Plasmodium falciparum Biomass and Haematological Changes during Treatment of HIV/AIDS Patients in Western Kenya

J. K. Kirinyet[1*] and C. S. Mulambalah[2]

[1]Department of Medical Laboratory Sciences, School of Medicine, College of Health Sciences, Moi University, P.O. Box 4606–30100, Eldoret, Kenya.
[2]Department of Medical Microbiology & Parasitology, School of Medicine, College of Health Sciences, Moi University, P.O. Box 4606-30100, Eldoret, Kenya.

Authors' contributions

This work was carried out in collaboration between both authors. Author JKK designed the study, collected data, did statistical analysis, and wrote the first draft of the manuscript while, author CSM managed the analyses and critiqued the manuscript. Both authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Background: Monitoring and evaluation (M & E) of anti-malarial treatment among people living with HIV/AIDS (PLWHA) is very important to assess the response and change in malaria parasite biomass in an endemic area. Published data have shown that HIV-related immunosuppression correlates with increased malaria biomass, treatment failure and complicated outcome despite an individual's immune status. We evaluated blood samples from PLWHA based on malaria parasite biomass and haematological changes during anti-malarial treatment
Aim: To evaluate *Plasmodium falciparum* biomass and haematological changes during antimalarial treatment of PLWHA.
Settings and Designs: Cross-sectional and descriptive study design
Subjects and Methods: A randomized antimalarial treatment involving 126 subjects was carried out in a hospital setup in Western Kenya. Blood samples were collected and analysed to determine

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Corresponding author: Email: joelkirinyet@gmail.com;

malaria parasitaemia, changes in parasite biomass and haemoglobin levels in 28 days among PLWHA following treatment with Quinine and Coartem® antimalarial drugs. Descriptive and chi-square tests were used to determine the association of parasitaemia with gender and relevant haematological changes during treatment.

Results: A significant difference between females and males of those with parasitaemia on day-3 was noticed (p - 0.031). Quinine and Coartem® arms recorded a 100% parasite deletion/clearance by day-14 but showed recurrence on day-21 of 6.3% and 3.1% of day-14 respectively. On day-28 the Quinine arm had higher parasitaemia (306.3%). Mean Hb improved from 11.0 gm/dL to 11.6 gm/dL by day-28.

Conclusion: Recurrence of malaria parasite biomass was noticeable from day-21 to day-28 with Quinine arm of recipients and by day-28 haematological parameters had improved indicating recovery. Routine M &E of malaria cases and haematinic agent dosages to correct anaemia among PLWHA are recommended.

1. INTRODUCTION

According to WHO, the combined infection of malaria and HIV cause more than 2 million deaths each year globally and given the considerable geographical overlap between the two, a substantial number of co-infections do occur [1].

The goal of malaria case management is to rid infected individuals of parasitaemia and reduce chances of possible built-up of plasmodia resistance to the commonly used anti-malarial drugs. Secondarily, prompt, and effective treatment of malaria is to prevent the progression of uncomplicated malaria to severe disease, reduce mortality and other subsequent complications [2]. Interactions involving human host and plasmodium parasite often result in a host mounting an immune response to counteract an invading parasite [3]. This may not be easily achieved in certain individuals/patients, especially those with co-infections. For instance, people living with HIV/AIDS (PLWHA), however, tend to exhibit a prolonged and high malaria parasite biomass owing to their immunosuppression status. Malaria parasite biomass can affect an individual's immune response, consequently prolonging treatment period, particularly among the co-infected individuals.

Falciparum malaria parasites biomass is closely linked with various adverse effects including obstruction of microvasculature common in severe disease and often associated with death in endemic areas/regions. This being the case, higher biomass is likely to lead to more severe disease and mortality in the absence of prompt and appropriate intervention. The outcomes may be even worse in more severely immunocompromised individuals when infected with *Plasmodium falciparum.* Known for its high parasite multiplication rate (PMR), *P. falciparum generates* higher parasitaemia biomass in the severe stage of the disease. Falciparum malaria schizonts that sequester in the intravascular system is reported to drive a rapid and early increase in parasites biomass in a biological process [4].

