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Efficacy of Artesunate-amodiaquine Combination Therapy against *Plasmodium falciparum* Malaria in a Forty-two Day Follow-up in the Ikata-Likoko Area of Southwest Cameroon

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Authors' contributions

This work was carried out in collaboration between all authors. Authors CBE, IUNS, LGL and HKK conceived the study. Authors CBE, IUNS, JEY and HKK performed the field and laboratory work. Author CBE analyzed the data, provided the artesunate-amodiaquin tablets and wrote the manuscript. Authors IUNS, LGL and HKK supervised while author CBE revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The main aim of this study was to monitor the efficacy of artesunate-amodiaquine (AS-AQ) against *Plasmodium falciparum* malaria in a 42 day follow-up in the Ikata-Likoko area of Southwest Cameroon. Specifically the late clinical failure (LCF), late parasitological failure (LPF), adequate

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clinical and parasitological response (ACPR) and variation in haematological parameters were assessed.

Study Design: Participants positive for *P. falciparum* parasitaemia in a baseline study were enrolled into the study and treated with AS-AQ following the WHO 2010 guidelines for the treatment of uncomplicated malaria. Follow-up was done according to WHO 2009 protocol for efficacy testing of antimalarial drugs. The study period was from June to September 2014.

Methods: During the follow-up, temperature, malaria parasitaemia (MP) and haematological parameters were recorded. Axillary temperature was used to monitor fever, MP through microscopy of Giemsa stained blood smears while haematological parameters were monitored through full blood count measured using an electronic blood cell analyser.

Results: The results showed LCF of 4% (4/101). The ACPR was 96.0% (97/101). Mean values of haematocrit (HCT) reduced significantly (P=.04) by day 14 before increasing. A constant reduction in mean cell volume (MCV) values were observed with the initial value being the highest (P=.04). Mean corpuscular haemoglobin concentration (MCHC) values increased significantly (P=.02) by day 28 and remained comparable. Fever reduced to 3% (3/101) by day 7, while mean body temperature remained below 37.5ºC after treatment. The mean temperature on day 0 (37.89ºC) was significantly highest (P=.03). Mild adverse events including abdominal pain, nausea, dizziness, anorexia and fatigue were observed in 21 (15.4%) participants.

Conclusion: AS-AQ remains effective against *P. falciparum* in the Ikata-Likoko area. However, there is need for continuous monitoring of its efficacy.

Keywords: Efficacy; artesunate-amodiaquin; Plasmodium falciparum; follow-up; Ikata-Likoko; Cameroon.

ABBREVIATIONS

ACT : Artemisinin based combination therapy
ACPR : Adequate clinical and parasitological response,
AE : Adverse events
AL : Artesunate-lumefantrine
AS-AQ : Artesunate-amodiaquine,
AS-MQ : Artesunate-mefloquine
AS-SP : Artesunate-sulphadoxine pyrimethamine
CDC : Centre for Disease Control and Prevention
DHAPQ: Dihydroartemisinin piperaquine
GMPD : Geometric mean parasite density
Hb : Haemoglobin
HCT : Haematocrit
IPTi : Intermittent preventive treatment for infants
IPTp : Intermittent preventive treatment for pregnant women
IRS : Indoor residual spraying
ITN : Insecticide treated bed nets
ITT : Intention to treat population
LCF : Late clinical failure
LLINs : Long-lasting insecticide treated bed nets
LPF : Late parasitological failure
MCH : Mean cell haemoglobin
MCHC : Mean corpuscular haemoglobin concentration
MCV : Mean cell volume
MP : Malaria parasitaemia
PCR : Polymerase chain reaction
Pf : Plasmodium falciparum
PP : Per protocol population
RBC : Red blood cell
SP : Sulphadoxine pyrimethamine
WBC : White blood cell
WHO : World Health Organization

1. INTRODUCTION

The control of *Plasmodium falciparum* malaria remains indispensable for the reduction of morbidity and mortality linked to the disease. Although there has been a scale-up in antimalarial activities, malaria persists as a major public health problem especially in Sub-Saharan Africa [1,2]. Several measures including the use of indoor residual spraying (IRS), sulphadoxine-pyrimethamine (SP) for intermittent preventive treatment (IPT) in pregnant women (IPTp) and infants (IPTi), window screens, insecticide-treated bed nets (ITNs) as well as prompt diagnosis and treatment are recommended by the WHO [3]. In this light, following the Abuja declaration of April 2000 on Roll Back Malaria in Africa by African Heads of State and governments,

measures have been put in place by governments to reduce malaria prevalence and burden.

