HIV, malaria parasites, and acute febrile episodes in Ugandan adults: a case–control study

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Background: In sub-Saharan Africa, co-infection with HIV and malaria is probably very common. Although an interaction between the two infections is biologically plausible, it has not been investigated thoroughly.

Objectives: To evaluate the association firstly between co-infection with HIV and malaria parasites and the occurrence of acute fever, and secondly between HIV infection and clinical malaria, defined as the presence of acute fever and malaria parasites.

Methods: A hospital-based case–control study was conducted in Gulu District (northern Uganda), an area endemic for malaria and with a high HIV prevalence. HIV testing and malaria parasite quantification were performed on 167 consecutive adult outpatients with acute fever and no signs or symptoms of localized infection, and on 134 consecutive adult in-patients without fever who were admitted for non-HIV-related trauma or elective surgery.

Results: No significant association with acute fever was observed for single infection with either malaria parasites [adjusted odds ratio (AOR), 1.75; 95% confidence interval (CI), 0.73–4.21] or HIV (AOR, 1.01; 95% CI, 0.51–2.03), whereas a significant association was observed for co-infection (AOR, 9.75; 95% CI, 1.19–80.00). An association was found between HIV infection and clinical malaria (AOR, 2.34; 95% CI, 0.89–6.17); the association became statistically significant when the definition of clinical malaria included a cut-off for parasite density (50th percentile; i.e., 586 parasites/μl; AOR, 3.61; 95% CI, 1.04–12.52).

Conclusions: Despite the limited statistical power, the results of our study show an association between HIV infection and clinical malaria; if confirmed, this finding could be important for public health in sub-Saharan Africa.

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Keywords: HIV, malaria, co-infection, Africa
Introduction

In sub-Saharan Africa, infection with HIV, AIDS, and malaria are among the greatest health problems. An estimated 25.3 million persons are currently infected with HIV [1], and more than 70% of the population lives in areas of intense malaria transmission, where almost 300 million episodes of clinical malaria are reported each year [2]. Although co-infection with HIV and malaria is probably very common, and understanding if and how the two infections interact could be important for the control of both diseases, few studies have been conducted on this potential association.

In areas where malaria is endemic, the population acquires some degree of immunity against the clinical manifestations of the infection (i.e., anti-toxic immunity) and subsequently against the infection itself (i.e., anti-parasite immunity). Because both T cells and B cells are thought to be essential in acquiring immunity, HIV-induced immunosuppression is expected to increase the risk of having parasitaemia and developing clinical malaria. Although the findings of early studies on the interaction between HIV infection and malaria were inconclusive [3], recent studies have shown a significantly lower prevalence of malaria among HIV-negative pregnant women as compared with HIV-positive pregnant women [4,5] among whom the efficacy of malaria treatment also appeared to be reduced [6]. More convincing evidence has been provided by two recent investigations: in a cohort of HIV-infected individuals, the incidence of clinical malaria was found to have increased with decreasing CD4 cell counts [7], and in a population-based cohort, HIV-infected individuals had a significantly higher risk of having parasitaemia and developing clinical malaria than HIV-negative individuals, and this risk tended to increase with decreasing CD4 cell counts [8].

Considering the effect of malaria on HIV infection, because malaria induces CD4 cell activation [9] and increases the concentration of proinflammatory cytokines in plasma [10] it is expected to enhance HIV replication [11] and thus accelerate HIV-disease progression [12]. Studies conducted in Uganda have shown that the population presents an immune hyperactivation and that this characteristic is environmentally driven (it is also present in Europeans who have been living in Uganda for a long period of time) [13–15]. Among the environmental factors involved in immune hyperactivation, numerous and recurrent infections with malaria could play a prominent role. This hypothesis is supported by studies that have shown increased HIV replication both in blood mononuclear cells exposed to malaria antigens in vitro [16] and in transgenic mice carrying complete DNA copies of the HIV genome and infected with Plasmodium chabaudi [17]. Finally, a recent study among HIV-infected individuals showed that proviral loads were significantly higher among persons co-infected with clinical malaria, compared to controls, and that these levels remained high for at least 4 weeks after treatment [18].

To understand better the relationship between malaria and HIV infection, we conducted a hospital-based case–control study in an area of northern Uganda that is endemic for malaria and which has a high prevalence of HIV infection [19,20].

Methods

Study setting and design

The study was conducted at St Mary’s Hospital Lacor (referred to as ‘Lacor Hospital’), the major hospital in northern Uganda and located in the Gulu District. The Hospital serves a large population, and most patients are from the nearby municipality of Gulu and the surrounding rural areas. The hospital’s catchment area is endemic for malaria, and P. falciparum is the cause of more than 95% of the reported cases [19]. The prevalence of HIV infection is also high in the catchment area (an estimated 17.8% among the general female population) [20].

