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RESEARCH ARTICLE

The Association of Falciparum Malaria and HIV/AIDS Infection in Relation to Parasitaemia and Anti-Malarial Treatment Outcome as seen at the Moi Teaching and Referral Hospital in Eldoret, Kenya

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Abstract

This paper examines the effect of HIV infection on falciparum malaria parasitaemia based on a study conducted at the Moi Teaching and Referral Hospital in Eldoret, Kenya. One hundred consenting patients with malaria were recruited into the study, malaria parasitaemia levels, HIV status and CD4 counts were determined for each patient, and the outcome of anti-malarial drug treatment with quinine or artemisinin derivative drugs observed. Malaria parasitaemia was monitored for each patient after antimalarial drug administration, for up to 7 days. Of the 100 patients recruited with falciparum malaria, 60% were children (<18 years) and 40% were adults. Of the total admitted subjects to study, 17% (5 children and 12 adults) were HIV positive. Malaria parasitaemia levels were higher in HIV positive patients than in HIV negative individuals and that HIV-negative individuals had a better parasitaemia clearance outcome by day seven (pday7) compared to HIV-positive ones whose parasitaemia clearance went beyond seven-day treatment period.

This study strongly recommends investigative work to be done in view of the recent Kenya Government launch of ACT as a first line course of treatment for uncomplicated malaria.

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Introduction

Malaria is an important cause of morbidity in HIV-infected persons in areas where the two infections coexist. Malarial episodes can transiently increase viral load, and, theoretically therefore have an impact on HIV disease progression and HIV transmission. Two studies (Kublin *et al.*, 2005; Whitworth *et al.*, 2005) observed that the increase in viral load was observed to be greatest in patients with higher clinical malaria, high parasitaemia, and relatively high CD4 counts [$>300/\mu\text{L}$]. However, a study conducted in adults in western Kenya showed that HIV infection with low CD4 counts (<200 cells/ μL) was associated with higher parasite density, and lower haemoglobin values at presentation (Shah *et al.*, 2006).

Other studies have observed that HIV-infection with low CD4 counts in association with anaemia increased the risk of poor response to anti-malarial therapy (Shah *et al.*, 2004). Studies also have indicated that individuals with asymptomatic malaria infection experience about 0.25 log increase in viral load, and up to about 0.89 log if they have a fever and parasite density $> 2000/\mu\text{L}$, but these levels return to baseline values about 8-9 weeks following effective anti-malarial treatment (Hoffman *et al.*, 1999; Kublin *et al.*, 2005). *P. falciparum* infection may stimulate HIV-1 replication through the production of cytokines (IL-6 and TNF-alpha) by activated lymphocytes (Xiao *et al.*, 1998; Piselli *et al.*, 2002; Rowland-Jones *et al.*, 2002; Froebel *et al.*, 2004). Other studies have shown that placental malaria (PM) is associated with increased expression on placental macrophages and foetal Hofbauer cells

of CC chemokine receptor 5 (CCR5), a major fusion co-receptor for HIV-1 cell entry (Tkachuk *et al.*, 2001; Salvatori *et al.*, 2001). The findings suggest that placental malaria may lead to increased mother to child transmission (MTCT) of HIV (De Cock *et al.*, 2000; Tkachuk *et al.*, 2001; Ayisi *et al.*, 2004). Placental HIV-1 viruses are transmitted from mother to child are predominantly CCR5 trophic (Wolinsky *et al.*, 1992). The true clinical impact of HIV on malaria infection and the overall impact of dual infections have not been determined (Whitworth *et al.*, 2005; Slutsker *et al.*, 2007).