In Cameroon, the government introduced the free distribution of insecticide-treated bed nets (ITNs) to pregnant women and children less than five years in 2003, the systematic distribution of ITNs and presently, the free distribution of long-lasting insecticide-treated bed nets (LLINs) to all households to prevent contact with the *Anopheles* vector. Currently, there is also free treatment of malaria for children under five introduced in 2010. Although these measures would reduce the morbidity and mortality linked to malaria, they may not be effective in some areas such as the Ikata-Likoko area as observed during our preliminary studies in the area. This persistence in malaria especially due to *P. falciparum*, may be attributed partly to antimalarial drug inefficacy.

Following inefficacy to effective antimalarial drugs such as chloroquine and SP, WHO recommended artemisinin-based combination therapies (ACTs) as first-line treatment of uncomplicated *P. falciparum malaria* in endemic regions [4]. ACTs consist of two active molecules, an artemisinin derivative and another antimalarial with longer half-life that is meant to clear malaria parasites not eliminated by the short-lived artemisinin component [5]. ACTs have a short treatment course which increases patient adherence. The artemisinin derivative is effective against the different blood stages [6,7] including multi-resistant *P. falciparum parasites*. These characteristics are susceptible to increasing their efficacy against *Plasmodium falciparum* malaria. Artemether-lumefantrine (AL), artesunate-mefloquine (AS-MQ), artesunate-sulfadoxine-pyrimethamine (AS-SP), dihydroartemisinin-piperaquine (DHAPQ) and artesunate-amodiaquine (AS-AQ) are the five recommended ACTs for uncomplicated malaria treatment [4].

AS-AQ was adopted for the treatment of uncomplicated malaria in Cameroon in 2004. It has been reported to be efficacious, safe and well tolerated in several studies [4,5,8-12]. It has also been shown to produce faster relief of the disease, rapid parasite clearance [13,14] and improvement in haematological parameters [15].

However, despite its efficacy, a decline in parasite clearance rates in ACT treated patients has been reported in Cambodia, the Lao People's Democratic Republic, Myanmar, Thailand, and Vietnam, [16-17]. Schramm et al. [18] also reported a reduced efficacy of AS-AQ in Liberia. In Cameroon, Ali et al. [12] reported the presence of resistant alleles against AS-AQ. Monitoring of therapeutic efficacy of ACTs in malaria-endemic countries is recommended every two years by the WHO [1] in a bid to detect a reduction of efficacy at early stages. Efficacy could be assessed through therapeutic efficacy studies [19].

Therapeutic efficacy studies measure patient response to treatment through parasite clearance and clinical response. It includes clinical and parasitological assessment within the first three days following treatment with ACTs. However, the usually slow response linked with haematological parameters may not be reliably assessed. Weekly follow-up of treatment efficacy will not only provide information on the long-term efficacy of the drug, but also the haematological changes that occur following treatment.

The Ikata-Likoko area is plagued with difficult accessibility and insufficient health care institutions. Consequently, malaria continues to pose a hindrance to the inhabitants' wellbeing. Access to adequate antimalarial is a problem and so the individuals in need are exposed to roadside drugs which are usually of doubtful quality and efficacy. The aim of this study was to determine the efficacy of AS-AQ against uncomplicated *P. falciparum* malaria in the Ikata-Likoko area. Specifically, the late clinical failure (LCF), late parasitological failure (LPF), adequate clinical and parasitological response (ACPR) and haematological changes that occur in participants after treatment were assessed.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in the Ikata-Likoko area of southwest Cameroon in continuation of a baseline study on the epidemiology of *Plasmodium falciparum* malaria. The area is characterized by typical tropical forest vegetation and climate. It has two seasons, the dry season which spans from October to March and the rainy season from March to September. Details of the description of the study area were published in the baseline study by Ebai et al. [20].

2.2 Study Design

This community based study was a 42 day therapeutic efficacy assessment of artesunate-

amodiaquin combination for the treatment of uncomplicated *Plasmodium falciparum* malaria using clinical, parasitological and haematological parameters. It constituted of weekly follow-up based on the WHO 2009 protocol for therapeutic efficacy [21]. The study was carried out between June and September 2014. Despite the unstable nature of the population, the 42-day protocol was chosen to assess haematological changes for a longer period.

