus infection is a risk factor for cerebral malaria in children in Uganda: a case-control study

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Author's response to reviews: see over
Human immunodeficiency virus infection and cerebral malaria in children in Uganda: a case-control study

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Authors’ responses to reviewers’ comments:

Version: 3  Date: 29 September 2010
Reviewer: Stephen Graham
Reviewer's report:

This paper addresses an important interaction, likely to be highly prevalent and still lacking in clear data in children.

Major comments
An important potential confounder here is age. HIV prevalence falls significantly with advancing age in children up until around 14 years as most are infected perinatally - and survival is poor esp. in first 5 years. Indeed, those with cerebral malaria are significantly younger than the comparison groups - as expected as cerebral malaria especially common around 2-4 years in endemic settings. Could the greater HIV prevalence in CM patients simply reflect the age association? It is stated in methods that multivariate analysis was done for independent risk, but it is not clear to me whether HIV infection was an independent risk factor.

HIV was an independent factor and because age is associated with both HIV and cerebral malaria, it is an important potential confounder. We have adjusted for age and age-adjusted ORs, reflected in the paper. Crude OR= 4.05 (95% CI 1.24-14.19), p-value=0.0056, and age-adjusted OR (aOR) =4.98 (1.54-16.07), p=0.0028.

Comparing CM-UM: aOR=5.94 (95% CI 1.36-25.94), p=0.0118
Comparing CM-UM: aOR=3.85 (95%CI 0.99-14.93), p=0.0372

These have been reflected in the manuscript

It is incorrect to conclude that HIV is associated with "development" of CM as this was not a longitudinal cohort study - rather with the clinical presentation of CM as it was cross-sectional.

Changed to association with clinical presentation of cerebral malaria instead of “development” of cerebral malaria

What is the HIV prevalence in the subset of uncomplicated malaria or no malaria (n=166) that are less than 60 months of age compared to the 81 CMs of that age?

Prevalence of HIV among children with uncomplicated malaria or no malaria was 1.2%.

Among children below the age of 5 years, the HIV prevalence in cerebral malaria cases was 8.6% (7 of 81) compared to 1.2% (2 out of 166) among all controls. The odds of presenting with clinical cerebral malaria below the age of five was higher among children who were HIV positive compared to the HIV negative counterparts OR=7.76 (95% CI; 1.42-77.6); p-value 0.003

Are there any outcome data for CM cases? Is HIV associated with poor outcome?
Patients were not followed up to assess outcome.

Minor points:
First para of methods, fifth sentence: "Severe anaemia.....were taken as having cerebral malaria”. I presume that they had coma as well? Please clarify.

Sentence clarified and reads: Severe anemia was not an inclusion criterion per se but children with severe anemia, who had a history of convulsions and were admitted with a BCS ≤2 (in coma), with a
relatively normal blood sugar (at least above 2.2mmol/l), normal electrolytes and a normal CSF with a positive blood smear for malaria parasites were taken as having cerebral malaria.

Last three paragraphs of results could be better structured and presented. For example, mentions HIV staging and CD4, and then next sentence starts with "However,..." and talks about anaemia. One could assume the inference but better to present more clearly. The last few paras of results reads as if a bunch of sentences thrown together without logical flow.

The entire results section was re-written to flow better.

A question mark after malaria in second para of discussion.

The question mark was removed and sentence revised

Version: 3 Date: 11 October 2010
Reviewer: James Berkley

Reviewer's report:
The manuscript deals with an important topic. Whilst there is reasonable understanding of the interactions of HIV and malaria in adults, there are few well conducted studies among children. The manuscript is clear and well written, the hypothesis is clear and the study design is appropriate. Definitions used are sensible and appropriate. Limitations are appropriately discussed.

Essential Minor Revisions:
The children with cerebral malaria were younger than those uncomplicated or no malaria. Since both incidence of cerebral malaria malaria and parasite density has a strong association with age, the odds ratios for these should be adjusted for age as a confounder. I see in the tables that this has been done, but the adjusted odds ratios rather than unadjusted odds ratios should be given in the abstract.

Age-adjusted odds ratios are now indicated in the abstract and in table 2.

The parasite density results in table 4 should also be adjusted for age and both the unadjusted and adjusted odds ratios presented in the text. The discussion should focus on the adjusted odds ratios

Both unadjusted and adjusted ORs shown in table 2

Were the actual white blood cell counts available to calculate parasite density? If so, they should be used rather than using 8000 as recommended WHO, since this may be an additional source of bias if counts differ between HIV+ and HIV- children.

Unfortunately, the actual white blood cell counts are not available to calculate the parasite densities.