Malaria Diagnosis

Conventional malaria diagnosis still uses the skilled but laborious and time-consuming microscopic examination of thin and thick blood films stained with Giemsa's or Field's stain. Newly developed tests include the quantitative buffy coat method (Becton Dickinson, Sparks, Md.) for the fluorescent staining of parasites after an enrichment step for the infected erythrocyte (reported to be as good as thick films for *P. falciparum* but not for the other species (Baird *et al.*, 1992); the ParaSight F (Becton Dickinson [Schiff *et al.*, 1993]) and the Malaquick tests (ICT Diagnostics, Sydney, Australia), based on the immunological capture of the *P. falciparum* histidine-rich protein 2 in whole blood (Kumar *et al.*, 1996); and the OptiMal (Flow Laboratories, Portland, Oreg.) assay, which is antibody-based detection of parasite lactate dehydrogenase (Makler *et al.*, 1998). These antibody-based dipstick tests are still being evaluated. PCR-based diagnostic tests for human malarias have been developed (Morgan & Thompson, 1998), but these are more applicable to large-scale surveys than to clinical diagnosis due to costs. PCR has been especially effective at detecting sub-microscopic levels of malaria parasitaemia (Makler *et al.*, 1998; WHO, 2000).

Impact of HIV on Malaria Parasitaemia and Clinical Severity in Adults and Children

HIV disease impairs the acquired immunity to malaria seen in older children and adults in endemic areas (1- to 2-fold higher of both parasitaemia and clinical malaria). HIV is also associated with increasing risk and higher density parasitaemia. The more advanced immuno-suppression occurs during the co-infection episodes (Steketee *et al.*, 1996; Whitworth *et al.*, 2000; Patnaik *et al.*, 2005). Adults with HIV also have a higher risk of severe clinical malaria, both in areas of endemic and unstable transmission (2003; Grimwade *et al.*, 2004). In Zimbabwe, Chirenda *et al.* (2003) have found out in their review that HIV-1 positive people staying in malaria endemic areas are at risk of developing

severe malaria. This was corroborated in South Africa by Cohen *et al.* (2005), who found out that people with HIV were more likely to have severe malaria, and the risk was increased in HIV-infected patients with a CD4 cell count below 200/cu.mm.

In Uganda, rates of parasitaemia among HIV infected children under the age of 5 were found to be 1.7-fold higher than in those without HIV, and they had greater parasite density as well (Mermin *et al.*, 2004). In another Ugandan study involving adults, it was found that HIV-1 increase malaria incidence by 1.2, 3, and 5 times for CD4 counts of $\geq 500/\mu\text{L}$, 200-499/ μL , and 200/ μL respectively (Korenromp *et al.*, 2005). Data from western Kenya suggested that severe anaemia and hospitalization due to malaria was more common in HIV infected infants (van Eijk *et al.*, 2002), which was also true with older children (Grimwade *et al.*, 2003). More importantly, it is now clear that infected pregnant women experience more malaria and higher density malaria parasitaemia, have more febrile illnesses, anaemia, and adverse birth outcomes (such as low birth weight (LBW), prematurity, and intra-uterine growth retardation (IGR), than women in single infections with malaria or HIV (Ter Kuile *et al.*, 2004).

Inter-villous blood mononuclear cells (IVBMC) normally produce interferon- γ (IFN- γ), which is implicated in the protection of placenta during pregnancy against falciparum malaria, but in HIV-infection this production is reduced in response to malarial antigen stimulation due to the severe impairment of interleukin-12 (IL-12) by HIV-1. This impairment is thought to potentially cause the increase in placental malaria (PM) in pregnant women (Bloland *et al.*, 1995; Chandramohan *et al.*, 1998; Ter Kuile *et al.*, 2004). Korenromp *et al.* (2005) have recently evaluated the overall impact of HIV on malaria and reported overall excess cases of three million and a 5% increase in malaria deaths due to HIV, translating to 65,000 excess deaths annually. The interactions would seem to be most compelling in regions with very high rates of HIV and unstable malaria transmission in areas such as Botswana, Zimbabwe, Swaziland, northern South Africa and Namibia, with malaria incidence increased as much as 28% and the number of deaths almost doubled.

Limitations of the Study

The limitation encountered in the study was the small number of HIV cases (n=17), as well as the limited number of child patients enrolled to the study presenting with co-infection of both malaria and HIV/AIDS compared to the adult patients. The authors therefore acknowledge that the results may not be sufficiently comprehensive. However, the

paper provides a framework upon which more studies could be conducted to obtain better results.