The study was designed as an unmatched hospital-based case–control study with two objectives: firstly to determine the effect of co-infection with HIV and malaria (defined as the presence of malaria parasites) on the occurrence of acute febrile episodes, and secondly to determine whether infection with HIV may be a risk factor for clinical malaria (defined as the presence of both malaria parasites and acute fever).

The study was conducted between February and August 2000. To achieve the first objective, the cases consisted of consecutive outpatients, 18–49 years of age, who reported to Lacor Hospital because of an acute febrile episode (fever > 38 °C starting not earlier than 1 week previously), without signs or symptoms of localized infection. The control group consisted of consecutive inpatients, 18–49 years of age, who had been admitted to Lacor Hospital for trauma or elective surgery for non-HIV-related diseases and who had neither current nor recent fever.

To achieve the second objective, the cases consisted of persons with clinical malaria (i.e., both malaria parasites and acute fever). The controls were those defined as for the first objective.

The criteria for exclusion from the study were: having undergone any type of anti-malaria treatment since the beginning of the current febrile episode, living outside
of northern Uganda, clinical diagnosis of AIDS (according to the case definition of the Ugandan Ministry of Health) [21], and being in the army. All participants provided informed consent to participate in the study.

The results of malaria testing were made known to the patient and to the clinician as soon as possible. HIV counselling was offered at no cost to all study participants, as done routinely for all patients who agree to undergo HIV testing. The study protocol was approved by the Ethics Committee of Lacor Hospital.

**Laboratory methods**

HIV antibodies were detected using an enzyme-linked immunosorbent assay (ELISA) (Wellcozyme Recombinant HIV 1 VK57; Murex Diagnostics, Dartford, UK). Positive and borderline samples were re-tested using another ELISA (Recombigen HIV 1-2; Cambridge Diagnostics, Galway, Ireland); when the results of the second ELISA were discordant, Western blot (New Lav Blot I; Sanofi Diagnostics Pasteur, Marnes La Coquette, France) was performed. Malaria parasites were detected and quantified using quantitative thick-film examination, according to standard procedures [22]; the technician was blinded with respect to the status of the patient (i.e., whether having fever or not).

**Statistical methods**

The sample size was calculated focusing on the second objective. Requiring a power of 80%, a ratio of controls to cases of 4 : 1, and expecting an HIV seroprevalence among controls of 17.5%, 67 cases and 268 controls were needed to allow detection of an odds ratio of >2.5 as statistically significant at a confidence level of 95%. However, because of the occurrence of an epidemic of *Ebola* Haemorrhagic Fever in September 2000 in Gulu District, study enrolment was discontinued before reaching the desired number of malaria cases, thus limiting the study power to 54%.

The association between co-infection with HIV and malaria parasites and acute febrile episodes was evaluated using a logistic regression model, which included age, sex and area of residence (urban versus rural) as potential confounders. Logistic regression models were also used to evaluate the association between clinical malaria and HIV infection, controlling for the potential confounders specified above. The adjusted odds ratios (AOR) and their 95% confidence intervals (CI) were used to describe the strength of the associations. All statistical analyses were performed using the SPSS statistical package [23].

**Results**

A total of 167 out-patients with an acute febrile episode (referred to as ‘fever cases’) and 134 in-patients who had been admitted for trauma or elective surgery without current or recent fever (referred as to ‘controls’) were enrolled in the study. Of the 167 out-patients with acute fever, 36 had malaria parasites and were thus considered as having clinical malaria (referred to as ‘clinical malaria cases’).

Table 1 shows the sex, age and area of residence (urban versus rural) of the three groups (i.e., fever cases, clinical malaria cases, and controls). No statistically significant differences were observed, although the control group consisted of a slightly higher percentage of males and had a higher median age as compared with the other two groups.

Of the 167 fever cases, 41 (24.6%) were HIV-positive, 36 (21.6%) had malaria parasites with a median parasite density of 586 parasites/μl (interquartile range, 152–4828 parasites/μl), and 13 (7.8%) were co-infected with HIV and malaria parasites. Among the 36 clinical malaria cases, 13 (36.1%) were HIV-positive. Among the 134 controls, 24 (17.9%) were HIV-positive; 14 (10.4%) had malaria parasites with a median parasite density of 234 parasites/μl (interquartile range, 79–424 parasites/μl), and 1 (0.7%) was co-infected with HIV and malaria parasites.

Neither single infection with malaria parasites (AOR, 1.75; 95% CI, 0.73–4.21) nor single infection with HIV (AOR, 1.01; 95% CI, 0.51–2.03) were significantly associated with the occurrence of acute febrile episodes, whereas a statistically significant association was observed for co-infection (AOR, 9.75; 95% CI, 1.19–80.00; *P* = 0.034) (absence of both infections was used as the reference category; Table 2).

