Journal of Infection (2016) xx, 1–11

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HIV coinfection influences the inflammatory response but not the outcome of cerebral malaria in Malawian children

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Accepted 28 May 2016
Available online ■ ■ ■

KEYWORDS
Cerebral malaria;
HIV;
Paediatric;
TNF

Summary *Objectives:* Study of the effect of HIV on disease progression in heterogeneous severe malaria syndromes with imprecise diagnostic criteria has led to varying results. Characteristic retinopathy refines cerebral malaria (CM) diagnosis, enabling more precise exploration of the hypothesis that HIV decreases the cytokine response in CM, leading to higher parasite density and a poor outcome.
Methods: We retrospectively reviewed data on clinical progression and laboratory parameters in 877 retinopathy-positive CM cases admitted 1996–2011 (14.4% HIV-infected) to a large hospital in Malawi. Admission plasma levels of TNF, interleukin-10, and soluble intercellular adhesion molecule (sICAM-1) were measured by ELISA in 135 retinopathy-positive CM cases.
Results: HIV-infected CM cases had lower median plasma levels of TNF ($p = 0.008$),

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http://dx.doi.org/10.1016/j.jinf.2016.05.012

Please cite this article in press as: Mbale EW, et al., HIV coinfection influences the inflammatory response but not the outcome of cerebral malaria in Malawian children, J Infect (2016), http://dx.doi.org/10.1016/j.jinf.2016.05.012

interleukin-10 ($p = 0.045$) and sICAM-1 ($p = 0.04$) than HIV-uninfected cases. Although HIV-infected children were older and more likely to have co-morbidities, HIV-status did not significantly affect parasite density ($p = 0.90$) or outcome (24.8% infected, vs. 18.5% uninfected; $p = 0.13$).

Conclusion: In this well-characterised CM cohort, HIV-coinfection was associated with marked blunting of the inflammatory response but did not affect parasite density or outcome. These data highlight the complex influence of HIV on severe malaria and bring into question systemic inflammation as a primary driver of pathogenesis in human CM.

Introduction

In sub-Saharan Africa over 3 million children are infected with the Human Immunodeficiency Virus (HIV).[1] There are in excess of 100 million cases of *Plasmodium falciparum* infection per year, leading to approximately half a million deaths, mainly in African children. While the overlap between the two diseases is considerable, with many malaria infections occurring in HIV-positive children,[2] determining the effect of HIV on the severity and outcome of malaria has been problematic, leading to variable and apparently contradictory results.[3–6] Some studies have found increased parasite density, an association with more severe malaria and worse outcome, and others have not (See Table 1 for a summary of published literature). We propose that at least in part, the use of insufficiently stringent diagnostic criteria for cerebral malaria (CM), could have led to misclassification of cases and therefore variability in the associations identified.

CM is a prominent severe malaria syndrome defined by the WHO as unrousable coma (Blantyre Coma Score[7] ≤ 2) in the presence of *P. falciparum* parasitaemia, with no other cause of coma found.[8,9] In the absence of additional criteria this clinical definition leads to over diagnosis of CM, leaving uncertainty as to whether coma is truly caused by parasitaemia or whether a person has an uncomplicated malaria infection and coma due to another aetiology. This is particularly problematic in high transmission settings where a high proportion of apparently well children in the community are parasitaemic. This was highlighted by a study at our centre in Malawi where a quarter of children diagnosed as having WHO-defined CM were found to have a non-malaria cause of coma and death at autopsy in the context of a peripheral parasitaemia.[9] This mis-classification may be exacerbated by HIV co-infection which may increase the risk of other non-malarial co-morbidities causing coma and thus confound the ability to detect true associations between HIV, CM and outcome (e.g. peripheral parasite density, the inflammatory response or mortality).

Characteristic retinal changes that are indicative of sequestration of *P. falciparum*-infected red blood cells (iRBC) in the neurovasculature[10] distinguish with high specificity and sensitivity those children with histological evidence of CM, from those with a non-malarial coma.[9] In order to re-examine the impact of HIV on CM, we have therefore used this refined diagnosis to classify a large cohort of Malawian children with CM, with and without HIV co-infection. Following the observation that peripheral blood mononuclear cells from HIV-infected individuals have impaired tumour necrosis factor-alpha (TNF) and interleukin 10 (IL-10) production *in vitro* in response to iRBC challenge,[11] we addressed the specific hypothesis that HIV-infection results in lower levels of systemic TNF and IL-10 in CM *in vivo* and that this is associated with a higher peripheral parasite density and a higher mortality.

Methods

Location

This study was conducted at Queen Elizabeth Central Hospital (QECH), Blantyre, Malawi. In 2010 HIV prevalence in pregnant women in this region was 18% and overall seroprevalence in Malawian children less than 14 years old was estimated to be 2.7%.[12] Malaria transmission in rural communities around Blantyre occurs year-round peaking during the rainy season (November—June).

Children diagnosed with HIV were followed up in paediatric HIV clinics, received daily preventive co-trimoxazole and, from 2001 and when eligible, combination antiretroviral therapy ([ART] lamivudine, stavudine and nevirapine; Triomune, Cipla). Routine CD4 quantification and WHO staging were introduced in 2006.

Patients

As part of a longstanding clinico-pathological study of CM in Blantyre,[13] Malawian children aged 6-months to 12-years presenting to QECH with clinical CM were recruited and managed on a paediatric research facility during consecutive rainy seasons from February, 1996 to June, 2011.

Management

Patients with CM were treated with intravenous quinine for at least 24 h and then switched to oral drugs (Sulphadoxine-pyrimethamine pre-2007 or Lumefantrine-artemether). Ward rounds by experienced clinicians were conducted twice daily.

From 2001 all patients whose HIV status was unknown were tested for HIV after a parent or legal guardian gave consent. Prior to 2001 HIV tests were conducted retrospectively on stored samples. In fatal cases where HIV-status was unknown, it was done posthumously. Ethical approval

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