2.3 Study Population

Following the prevalence of 33.5% obtained in the baseline study [20], the protocol for populations with moderate endemicity was applied as indicated in WHO, 2009 [21]. Participants aged six months and above with only *Plasmodium falciparum* asexual parasitaemia between 1000–100000 parasites/µL of blood, axillary temperature ≥37.5ºC or history of fever during the 24 h before recruitment, were able to swallow medication and follow the 42 day protocol were admitted into the study. Participants with signs of chronic or severe illness such as cardiac, renal or hepatic disease, HIV/AIDS, severe diarrhoea, dehydration, and those with history of treatment with AS-AQ in the past month, hypersensitive reaction to AS-AQ, were pregnant or breast feeding, were excluded from the study [21]. Also, participants who used any other antimalarial drug were excluded.

2.4 Sample Size

Due to an unknown AS-AQ failure rate in the area, a minimum of 50 participants was set. An additional 20% was added to account for participants who were likely to be either lost to follow-up or withdrew [21]. A minimum of 60 participants was expected to complete the study. However, due to the unstable nature of the population, their commitment to cocoa farming whose peak season coincided with the period of this study, up to 136 participants who qualified for the study were admitted.

2.5 Clinical Assessment of Participants

Participants were clinically examined by a nurse. History of fever and malaria was taken and recorded in a structured questionnaire. Axillary temperature of participants was measured using a clinical thermometer. Body weight was obtained using a floor scale.

2.6 Laboratory Procedures

2.6.1 Specimen collection and preparation

Venous blood was collected and two drops were used to prepare thick and thin blood films while four mLs were poured into EDTA tubes. The air-dried slides were transported in slide boxes alongside the blood in a cooler from the field to the Malaria Research Laboratory of The University of Buea. Thin blood films were fixed using absolute methanol for two minutes and stained with buffered Giemsa (pH 7.1) alongside thick films for 10 minutes and air dried [22].

2.6.2 Malaria parasite density determination and speciation

Examination of stained films was done on a binocular light microscope (Olympus, NY-USA) using the oil immersion objective (X100) and X10 eye piece. The number of parasites /µL of blood was estimated by counting asexual parasites against 200 white blood cells using a hand tally counter. The count was repeated in two other areas of the film and an average of the three counts was taken. Each slide was read by two parasitologists, in the case of a disparity the reading of a third parasitologist was considered. Using the number of parasites counted and the WBC count of participants, the parasitaemia per microliter of blood (MP/µL) was calculated. The determination of *Plasmodium* species was done on the thin blood film and confirmed using an identification chart as described by The Centre for Disease Control and Prevention [23].

2.6.3 Measurement of haematological parameters

Haematological parameters were measured using an electronic blood cell analyser (URIT 3300 ANALYSER). These included white blood cell count (WBC), red blood cell count (RBC), haemoglobin (Hb) and red cell indices. The red cell indices included Mean Cell Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Cell Haemoglobin (MCH). Anaemia was defined as Hb < 11 g/dL [22]. Values that were obtained during weekly controls were used to monitor changes in haematological parameters.

2.7 Treatment and Follow-up of *Plasmodium falciparum* Malaria

Participants were treated with artesunate-amodiaquine (AS-AQ Winthrop®) combination, 4

mg/kg body weight of artesunate and 10 mg/kg body weight of amodiaquine orally packaged in fixed dose combination tablets, once daily for 3 days [4]. Treatment was served alongside snacks (biscuits) to improve absorption. Antipyretic (paracetamol tablets at 15 mg/kg body weight every eight hours, for 24 h) was given on days 0, 1 and 2 to participants with temperatures >38°C alongside the antimalarial treatment, as prescribed by national guidelines. Participants were observed for at least 30 minutes after treatment and those who vomited were served another complete dose of the treatment. In case vomiting continued, the participant was withdrawn from the study and referred to the hospital. Instructions were given to participants, parents or guardians to serve the treatment on days 1 and 2 with food or snacks. There was no AS-AQ treatment supervision on days 1 (2^{nd} dose) and 2 (3^{rd} dose), no clinical assessment and blood examination on days 1, 2 and 3 as prescribed by the WHO protocol. Participants were however contacted on phone at least once on days 1 and 2 to remind and confirm drug administration, and up to day 6 to find out any adverse events (AE) or ill health. AE was defined as any sign or symptom that was not observed during admission into the study but appeared following treatment. Follow-up visits were done on days 7, 14, 21, 28, 35 and 42 from 7am to 6:30pm. This included the measurement of axillary temperature, recording of reported adverse events and collection of blood samples for parasitaemia and haematological parameters. All participants who did not come for follow-up at the collection sites were contacted and attended to at home on the same day.