Malaria treatment outcome may vary depending on several factors such as age, previous exposure to the infection, and nature of host-parasite interaction and presence of co-infections. Parasite biomass, endothelial activation, and microvascular dysfunction among the aging members of society in malaria low-endemic regions may account for the greater severity of the disease observed in certain studies [5]. Individuals, however, with previous exposure to falciparum malaria in a malaria endemic area tend to have lower parasite biomass [6]. In children, regardless of malaria endemicity, parasite biomass tends to be significantly greater in those with uncomplicated malaria [7].

Studies on host-parasite interactions have demonstrated interplay between parasite biomass and specific *Plasmodium falciparum* erythrocyte membrane protein-1(PfEMP1). In the development of adult severe malaria, the adhesion protein-1(PfEMP1) types indicate that low impairment of endothelial protein C receptor (EPCR) functions could play a role in parasite virulence [8].

Globally, studies on malaria-HIV/AIDS co-infection indicate that HIV-related immuno-

suppression correlates with increased malaria infection, burden, treatment failure, and complicated malaria despite the individual's immune status [9]. In sub-Saharan Africa, related studies indicate that HIV-related immune-suppression not only increases malaria parasite biomass but, also exacerbate the emergence of anti-malarial drug resistance [10]. In East Africa, a cross-sectional study undertaken in Tanzania examined the relationships between malaria parasitaemia (biomass) and CD4 T cell count and viral load among human immunodeficiency virus (HIV)-infected pregnant women [11]. In this study, women with low baseline parasitaemia, versus none, had higher viral loads at both time points, which predicted an increased rate of Aids-related disease (ARD) among studied women [11]. These findings have generated a lot of interest amongst scientists and international health/ research institutions including the World Health Organization.

As a follow-up, the World Health Organization (WHO) recommended that therapeutic efficacy studies (TES) addressing malaria-HIV/AIDS co-infections should be conducted in a controlled environment in which drug administration is supervised [2]. In such studies, the results of microscopic examinations of blood films are validated, and the origin and quality of the drugs are verified; and those prospective patients' clinical and parasitological responses to directly observed treatment (DOT) for uncomplicated malaria should be evaluated [2].

Assessment of therapeutic outcomes, principally, is done on the final day of a study (day 28, or day 42 for drugs with longer elimination half-lives). Any occurrence of malaria infection appearing during follow-up must be accompanied by genotyping to distinguish new infections from recrudescence [2].

There are reports indicating a decline effect in artemisinin combined therapy (ACT) being manifested by slowed clearance of parasite biomass in treated malaria patients, particularly in the Eastern World [12-15]. In such circumstances, there is need to constantly monitor the efficacy of antimalarial drugs in use by observing the changes in malaria parasite biomass [16], and that changes in parasite biomass that occurs following anti-malarial treatment can be used to assess the therapeutic response to anti-malarial drugs [12,17]. There is a paucity of data regarding performance of the first-line antimalarial drug - artemisinin combined

therapy (ACT) in Kenya, which this study sought to address.

We evaluated blood samples from PLWHA based on changes in malaria parasites biomass following administration of two routinely used antimalarials to assess their response, which could guide decisions in the management of malaria cases in this region.

2. SUBJECTS AND METHODS

2.1 Study Area and Site

The study was conducted in a rural area of Western Kenya, some 24km North-west of Kisumu City at Chulaimbo Health Centre. The site is located at latitude: 0^0 06' 07'' S and longitude: 34° 45' 42'' E. The site also is situated at 1,174 m above sea level and has mean annual temperature of 25°- 35° Celsius with a mean annual rainfall of 1800 mm.

2.2 Study Design

This was a health facility-based cross-sectional and comparative study carried out from November 2014 to October 2015 in an area known to be both malaria and HIV/AIDS endemic [18].