MATERIALS AND METHODS

The study was conducted in a hospital set-up at the Moi Teaching and Referral Hospital (MTRH), Eldoret, located in the Uasin Gishu District in the North of Rift Valley Province, Kenya. Eldoret town is situated at an altitude of 2300 metres above sea level and has a population of approximately 400,000, with an annual rainfall of 1140mm. It is situated about 360km North West of Nairobi, on the Uasin Gishu Plateau, punctuated with swamps of papyrus reeds, conditions that favour breeding of mosquito vectors. It is considered one of the malaria epidemic prone districts in Western Kenya (DOMC, 2006.). In addition to malaria, the District has had its own share of the HIV epidemic. The MTRH has a bed capacity of 446. The hospital houses the School of Medicine of Moi University that, on average, receives about 150,000 referred patients who come from the North of the Rift Valley, Western and Nyanza Provinces for medical attention. About 123,000 of the referred patients receive laboratory services annually for various tests, and many are admitted for further care and management.

The study subjects comprised patients (males and females) two years and above that were recruited from among the clinical patients admitted to the wards for better management of various conditions. Recruitment of study patients was done at the medical wards (Paediatrics and Adult) among patients sent from Out-Patient Department presenting with febrile conditions among other ailments. A total of 1,344 in-patients were screened for malaria parasitaemia and 100 of them were recruited into the study after signing the informed consent form administered to them. All the study subjects were treated with anti-malarial drugs (either quinine I.V. or artemisinin derivatives I.M.) and given professional attention along with the other in-patients at their respective wards. Apart from the blood slide test, all the patients admitted to the study in the wards were screened to determine HIV-sero-status after counseling by trained AMPATH (Academic Model for the Prevention and Treatment of HIV/AIDS) personnel within the admitting wards. AMPATH Laboratories are jointly run by Moi University and MTRH. HIV-sero-positive results were made readily available in the patients' files only to professional medical personnel handling the patients. A 3ml venous blood sample from all HIV-infected patients was obtained for CD4 cell count at the AMPATH reference laboratory.

Only patients admitted to Paediatric and adult medical wards of MTRH, Eldoret, with symptoms of

fever (temperature $>38^{\circ}\text{C}$) and/or diagnosed at the OP with malaria positive blood slide, aged ≥ 2 years and were willing (or their guardians or parents) to give a written consent to join the study were recruited. At the baseline, eligibility to the study was a positive blood slide with a minimum falciparum malaria parasitaemia of $1000/\mu\text{l}$ of blood ($=25$ trophozoites/200 white blood cells) and above. In addition, eligible subjects were required to accept to be screened for HIV. Any patient who declined to give consent on the above conditions or was considered too ill by admitting clinicians to have any meaningful monitoring of the response to anti-malarial treatment within the duration of confinement was excluded from the study.

Malaria Diagnosis

Pre-treatment and post-treatment blood samples were obtained from recruited subjects. Both thick and thin blood smears were prepared from each patient on day zero (D0). Thin blood films were fixed in absolute methanol for 2 minutes and the two smears (thick and thin) air-dried and stained with 10% standard buffered Giemsa stain (pH 7.2) for 10 minutes. Thick blood smears were scanned for the presence of malaria parasite under oil immersion objective $\times 100$ and scored accordingly per microlitre (μl) of blood. On the other hand, thin blood smears were similarly examined to confirm *Plasmodium falciparum* species. Malaria parasites were counted in all positive blood smears and reported per 200 white blood cells (WBC) counted. Parasite load was then calculated per microlitre (μl) of blood using the following mathematical formula (McKenzie *et al.*, 2005),

Malaria parasites per μl blood =

$$\frac{\text{No. malaria parasites counted} \times 8\,000}{200 \text{ WBC}}$$

An average of 8000 leucocytes per μl blood (range 4 000-11 000 leucocytes per μl blood) was used as the standard for the population under study. However, in spite of the inaccuracies as a result of the variations in the number of leucocytes between individuals in normal and greater variations in ill health, the above figure allows for reasonable comparisons between individuals' white blood cell values. Blood smears for parasitaemia change were consecutively taken at day zero (D0) pre-treatment, day one (D1), day two (D2), day three (D3), and day four (D4) after admission. Some patients were discharged home on the second or third day of admission to continue with the same treatment they had been started on (smears were not taken at home). Upon discharge, arrangements were made for patients to come back for a day seven blood slides re-check. Overall, there was 95% turn-up on day 7th for re-check. Change in

parasitaemia levels after anti-malarial drug administration was calculated based on D7 parasitaemia rather than on D3 parasitaemia since 57% of the subjects had been discharged home by D2. Patients could not be kept longer in the wards by the clinicians due to cost of maintenance and congestion in wards. Fourteen subjects who remained parasitaemic on D7 were given artemisinin therapy before they were returned home to continue with treatment.