2.8 Outcomes

Outcomes of this study were clinical and parasitological parameters adopted from WHO protocol for 42 days follow-up [21]. These included: late clinical failure (LCF), defined as the presence of danger signs or severe malaria in the presence of parasitaemia on any day between day 4 and day 42; or the presence of parasitaemia on any day between day 4 and day 42 with axillary temperature ≥ 37.5ºC; late parasitological failure (LPF) was defined as the presence of parasitaemia on any day between day 7 and day 42 with axillary temperature <37.5ºC. Any participant with absence of parasitaemia on day 42, irrespective of axillary temperature, who did not previously meet any of the criteria of LCF or LPF was said to have adequate clinical and parasitological response (ACPR). Early treatment failure could not be evaluated since clinical assessment and blood examination for malaria parasite were not done on days 1, 2 and 3. Given that PCR confirmation was not done to distinguish between re-infection and recrudescence as recommended by the standard WHO protocol, higher parasite count/µL of blood than the previous count which was not negative, was considered as failures. A positive sample that was negative during the previous reading was considered PCR unconfirmed re-infection. The cure rate was defined as the proportion of participants who did not meet the criteria of either LCF or LPF on a follow-up day of the study. The cure rate was reported for both the per protocol population (PP), calculated without considering participants who were lost or withdrew in the course of follow-up and the intention to treat population (ITT), calculated taking into consideration all participants included in the study.

2.9 Statistical Analyses

Data was analysed using IBM Statistical Package for Social Sciences (IBM SPSS) version 20 (IBM Inc. 2012). Proportions were compared using the Cramer's V, while the Kruskall-Wallis test was used to compare GMPDs (geometric mean parasite density) and mean values of haematological parameters. P-values less than .05 were considered significant.

3. RESULTS

3.1 Baseline Data of Participants

One hundred and thirty-six participants were enrolled into the study. The distribution in terms of age, sex and locality of the 136 participants are presented in Table 1.

3.2 Treatment Outcome after Follow-up

A complete follow-up was done for 101 participants (Fig. 1). The overall adequate clinical and parasitological response rate was 96.0%. Four late clinical failures were recorded (Table 2). Three of the failures were detected on day 7 and one on day 14. Thirty five (35) participants did not complete the study (twenty-nine were lost to follow-up while 6 withdrew consent) as shown in Fig. 1. The PCR uncorrected cure rate in the ITT population on day 28 was 71.3% while that of the PP population on the same day was 96.0%. No case of re-infection was observed.

Table 1. Baseline information of the participants

Characteristic	Category	Frequency (%)/value (range)
Sex	Male	55 (40.44)
	Female	81(59.56)
Age group (years)	<5	5 (3.7)
	5-14	60 (41.1)
	>15	71 (52.2)
Locality	Bafia	35 (25.7)
	Ikata	33 (24.3)
	Likoko	37 (27.2)
	Mile 14	31 (22.8)
Mean age ± SD (years)		24.2±16.9 (range: 2-74)
GMPD (parasites/µL)		1074.12 (range: 1000-10000)
Mean temperature ±SD (ºC)		37.89± 0.96 (range: 37.5-40)
Mean weight ± SD(Kg)		47.8±20.8 (range:10-91)
Mean haemoglobin ± SD (mg/dL)		12.36±3.6 (range:7-24)

Table 2. Treatment outcome of artesunate-amodiaquine against *P. falciparum* in the Ikata-Likoko area of southwest Cameroon

Outcome	Frequency (%)
Late clinical failure	04 (4.0)
Late parasitological failure	00
Adequate clinical and parasitological response	97 (96.0)
Cure rate per protocol day 42 PCR uncorrected	96.0 (97/101)
Cure rate intention to treat day 42 PCR uncorrected	71.3 (97/136)
Cure rate per protocol day 28 PCR uncorrected	96.0
Cure rate intention to treat day 28 PCR uncorrected	71.3 (97/136)
Infection with different species of *Plasmodium*	0 (0.0)
Loss to follow-up	29 (21.3)
Withdrew from the study	06 (4.4)

3.3 Variation of Body Temperature and Fever during Follow-up

The proportion of participants with fever decreased significantly to 3% on day seven after treatment, and to zero on day 28 (Fig. 2). An increase in cases of fever was later observed, 3 on day 35 and 3 on day 42 although microscopy showed no parasites. The mean temperature on day 0 (37.89, range: 37.5-40°C) was significantly higher (P = .03), than any other day in the follow-up while mean temperature was comparable between all the days of follow-up (P = .3, range: 36.9-37.03°C).