2.3 Study Sample Size

The study sample size was calculated according to Lemeshow's formula [19]; with a recruitment of 121 participants into the study based on 95% and 90% confidence levels and margin of error of 5% and 10%, respectively for the two drugs (Quinine and Coartem®) in accordance with the formula:

$$ n = \frac{2(Z_{1-\alpha/2} + Z_{1-\beta})^2 \, \delta^2}{(M1 - M_2)^2} $$

Where:

$Z_{1-\alpha/2}$ = STD error = 1.96(95%)
$Z_{1-\beta}$ = STD error = 1.282(90%)

δ = Std. déviation of Quinine [(=12), Satti *et al*. 2002]
M_1 = mean parasite clearance of Quinine (41 hr)
M_2 = mean parasite clearance of Coartem® (36 hr)

$$ N = 2(1.96 + 1.282)^2 \times 12^2 / (41-36)^2 $$
$$ = 2(3.242)^2 \times 12^2/5^2 $$
$$ = 2(10.525) \times 144/25 $$
$$ = 21.05 \times 5.76 $$
$$ N = 121 $$

2.4 Quality Assurance

Both good laboratory and good clinical practices were observed. Anti-malarial drugs used in the study were supplied by the Kenya Medical Supplies Agency (KEMSA). Giemsa stain and chemicals used such as methanol and buffer salts were sourced from Sigma-Aldrich (Catalogue No: 65637-25G; M1775-1GA; P3288-12VL respectively). Training and quality assurance in microscopy for study site technologists was done continuously by the PI and all subjects' slides were taken to Malaria Unit, Centre for Biotechnological Research and Development, KEMRI, Nairobi for re-examination and validation.

2.5 Recruitment of Participants

2.5.1 Inclusion criteria

Individuals with co-infection of falciparum malaria and HIV/AIDS infection with asexual parasite density of between 1000 and ≤200,000 parasites/µL; febrile, of auxiliary temperature above 37.5^{o}C. and haemoglobin (Hb) of not less than 5 g/dL of blood. All participants were aged 2 years and above comprising of consenting males and females into the study.

2.5.2 Exclusion criteria

All individuals with multiple infections with *Plasmodium* species and aged below 2 years and severe anaemia below 5.0 g/dL were ineligible. All those with a history of anti-malarial therapy within 48 hours prior to visiting the study site were also excluded from the study.

2.6 Sample Collection and Processing

Finger-prick (thick and thin) blood smears were collected from prospective individual PLWHA to determine eligibility. Concurrently, 0.02 ml of blood from the same site was also removed for Hb estimation using Swedish equipment, HemoCue AB, Angelhom. Before initiation of staining with 10% buffered Giemsa stain (pH 7.2), thin blood smears were fixed in absolute methanol and both slides were stained for 10 minutes. Thick smears were quantitatively examined under the microscope x100 objective lens for parasite biomass level while the thin blood smears were examined for speciation. A similar procedure for quantifying parasite biomass was repeated on follow up days: - day-

3, day-7, day-14, day-21, and day-28 while Hb estimation was done on day-7 and day-28 of follow up.

All participants who gave consent and met inclusion criteria were recruited from among known seropositive clients at AMPATH clinic while attending management care. They were all randomized to receive routinely used anti-malarial drugs (either Quinine - Grouped A or Coartem® - Grouped B) using a pre-determined computer-generated randomization list (Random Sequence). They received respective anti-malarial drugs administered to them orally in accordance with the dosing schedules provided in treatment schedule (Table 1) and confined in the wards for three days under Directly Observed Treatment (DOT) method. Close observation was done within 30 minutes during anti-malarial loading dose and any participant who vomited the first dose of treatment was given a repeat dose and further observed until they became stable. On discharge day, they were all booked for follow-up on days 7, 14, 21 and 28 for blood slide check-ups and routine profiles. Participants who failed to turn up for follow-up on their scheduled days were visited at home for a blood slide sample.