HIV Diagnosis

Blood samples for HIV screening were tested at admission in the wards by trained AMPATH personnel using two rapid test kits (Determine, Abboth, Abott Park, IL, USA; and Unigold Trinity Biotech, Wicklow, Ireland - immunochromatographic band and Unigold - spot colour change methods respectively). CD4 counts from HIV-infected subjects were processed at AMPATH Laboratory using Flow Cytometric Method (BD FACSCount, Becton, Dickinson and Co, Franklin Lakes, NJ, and USA).

All the recruited subjects were treated with anti-malarial drugs (either quinine hydrochloride or artemisinin derivatives) and monitored for seven days. The change in parasitaemia levels after anti-malarial drug administration was compared between HIV-positive and HIV-negative patients. A comparison was also made on the same subjects to determine their response to anti-malarial therapy. On HIV subjects, based on their CD4 levels (below or above 200 cells/ μ L), and whether or not they were receiving anti-retroviral drugs (ARVS), a comparison was made to determine the change in parasitaemia levels and response to anti-malarial therapy.

Data were entered into Microsoft Excel programme. The original hard written copy was safely kept for reference by the project's Principal Investigator (PI). No unauthorized persons were allowed access to the data. Only the clinicians who were handling the subjects in their respective wards were given copies of sample request form results to use for treating or monitoring subjects. Analysis of data was performed using SPSS (Statistical Package for Social Scientists) version 12.0 for Windows. Validation of data was done before analysis that involved descriptive statistics such as means, standard deviation, median and frequency distributions. Fisher's Exact Test was used to test for significant difference between HIV-positive and HIV-negative patients.

RESULTS

Monthly distribution of Malaria among children under 18 Years of Age

The study begun with a draught spell that started in September 2005. In the six months period of the study between February 2006 and July 2006 (Figure 1), obtained data showed an overall slight elevation in malaria distribution among female children as compared to male children. Overall, malaria parasitaemia had a peak between the months of April and May with seemingly a declining trend in June and July regardless of gender.

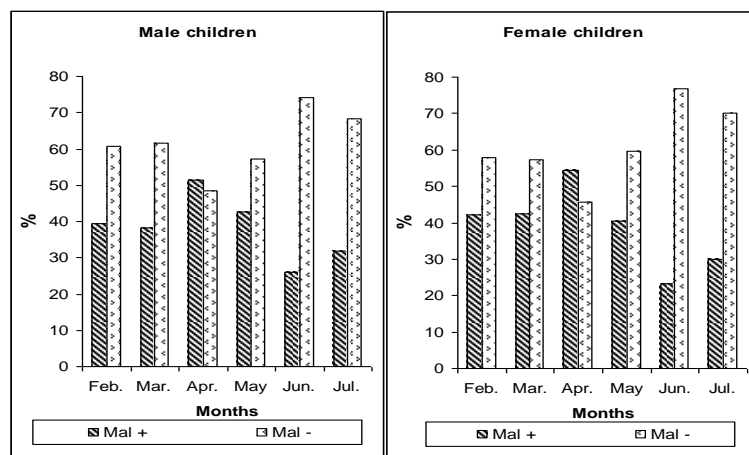


Figure 1: Monthly distribution of malaria among children

Legend

Mal⁺ - malaria positive

Mal⁻ - malarial negative

Monthly distribution of Malaria among Adults above 18 Years of Age

Figure 2 shows monthly distribution of malaria among adults (males and females) given in percentage (%) with malaria parasitaemia. Adult females were shortly more prone to malaria parasitaemia than their male counterparts in all the study months except in July.