3.4 Variation of Haemoglobin Concentration and Malaria Parasitaemia

There was a tendency for haemoglobin values to increase as GMPDs decreased following treatment. Mean haemoglobin value was lowest on day zero when compared to the values of the subsequent follow-up days although the difference was not statistically significant (P=.07) (Fig. 3). The values however increased on day 21 but were comparable with the other days following treatment (P>.05). All participants remained negative for MP (malaria parasitaemia) from day 21 till day 42 (Fig. 3).

3.5 Variation of Haematological Parameters Post-treatment with AS-AQ

The initial WBC count (9.8x 10^9/L) was significantly higher (P = .04) than all the values recorded during follow-up. The lowest WBC count (8.0x10^9/L) was recorded on day 14 after which the values increased successively up to day 28. However, the difference between the

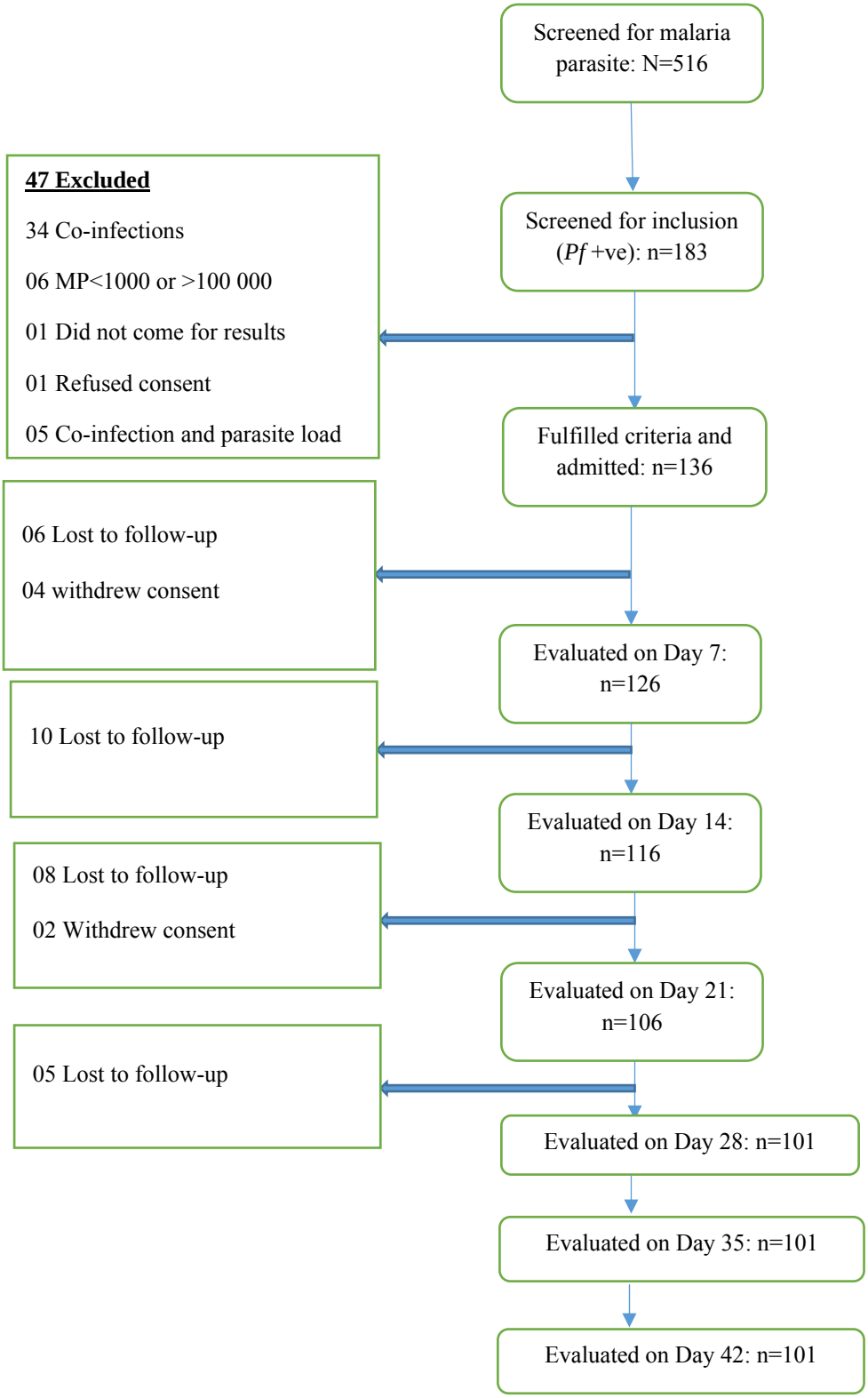

Fig. 1. Screening and follow-up profile for assessment of the efficacy of artesunate-amodiaquine combination therapy in the Ikata-Likoko area of southwest Cameroon