2.6.1 Determination of parasitaemia biomass

All blood smears for malaria parasitaemia determination were made in two sample-forms (thick and thin smears) from each participant finger-prick on day zero (Day-0) pre-treatment and stained with 10% buffered Giemsa stain (pH 7.2) for 10 minutes. Thick blood smears were microscopically scanned for the presence of malaria parasite under oil immersion objective x100 and reported accordingly per microlitre (µL) of blood. Thin blood smears were similarly examined to confirm the type of *Plasmodium* species [20]. The ultimate parasite biomass was calculated and expressed per microlitre (µL) of blood using McKenzie's mathematical formula [21].

Malaria parasites per µL blood =

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

An average of 8000 leucocytes per µL blood (range 4000–11000 WBC/µL blood) was used as the standard for the study population.

2.6.2 Determination of haemoglobin (Hb) concentration

Finger-prick blood samples were obtained. Measurement of haemoglobin (Hb) was done using HemoCue AB, Angelhom, Sweden equipment. The initial test results of the haemoglobin level were to rule out the cases of severe anaemia prior to inclusion in the study that had fixed a minimum Hb value of 5.0 g/dL and above. The subsequent haemoglobin readings (Fig. 3) were used to compare and monitor recovery over the study period between the two groups, after the initiation of anti-malarial therapy.

2.6.3 Evaluation of malaria parasite biomass post-anti-malarial treatment

After all the six blood slide samples (day-0, day-3, 7, 14, 21 and 28) had been microscopically read and quantified, data were used to compare the change in mean parasite biomass from the initial reading on day-0 (pre-treatment) and subsequent follow-up days. The overall change in mean parasite biomass level in all the six (6) blood smear samples was assayed (Fig. 4).

2.7 Data Management and Analysis

Collected data in the field was stored in Microsoft Excel Spreadsheet program, flash discs and hard written copy in analysis notebook. After validation, analysis of data was done using SPSS computer software version 23 that involved descriptive statistics and for categorical data such as sex, chi-square test was used to determine the levels of parasitaemia in gender.

3. RESULTS

3.1 Demographic and Clinical Characteristics

One hundred and twenty-six subjects comprising of both children aged ≥2 and ≤ 17 years and Adults ≥ 18 years old participated in the study. Of the total participants, 83.3% were adults, of whom 66.7% were females and 33.3% males; children represented 16.7% with a higher number of males (61.9%) than females (38.1%) (Fig.1).

The participants' mean age was 31.4, the median 31, and standard deviation was 14.495 with a range of between 2 and 72 years. The distribution curve seemed to shift to the right, more aged people than young.

Two routinely used anti-malarial drugs (Quinine and Artemether lumefantrine) against malaria cases at the study facility were administered according to the protocol (Table 1) and the recipients of each drug were categorized according to age groups (Table 2).

3.1.1 Haemoglobin levels and temperature values of participants

On day-0 the pre-treatment mean Hb level of the participants was 11.7 gm/dL (anaemic) and a further haemolysis state on day-7 with a mean of 11.0 gm/dL. After day-7, however, mean haemoglobin showed a slight improvement indicating recovery towards day-28 of follow-up with a mean of 11.6 gm/dL (Fig. 3). On the date of admission to study, the temperature of most participants was slightly febrile (mean of 37.6^o C), which declined in subsequent days during treatment. Mean temperature, however, showed an elevation of up to 39.0^o C on day-28 due to recurrence of malaria infection observed among some participants (Fig. 3).

3.1.2 Gender in relation to parasitaemia

Chi-square analysis of categorical data for genders in relation to parasitaemia showed a significant difference between females and males of those with parasitaemia (p - 0.031**)** on day-3 (Table 3). Overall, change in malaria parasitaemia cases tended to be faster among the male participants as compared to their female counterparts, but females showed more recurrence of malaria on day-28 though not statistically significant (p - 0.658) (Table 3).