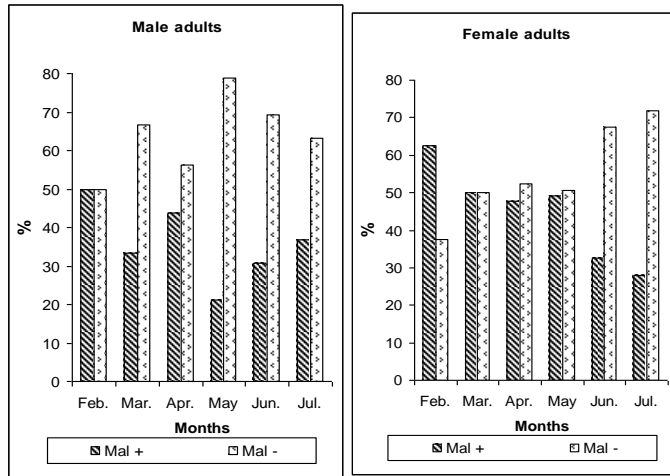


Figure 2: Monthly distribution of malaria among adults

Legend

Mal + - malaria positive

Mal - - malarial negative

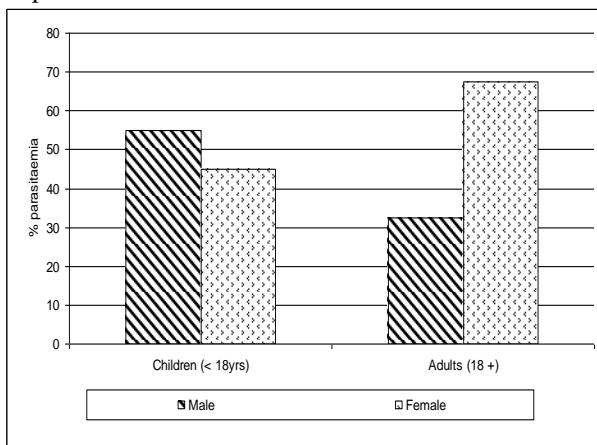
Malaria Parasitaemia in Children and Adults according to Gender

Figure 3 shows the percentage malaria parasitaemia (presence of malaria parasites in blood) in both children and adults. Among the children participating, it was observed that males tended to have more exposure to malaria than their female counterparts; but female adults showed more falciparum parasitaemia than their male counterparts, P=0.04. Statistical significance between gender and age in terms of acquisition of malaria was established.

Figure 3: Malaria parasitaemia in children and adults

Legend

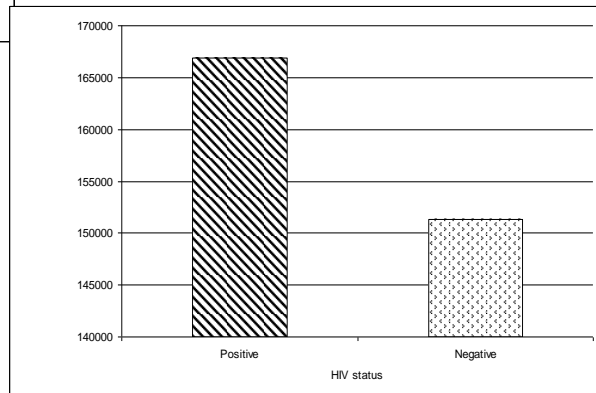
% parasitaemia - percentage subjects with parasitaemia



Malaria parasite load in HIV- positive and HIV-negative individuals

Of the study subjects (n=100), HIV-positive individuals had a higher mean parasite load (density) of 166,916.1/μL compared to HIV-negative individuals with a mean parasite load of 151,316.8/μL (p=0.839), Figure 4.11. This comparison was based on day zero (pday0) parasitaemia immediately after they were recruited to the study and before treatment was commenced.