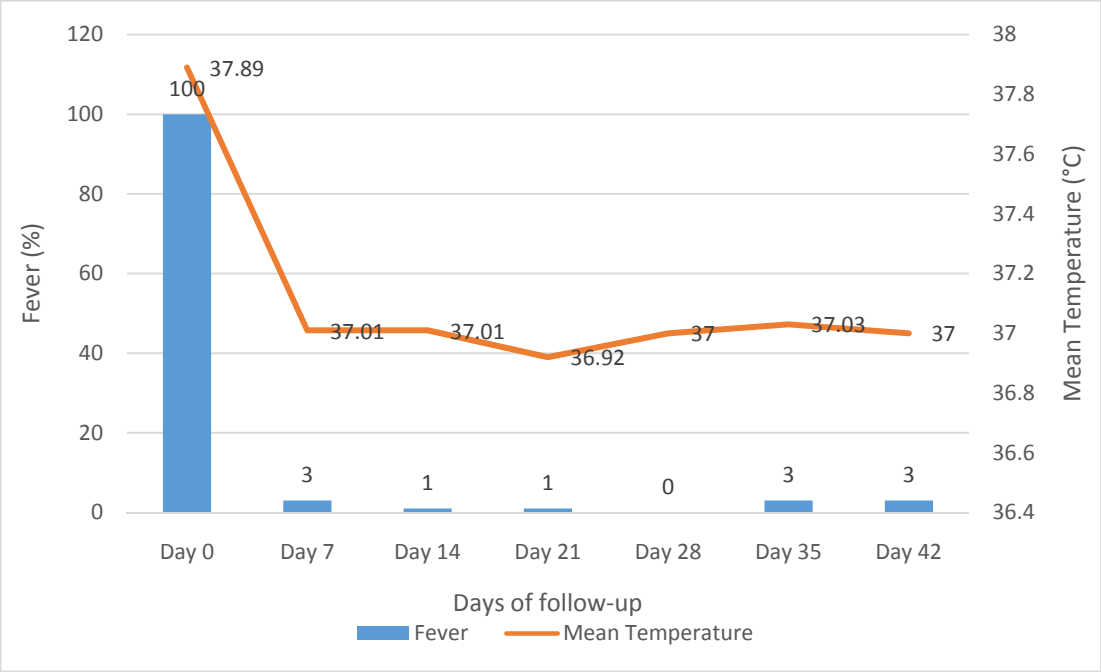

Fig. 2. Variation of fever and temperature during follow-up

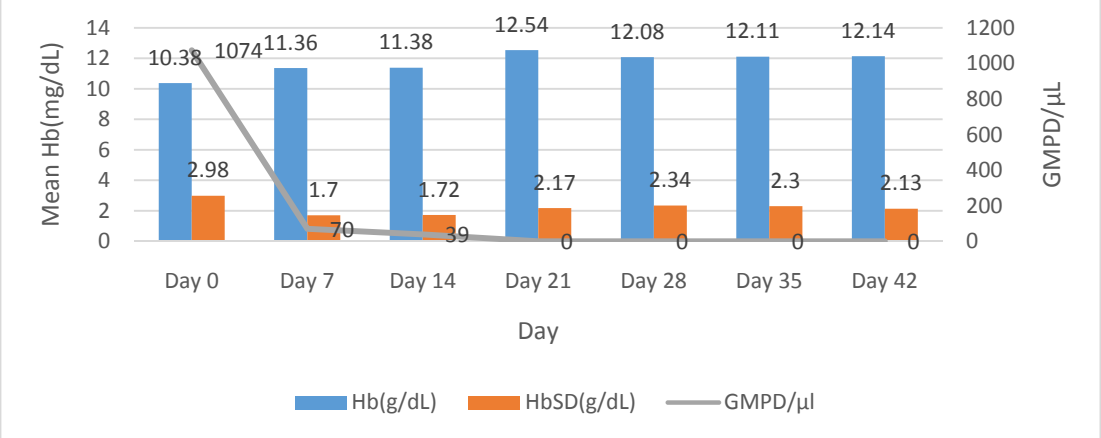

Fig. 3. Variation of mean haemoglobin concentration and GMPD post-treatment with artesunate-amodiaquine

WBC values from day 21 to day 42 was not significant (P>.05).

The mean RBC count on day 28 was similar to the initial count but significantly higher than values recorded on other days of treatment follow-up (P = .03). Mean values of HCT reduced significantly (P = .04) following treatment between day zero and day 14 and then remained comparable (P>.05) till day 42 (Table 3). A constant reduction in MCV values was observed following treatment with the initial value being the highest (P = .04). Mean values of MCH were comparable all along the treatment follow-up (P>.05), while mean values of MCHC depicted a constant gradual increase up to day 28 when the highest value (35.6 g/dL) was observed (P = .02).

3.6 Adverse Events

During follow-up, 21 (15.4%) of the participants presented with adverse events which included abdominal pain, nausea, dizziness, anorexia, and fatigue (Table 4). These events were mild and subsided soon after treatment was completed.

Table 3. Variation of haematological parameters post-treatment with AS-AQ

Day of follow-up	WBC±SD ($x10^9$/L)	RBC±SD ($x10^{12}$/L)	HCT±SD (%)	MCV±SD (fl)	MCH ±SD (pg)	MCHC±SD (g/dL)
0	9.8(20)	4.73(0.98)	35.87(8.29)	82.41(8.40)	26.80(3.02)	33.00(1.44)
7	9.6(4.8)	4.27(0.98)	33.04(8.27)	79.41(10.11)	27.16(3.07)	33.02(2.02)
14	8.0(4.6)	4.35(0.98)	33.04(8.27)	77.49(10.11)	26.56(3.08)	34.43(2.11)
21	8.5(4.3)	4.67(0.80)	35.00(4.96)	77.66(10.61)	27.12(3.10)	35.09(2.57)
28	8.6(5.1)	4.73(1.27)	34.76(8.50)	76.31(3.24)	27.21(3.24)	35.60(3.40)
35	8.3(4.6)	4.69(1.24)	33.87(8.54)	74.87(9.14)	26.42(3.22)	34.79(3.45)