3.1.3 Parasite biomass levels post treatment

Initial mean parasite biomass level on day-0 samples prior to initiation of treatment was 43,504.23(100%). Following treatment with either Quinine or Coartem®) in the subsequent evaluation days, the changes (decline/increase) in parasite biomass levels were recorded in the prevalence of existing (uncleared/cleared) parasite biomass at the time of evaluation with respect to each arm of treatment type (Fig. 4). On day-3, the parasite biomass of Quinine arm had experienced a decline change of 61.9% (from 100% to 38.1%) compared to 77.8% (100% to 22.2%) of Coartem® counterparts with a reported case of non-compliance in drug (Coartem®) taking on day-2. On day-7, the Quinine arm had recorded a decline change of 91.6% (from 38.1% to 3.2%) compared to 78.4% of Coartem® counterparts (from 22.2% to 4.8%). On day-14, the Quinine arm had recorded a

100% deletion of all parasite biomass compared to a decline of 33.3% (from 4.8% to 3.2%) with Coartem® counterparts. On day-21, the Quinine arm recorded an increase of 6.3% (from 0% to 1.6%) parasite biomass due to recurrence of infection compared to 3.1% (from 3.2 to 3.3%) Coartem® counterparts possibly due to non-compliance in drug-taking on day-2. On day-28, the Quinine arm recorded an even higher increase in parasite biomass of 306.3% (from 1.6 to 6.5%) due to further recurrence of parasite biomass compared to Coartem® counterparts which showed a steady decline of 3.2% (from 3.3 to 1.7%). Of the eight recurrent cases with heavy parasitaemia levels, five were on day 28 and all were taken to reference lab for genotyping (data not included here).

4. DISCUSSION

It is evident that malaria infection is associated with anaemia due to haemolysis caused by plasmodium parasites. At the time of recruitment to the study, there was slight anaemia observed which further progressed on the decline following antimalarial treatment up to day-7. After that period, it again began to build up as the parasites were eliminated from the vascular system. This outcome was comparable with data observed elsewhere where many patients experienced a drop in haemoglobin (Hb) 1-2 days following antimalarial treatment and then followed by a linear increase through follow-up [22]. In severe malaria cases that were treated with artemisinin derivatives, the observed outcome showed associated haemolysis with the drugs which could lead to life-threatening anaemia among the participants [23]. But severe malaria in our study, however, was one of the exclusion criteria. Available data show that in areas of high malaria transmission; infants, young children, older children, and adults with severe life-threatening malarial anaemia who sought blood transfusion services in health facilities was reported to form a major cause of hospitalized admissions [24].

Antimalarial drug monitoring and evaluation in a health facility are crucial in case management to assess the effectiveness of a specified drug in use. The primary objective of treatment, therefore, is to ensure full elimination of an aetiological agent, *Plasmodium* parasite from the patient's blood, in order to prevent progression of the disease to severe stage or death. Secondly, it is to reduce transmission of the infection to others, by reducing the infectious reservoir, and to prevent the emergence and spread of resistance to antimalarial medicines [2]. A related study carried out in a Bangladeshi tertiary hospital showed higher total parasite biomass in severe *falciparum* malaria patients than in uncomplicated malaria patients despite shorter fever duration [6]. In Kingston's findings, circulating and total, but not sequestered, parasite biomass estimates were significantly greater in children with severe malaria than in those with uncomplicated malaria. The same study findings were comparable with those of a related study involving Gambian children which established that parasite biomass estimates were significantly greater in children with severe malaria than in those with uncomplicated malaria [7]. This scenario calls for an effective antimalarial drug that can eliminate the parasite biomass from blood circulation as fast as possible. In adults, interplay between parasite biomass and specific PfEMP-1 adhesion types in the development of severe malaria indicates that low impairment of endothelial protein C receptor (EPCR) function may contribute to parasite virulence [8]. And in old age, the association of related processes with ageing may account for the greater severity of malaria observed in low-endemic regions [5].
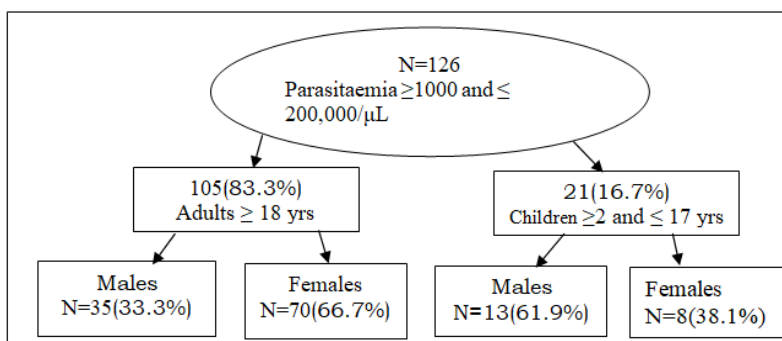