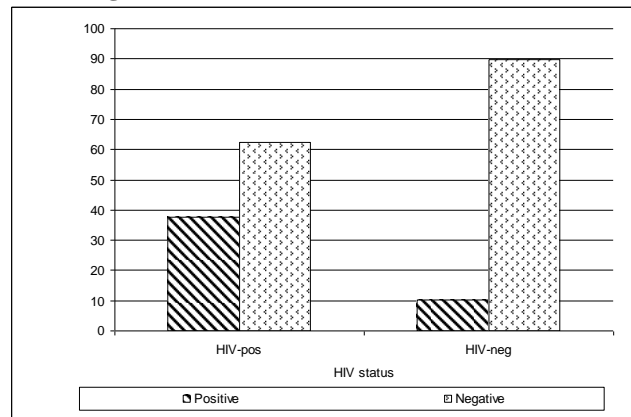
Figure 4 Malaria parasite load in HIV- positive and HIV- negative HIV and malaria co-infection and response to anti-malaria therapy



All the study participants (n=100) were challenged with anti-malarial chemotherapy (both Artemisinin and Quinine) and their parasitaemia response to treatment was compared in relation to HIV-status (Figure 4.13). It was observed that HIV-negative individuals had a better parasitaemia clearance of about 90% by day seven (pday7) compared to HIV-positive ones whose parasitaemia clearance was about 62% (p=0.012); OR=5.33(1.53-18.56).

Figure 5 Co-infection and response to anti-malaria therapy

Change in parasitaemia levels after anti-malarial drug administration.



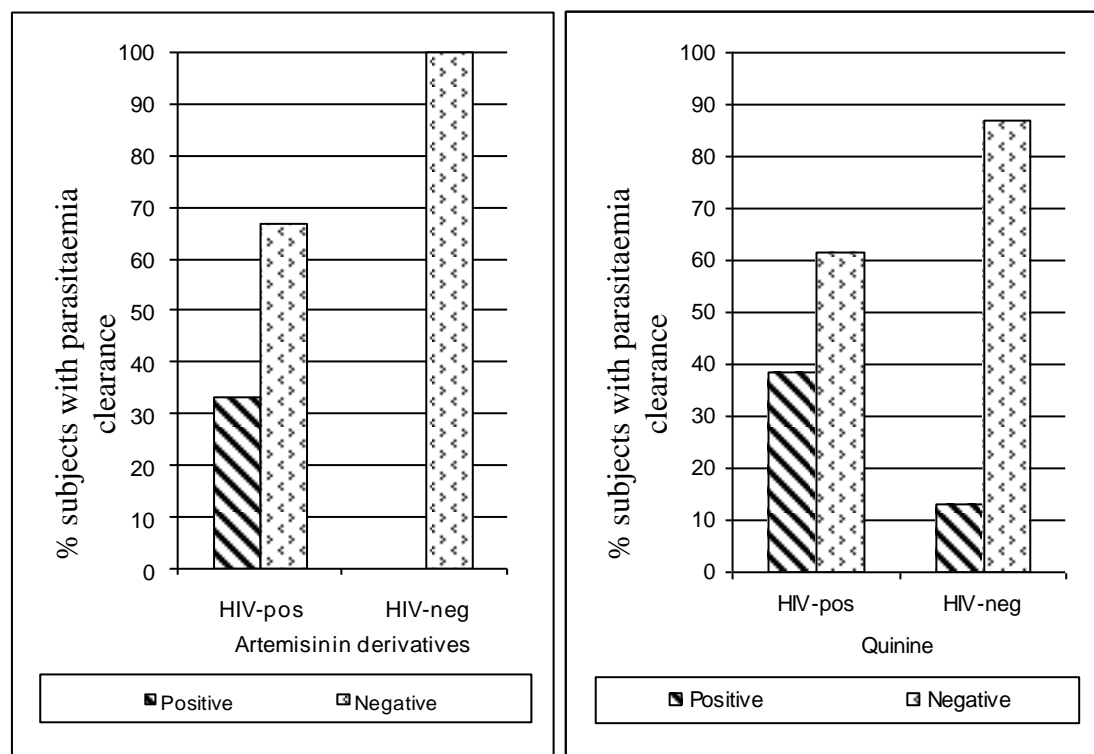


Figure 6 Effect of Artemisinin derivatives and Quinine therapy among subjects.