42	8.4(4.3)	4.70(1.39)	33.895(8.16)	74.88(7.77)	26.42(4.56)	34.85(5.63)
Level of significance*	**P = .04**	**P = .03**	**P = .04**	**P = .04**	**P>.05**	**P = .02**

**Level of significance is the difference between day 0 and day 7*

Table 4. Adverse events reported on participants in the Ikata-Likoko area of southwest Cameroon

Adverse event	Frequency (%)
Abdominal pain	10 (7.3)
Nausea	6 (4.4)
Dizziness	17 (12.5)
Anorexia	3 (2.2)
Fatigue	14 (10.3)
Total with at least one event	21(15.4)

4. DISCUSSION

The use of artemisinin based combination therapy as first-line treatment for uncomplicated malaria in endemic areas was recommended by WHO following reports on widespread resistance of malaria parasites to antimalarial drugs [4]. This resistance to antimalarial drugs is globally on the rise, prompt and adequate treatment remains the most effective measure to fight against the disease morbidity. Since 2004, AS-AQ has been used in the treatment of uncomplicated *Plasmodium falciparum* malaria in Cameroon. However, continuous monitoring remains indispensable for the detection of reduced efficacy.

The observed efficacy in this study shows that AS-AQ remains adequate in the treatment of *Plasmodium falciparum* malaria, given that the efficacy is well above the 90% recommended by WHO [21]. The ACPR of 96.0% on day 42 shows that it is effective against blood stages of *Plasmodium falciparum*. This finding is higher than that of Nji et al. [24] (ACPR: 89.4%) in Cameroon and 52.8% recorded by Schramm et al. in Liberia [18] but lower than 98.9% reported by Yeka *et al*. [25] in Uganda. In like manner, the cure rate (PCR uncorrected) in the PP population on day 28 of this study (96.0%), confirms similar efficacy reports (90.2%) by Apinjoh et al. [26] in a trial in southwest Cameroon, but higher than 52% recorded by Yeka et al. [25] in Uganda.