Fig. 1. Distribution of participants according to gender

Table 1. Anti-malarial treatment schedule protocol

Treatment	Medication	D0(Hr.)	D1(24 Hr)	D2 (48 Hr)	D3(72 Hr.)-D6(156 Hr.)
Quinine (Drug A)	Quinine base	8mg/kg x 3	8mg/kg x 3	8mg/kg x 3	8mg/kg x 3 daily for 7 days
Coartem® (Drug B)	Artemether/	2mg/kg x 2 (8Hr. apart)	2mg/kg x 2 (8Hr. apart)	2mg/kg x 2 (8 Hr. apart)	/
	Lumefantrine	12mg/kg x2 (8Hr.apart)	12mg/kg x 2 (8Hr. apart)	12mg/kg x 2 (8Hr.apart)	/

Key: D0-Day-0; D1-Day-1; D2-Day-2; D3-Day-3; D6-Day-6; Hr-hour; H-hour; mg-milli-grammes; kg - kilogramme

Table 2. Antimalarial drugs used & age groups

	Age groups (yrs)												
Antimalarial drug used	0-4	5-9	10-14	15-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	≥ 55	Total
Quinine	4	3	1	3	5	7	13	6	8	8	1	4	63
AL	3	5	3	1	6	10	9	9	6	5	3	3	63
TOTAL	7	8	4	4	11	17	22	15	14	13	4	7	126

Key: AL - Artemether lumefantrine

Table 3. Gender and parasitaemia

	Day0			Day3			Day7			Day14			Day21			Day28		
Parasit. Status	F	M	F+M	F	M	F+M	F	M	F+M	F	M	F+M	F	M	F+M	F	M	F+M
-ve	0	0	0	57	28	85	71	45	116	71	47	118	71	44	115	66	43	109
+ve	78	48	126	18	20	38	3	2	5	2	0	2	1	2	3	3	2	5
Total	78	48	126	75	48	123	74	47	121	73	47	120	72	46	118	69	45	114
p-v						**.031**			.647			.368			.336			.658