In the discussion the statement that '... There are no similar studies with which to compare these results....' is not correct. The finding of an association between HIV infection and severe malaria in Kenyan children against community controls, and an association with the density of parasitaemia was was reported last year (Berkley et al. Clin Infect Dis. 2009 Aug 1; 49 (3):336-43.). The findings of this study should be discussed in relation to that paper.

Findings from Berkley et al. included in the discussion.
I suggest dropping the suggestion under 'limitations' in the discussion for a prospective cohort study because cotrimoxazole prophylaxis would be required, reducing subsequent malaria episodes dramatically.

Dropped suggestion of a prospective study.

Surely a more important statement in the abstract conclusion is the importance of starting cotrimoxazole prophylaxis, rather than more research on the correlation?

Conclusion revised:

**Conclusions and recommendations**

HIV-1 infection is associated with clinical presentation of cerebral malaria in children. HIV positive children should be initiated on cotrimoxazole prophylaxis as soon as they are diagnosed with HIV and should be counselled on adherence. In addition, use of mosquito nets in children especially those below the age of five should be emphasized during counselling sessions.
Reviewer's report
Version: 3 Date: 15 October 2010
Reviewer: Jean-Pierre Van geertruyden

Reviewer's report:
The question posed by the authors is well defined. Severe malaria is not so uncommon in children as in adults and the research question might be more accurately answered through a prospective cohort study (i.e. in a demographic surveillance site where both diseases are prevalent). Conversely, the main risk factor, HIV prevalence, is much lower in children as in adults. This said, there is no objection to use a case-control as research methodology which can give a preliminary indication. The findings are in line with the findings found in adults and adds to the body of knowledge.

The authors need to reorganise the result section (including tables)

I’ve attached the manuscript and you can find my comments within the text. All comments as such can be considered as Discretionary Revisions or Minor Essential Revisions except for the editing.

Abstract
Revised results section
Results: HIV-1 infection was present in 9% of children with cerebral malaria compared to 2.3% in uncomplicated malaria (age-adjusted odds ratio (aOR) 5.94 (95% confidence interval (CI) 1.23-28.72, p=0.012); and 2.5% in children with no malaria (aOR 3.85 (95% CI 0.98-15.07, p= 0.037). The age-adjusted odds of being HIV-positive among children with cerebral malaria compared to the control groups (children with uncomplicated malaria and no malaria) was 4.98 (95% CI 1.54-16.07). The association was seen among HIV-positive children with a relatively normal or mild immune suppression compared to those with moderate to severe suppression. Other factors associated with cerebral malaria were age and parasite density.

Background
References inserted in background
Malaria and Human immunodeficiency virus (HIV)-1 are two of the most common global health challenges today and the two infections commonly overlap in distribution in most countries especially in sub-Saharan Africa(1). Studies have demonstrated interaction between these two infections with the majority of studies conducted in adults (2-6). HIV-1 infection has been found to be associated with severe forms of malaria and particularly cerebral malaria in adults but there is still a paucity of information on the interaction between the two infections in children (2, 7, 8).


Methods
Because samples were sent to different laboratories for blood work up, more blood was collected from the children who had cerebral malaria. Clarified this in paragraph:

“Five millilitres of blood were drawn from each child with cerebral malaria and 2-3 millilitres in children with uncomplicated malaria for a malaria smear, *P. falciparum* parasite density, HIV serology and CD4 cell count, blood glucose and serum electrolytes which were done in different laboratories. A finger prick was done for children with no malaria for the blood smear and rapid HIV test and a blood sample for CD4 count taken if the child was found to be HIV positive.”


**Results**

Tables and results section were reorganized
Table 4 merged with table 1
Table 2: Comparing cerebral malaria and uncomplicated malaria
Table 3: Comparing cerebral malaria and no malaria groups
Table 4: Association between cerebral malaria and HIV infection comparing CM group to the control groups

Results section also reorganized and description of the study and description of the cases (including CD4) and controls made clearer, only important parameters indicated.

Typos and minor corrections changed as suggested

Multivariate analysis included in the results section

**Discussion**

In discussion, focused more on increased risk rather than prevalence. Study findings are of both clinical and public health importance and this has been mentioned in the discussion.

Our findings support the hypothesis that HIV infection is a risk factor for cerebral malaria in children. The findings from this study add to the existing knowledge of interaction between HIV infection and *P. falciparum* malaria in children and are of both clinical and public health importance.

**Conclusions and recommendations**

HIV-1 infection is associated with clinical presentation of cerebral malaria in children. Clinicians should ensure that children diagnosed with HIV infection are initiated on cotrimoxazole prophylaxis as soon as the diagnosis is made and caretakers counselled on the importance of adherence to the cotrimoxazole towards reducing the risk of acquiring *P. falciparum* malaria and associated complications such as cerebral malaria. Other malaria preventive measures such as use of insecticide-treated mosquito nets should also be emphasized during counselling sessions.