The low cure rate (PCR uncorrected) in the ITT population in this study (71.3%) may have resulted from the several participants lost to follow-up and those who withdrew their consent. This was lower than 81.4% reported by Nji *et al.* [24], in a study carried out between 2009 and 2013. Considering the time past, the efficacy might have reduced though the studies were not carried out in the same population. Similarly, the PCR uncorrected cure rates with ITT on day 28 in this study (71.3%) was lower than the 86% obtained by Apinjoh et al. [26] possibly due to a higher loss to follow-up during this study or due to a possible reduction in efficacy with time.

With respect to the two participants who presented with parasitaemia greater than the previous follow-up value on day 14, this may be due to the variability in pharmacokinetic profiles of antimalarial drugs (including AS-AQ) between individuals [27], which has been linked with differences in the level of drug response with outcome including incomplete cure and recrudescence.

There was a general decrease in cases of fever in the study population following treatment. Given that fever is a common symptom in *falciparum* malaria, it is possible that the clearing of the parasites reduced the rate of fever in the population. The rapid decrease in overall fever prevalence in AS-AQ treatment was also reported in the study by Apinjoh et al. [26]. However, fever in some participants on days 7 to 21 may have been due to other factors including other infections or level of parasitaemia.

Haematological parameters showed a general increase following treatment except for WBCs. The decrease in mean RBC and HCT values (P=.04) on day 7 following treatment could be due to an attenuation effect of amodiaquine on artemisinin. Artemisinin derivatives have been reported to selectively kill parasites without the spleen destroying the RBCs containing the dead parasites in a process called spiting [28]. In this study, it is likely that the decrease in red cell count may be due to an attenuation effect of amodiaquin on artemisinin favoured spiting on red cells containing dead parasites as demonstrated by Sowunmi et al. [29]. The constant increase in Hb values to day 21 post-treatment is a confirmation that infection with *P. falciparum* parasites is associated with reduced Hb levels in blood and consequently anaemia [30-33]. The increase here is an indication that AS-AQ effectively cleared the parasites and favoured the increased levels of Hb. This observation corroborates others in Cameroon [15,26,34]. The observed general decrease in values of MCV could be associated with a reduction in red cells size as they mature following treatment. It is known that production of RBCs is stimulated in anaemic conditions due to the increase in oxygen demands. The numerous immature red cells newly produced could account for the high values of MCV due to their greater size. As the cells mature following treatment their sizes reduce and consequently the MCV values [22]. Increase in values observed in MCH and MCHC up to the day 21 could be attributed to the corresponding increase of haemoglobin values since they are directly related.

Adverse events were reported in 15.4% of the participants with the most frequent being dizziness (12.5%) but didn't persist beyond day 14 of the follow-up. These events have also been reported in other studies [26,35,36].

Limitations of this study included the absence of clinical and parasitological assessment during days 1, 2 and 3 and the non-confirmation of parasitological analysis with PCR. Follow-up on days 1, 2 and 3 was difficult as most of the participants spent most of their days working in their farms and may only come home during weekends. That notwithstanding, the findings revealed that AS-AQ clears *Plasmodium falciparum* parasites in blood within the first week; and that haematological parameters and fever ameliorate within the first week following the start of treatment. The loss to follow-up was mainly due to individuals who spent days working in their farms and others who travelled for personal reasons.

5. CONCLUSION

Artesunate amodiaquin combination effectively treated *P. falciparum* malaria as it cleared the parasites in blood and ameliorated clinical and haematological parameters associated with the infection in the Ikata-Likoko area. However, patients should always be informed of the potential adverse events when treated with this drug. Continuous monitoring of AS-AQ efficacy should be carried out regularly to detect any decline in efficacy.

CONSENT

Informed consent information about the procedures, risks and benefits were read out to participants. Consent and assent forms were signed by adults, parents and legal guardians of children and minors who were participants. Participation in the study was voluntary. All participants consented that information collected in this study can be published.

ETHICAL APPROVAL

Administrative authorization was obtained from the Regional Delegate of Public Health for the South West, Cameroon. Ethical approval was obtained from the Institutional Review Board of the University of Douala.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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