Pearson Chi-square p-value ≤0.05

Key: -ve – individuals showing no malaria parasitaemia at test time; +ve - individuals showing malaria parasitaemia at test time; p-v – p-value, and of significance is that of ≤0.05; Parasit. - parasitaemia
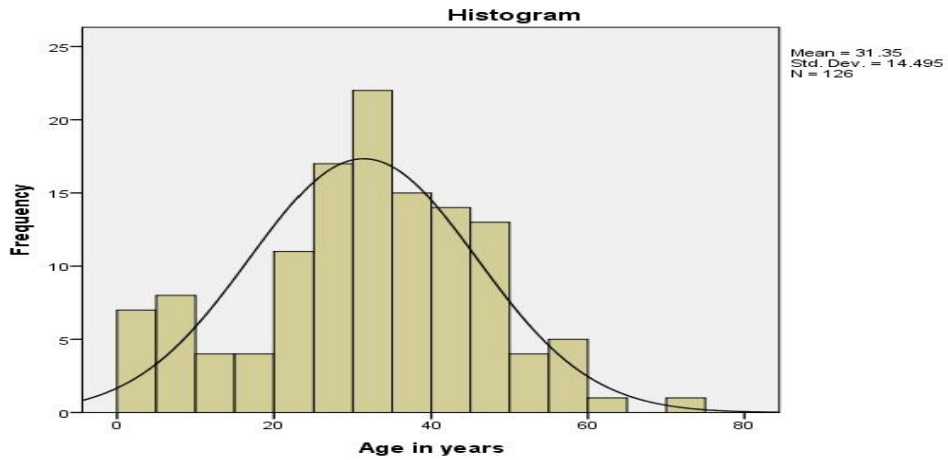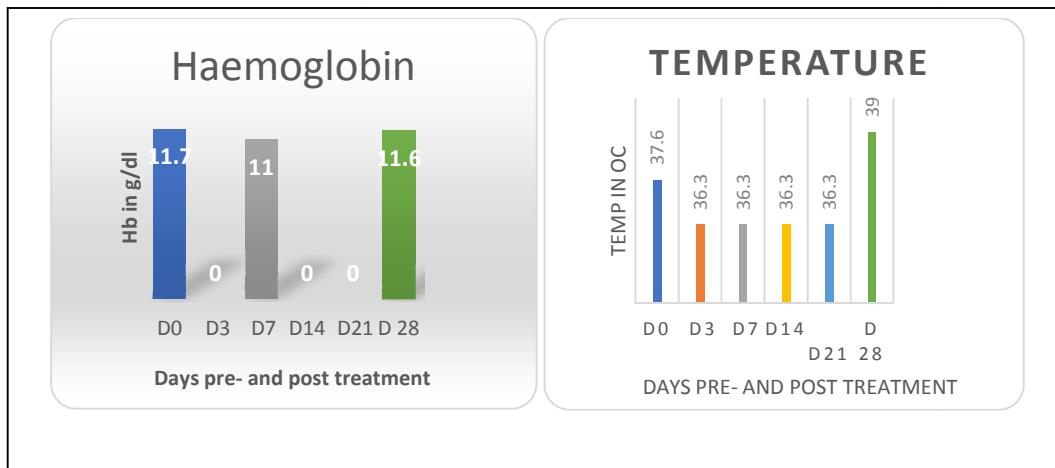
Fig. 2. Age of the participants

Fig. 3. Haemoglobin (Hb) and temperature during follow up

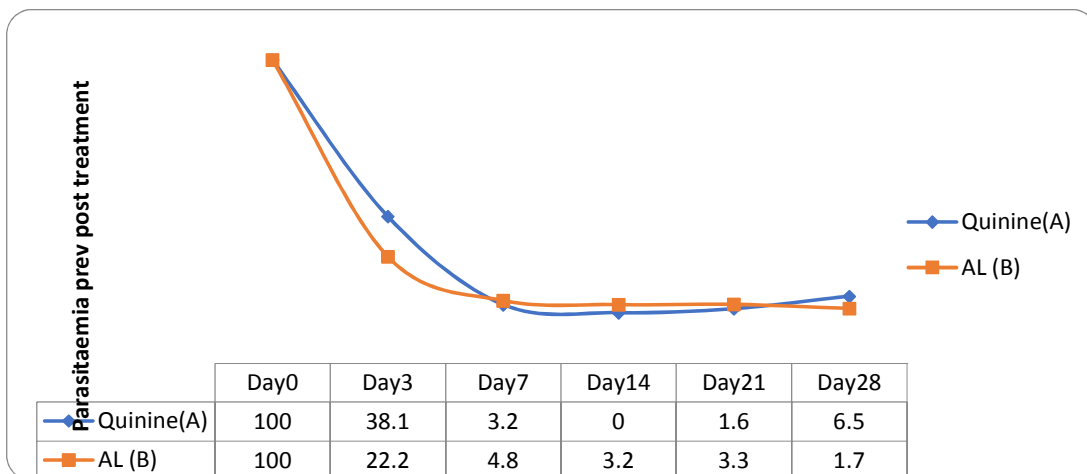|	Day0	Day3	Day7	Day14	Day21	Day28
Quinine(A)	100	38.1	3.2	0	1.6	6.5
AL (B)	100	22.2	4.8	3.2	3.3	1.7