All the patients were treated with either artemisinin derivatives or quinine hydrochloride on admission (n=100) and were monitored up to day seven (Figure 4.14). Their parasitaemia levels were checked while in the wards, and comparison was made in relation to HIV-status and the effectiveness of each treatment group. Overall, 80 individuals were treated with quinine while 20 others were treated with artemisinin derivatives. Of the 13 HIV-positive individuals who were treated with quinine and lived up to day seventh, 5 (38.5%) had remained parasitaemic while 8 (61.5%) had been cured. Of the 62 HIV-negative individuals who were treated with quinine and lived up to day seventh, 8 (12.9%) had remained parasitaemic while 54 (87.1%) had been cured (p=0.042). Of the four HIV-positive individuals who were treated with artemisinin derivatives and lived up to day seventh, 3 (75%) were cured while 1 (25%) remained parasitaemic due to non-compliance in taking treatment at home. All the sixteen HIV-negative individuals who were treated with artemisinin derivatives were cured of malaria infection.

DISCUSSION

All the n=100 participants in the study had malaria at the baseline, and comprised both children and adults who had been recruited out of 1,344 screened patients. In total, there was 38.8% (521/1344) malaria

parasitaemia prevalence. During the study period, there was a malaria peak season between the months of April and May, 2006, with females experiencing higher malaria episodes than the males among all the screened patients at the facility. According to Gilks *et al.* (1990), the reasons malaria could be more severe in adults with HIV-infection could be: i) relative failure to control parasite multiplication; ii) could be related to an aberrant host response triggered by malaria infection, iii) could also be due to secondary complications of malaria such as bacteraemia, itself a well recognized HIV-related problem in Africa.

Effect of HIV on Malaria Infection

Overall the study recorded 17% prevalence of HIV infection, comprising children (29%) and adults (70.1%). These results seem to show HIV infection to up-regulate malaria parasitaemia (Fig.4.11) with a mean density of 166,916.1/ μ L compared to HIV-negative individuals with a mean parasite load of 151,316.8/ μ L, p=0.839.

Effect of HIV on Anti- Malarial Treatment Response.

After seven days treatment, the participants (n=100) were compared on their response to anti-malarial treatment. HIV-positive individuals (about 38%) had not cleared malaria parasitaemia by day seven compared to about 10% of HIV-negative individuals who were observed in the same period (Fig.4.13).

There was a significant association between HIV sero-positive status with malaria ($P=0.012$), which was confirmed by the Odds ratio of 5.33(1.53-18.56), that implied that HIV-positive patients were 5.33 times likely to have malaria parasitaemia clearance prolonged than HIV-negative patients. It is suggested, therefore, that this delay in parasitaemia clearance may cause HIV disease progression due to continued production of cytokines (IL-6 and TNF- α) required for the HIV-1 RNA replication (Xiao *et al.*, 1998; Piselli *et al.*, 2002; Froebel *et al.*, 2004; Rowland-Jones *et al.*, 2002), causing some rise in viral load and a decrease in CD4 cells. This scenario may impact negatively on the response to anti-malarial treatment among HIV-infected individuals.

The poor response among HIV-positive individuals to anti-malarial therapy (Fig.4.14) compares well with studies done elsewhere by Kanya *et al.*, 2001; Birku *et al.*, 2002; Patnaik *et al.*, 2004; and Vangeertruyden *et al.*, 2004. It appears, however, that HIV-positive individuals are better off with artemisinin derivatives therapy (Fig.4.15), as long as they do not have complicated malaria (CM).

CONCLUSION AND RECOMMENDATIONS

Plasmodium falciparum seems to show resistance to quinine treatment in both populations of HIV-positive and HIV-negative subjects. Of the 13 HIV-subjects treated with quinine, and lived to day seventh, 38.5% and 12.9% for HIV-positive and HIV-negative individuals respectively remained parasitaemic (resistant to anti-malarial treatment) beyond day seven.

In order to prolong life expectancy among HIV/AIDS patients in all malarial areas, personal protective measures against malaria mosquitoes including the use of ITNs and insecticide repellents are encouraged. Anti-malarial prophylactic measures might be important for all HIV-infected people who are not yet eligible to receive antiretroviral therapy (CD4 counts ≥ 200 cells/ μ L) living in malaria endemic areas.

In essence, all HIV/AIDS control programmes in malaria endemic and epidemic areas should have anti-malarial activities carried out in tandem (integrated) in order to provide double benefit to the public.

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