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<tr>
<th>Table 1. Demographic characteristics of the study participants (n = 301).</th>
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<td>Fever cases (n = 167)</td>
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<td>Male (%)</td>
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<td>Median age [years (range)]</td>
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<td>Residents of urban area (%)</td>
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*Thirty-six of the 167 fever cases had malaria parasitaemia and were thus considered to be clinical malaria cases.
The association observed between HIV infection and clinical malaria was not statistically significant (AOR, 2.34; 95% CI, 0.89–6.17), probably because of the low study power to detect such an odds ratio as statistically significant. By using as a cut-off for malaria parasitaemia the 50th percentile among the parasitaemic fever cases (586 parasites/μl), this association increased (AOR, 3.61; 95% CI, 1.04–12.52; P = 0.043) and reached statistical significance (Table 3).

Discussion

Our findings that co-infection with HIV and malaria parasites was associated with occurrence of acute febrile episodes, and that single infection with either HIV or malaria was not, suggests that there is an important interaction between these two infections in Ugandan adults. Specifically, the anti-toxic immunity to malaria, which protects persons with parasitaemia from clinical manifestation of the disease, is probably suppressed by HIV, resulting in clinical manifestation (i.e., acute fever). This hypothesis is supported by our finding that all but one of the 14 parasitaemic controls were HIV-negative. Furthermore, non-AIDS HIV-infected individuals usually report prolonged fever (i.e., lasting more than 1 month), as opposed to acute fever; thus the acute fever observed among HIV-infected individuals seems to have been due to co-infection with malaria parasites.

Clinical malaria appears to be associated with HIV infection. Although this association was not statistically significant initially, it became significant when a cut-off level for malaria parasite density was introduced (i.e., 50th percentile among parasitaemic fever cases). In fact, using a cut-off level for malaria parasite density increases the reliability of malaria microscopy [24] and the specificity of the case definition [25] thus increasing the level of any potential association between HIV infection and clinical malaria. HIV probably suppresses anti-parasite immunity, increasing the level of malaria parasitaemia, with an additional increased risk of malaria parasitaemia becoming clinical malaria due to the HIV-induced suppression of anti-toxic immunity, as mentioned above.

The most common problem with case–control studies is selection bias of the study participants. In our study, cases were out-patients while we only found a suitable control group among the in-patients. This is unlikely to have introduced a substantial selection bias as the decision to admit a patient is taken at the out-patient department and, given the extremely low in-patient fees and the exemptions applied at the Hospital, in-patient care is as accessible as out-patient care. We made concerted efforts to avoid a situation in which the main exposure of interest (i.e., HIV infection)
would affect the likelihood of an individual becoming a control. Specifically, we excluded persons admitted for elective surgery for diseases that were potentially HIV-related (certain tumours and infections), soldiers (often admitted for trauma and among whom the HIV seroprevalence could differ from that of the general population), and persons with AIDS (probably less likely to suffer from trauma than the general population). It is worth noting that the observed HIV prevalence among controls (17.9%) was consistent with the estimated prevalence among the general female population in the same age range and study area (17.8% in 1996–1997) [20]. Thus, the controls selected appear to be representative of the general population that produced the cases. Finally, to avoid the selection of drug-resistant cases of malaria, we excluded persons who reported that they had received any anti-malaria treatment. In our experience, patients reporting not having taken any anti-malaria treatment are reliable because only those who have undergone previous treatment are given second line anti-malaria drugs, which are much requested.

Although an Ebola epidemic led us to interrupt the study before reaching the pre-established sample size, thus reducing the power of the study, the objectives were substantially met. Whereas the results of early investigations were inconclusive [3], more recent studies have provided some evidence that HIV infection may increase the incidence of clinical malaria in adults [7,8]. Our study also contributed evidence of this effect of HIV infection, although this was probably underestimated because we excluded persons with AIDS—a group of HIV-infected individuals among whom the incidence of clinical malaria is expected to be highest [8]. Given the high prevalence of these two diseases, the effect of an association between HIV and clinical malaria could be very important for public health in sub-Saharan Africa. Further investigations, possibly designed as longitudinal studies and including an HIV-stage classification based on biological markers such as CD4 cell counts and viral load, are needed to gain an in-depth understanding of this association.

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References

17. Freitag C, Schito M, Near KA, Chounguet C, Langhorne J, Sher A. Malaria infection induces increased viral expression in HIV-1 transgenic mice possible role for malaria specific T cells in viral induction. XIII International Conference on AIDS. Durban, July 2000 [abstract TuPeA3105].