Fig. 4. Change in malaria parasite biomass during anti-malarial treatment

In co-infection of malaria and HIV/AIDS, the impact of HIV-1 epidemic has been reported to enhance malaria parasite biomass in sub-Saharan African countries. Persistence of the co-infections in endemic areas may increase the rate of emergence of antimalarial drug resistance in endemic areas for both HIV-1 and malaria [10].

The findings in this study seem to suggest that individuals living in the malaria-endemic area may possess partial immunity against falciparum malaria, regardless of their co-morbidity with HIV status. Experimental studies have demonstrated that innate immune sensing of malaria parasite liver-stage infection and the ensuing innate responses can eliminate parasite biomass [25]. Further, it has also been reported that the production of malaria-specific tumour necrosis factor (TNF)-α and production of interleukin (IL)-12 were associated with reduced risk of fever and parasitaemia during follow-up studies lasting 28 days [26]. In yet another experiment, the persistent parasite growth in wild-type (WT) mice was solely found to be as a result of schizont sequestration in intravascular tissues. This could be a major biological process driving rapid and early increases in parasite biomass during blood-stage *Plasmodium* infection [4]. As witnessed, this study recorded an increase in recurrence of malaria as exhibited by both arms of anti-malarial recipients during follow-up, which could either be recrudescence or new infection. The recurrence in parasite biomass was noted particularly among quinine recipient group, which showed a more exponential percentage increase that could possibly be an indication of drug resistance.

The use of co-trimoxazole prophylaxis and antiretroviral therapy in PLWHA have substantially shown to reduce the morbidity of malaria in the affected patients, but however, required a confirmatory diagnosis of malaria [9].

It is a fact that malaria will not be controlled without effective medication for treatment and prevention, and that the loss of antimalarial drug efficacy has led to the tragic persistence of malaria transmission in many regions of the world [27]. Monitoring and evaluation of treatment, especially in PLWHA, from the time of initiation of anti-malarial treatment up to day-28 is significantly important to establish recurrence of malaria parasite biomass. In this study, recurrence of malaria biomass was noticed from day-21 and exponentially increased beyond the period of study.

5. CONCLUSIONS AND RECOMMENDATIONS

Based on the study findings, we conclude that there was a recurrence of malaria parasite biomass from day-21 following anti-malarial treatment with an exponential increase in intensity beyond the study period, which was more noticeable among the recipients of Quinine hydrochloride anti-malarial drugs. By day-28 haematological parameters had improved indicating recovery of the subjects. We recommend a life-lasting routine monitoring and evaluation of malaria cases among PLWHA so to mount strategies to prevent any consequences occasioned by resurgence/resistance of plasmodium parasites to antimalarials in use.

CONSENT AND ETHICAL CONSIDERATION

Authority to conduct this study was granted by the following Institutions: a) Scientific Steering Committee (SSC)/National Ethical Research Committee (NERC), KEMRI, Nairobi – No: SSC PROTOCOL No 1495 b) Academic Model for Providing Access To Health care (AMPATH) Research Committee of Moi University/Indiana University collaboration - Ref: RES/STUD/12/2009 and c) Institutional Research & Ethical Committee (IREC) of Moi Teaching & Referral Hospital/Moi University, School of Medicine, Eldoret – No: FAN: IREC 000421.

All issues pertaining to study were professionally observed: dignity, safety, and welfare of the study participants. Every participant was assigned a study number at the point of recruitment for use in all subsequent tests and test results to conceal identities of participants. Assents from all children aged between 8 and 17 years of age were sought, followed by their guardians/ parents' permission by a signed consent. Children aged between 2 and less than 8 years were consented to participate in the study by their parents/guardians.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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