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Effect of Nevirapine-based Antiretroviral Therapy on the Treatment Outcome of Uncomplicated Malaria with Artemether-Lumefantrine among HIV-infected children

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ABSTRACT

Background: The geographical overlap of malaria and HIV in sub-Saharan Africa posed a major public health challenge, which is further worsened by the potential interactions between antimalarial and antiretroviral drugs when co-administered. This study aimed to compare the responses to artemether/lumefantrine (AL) treatment among HIV-infected children on nevirapine based highly active antiretroviral therapy (HAART) and HIV-non-infected children that were positive for *Plasmodium falciparum* malaria parasites.

Methods: This is a multi-centered, prospective, non-randomized, open-labelled study with two arms consisting of HIV- infected children on nevirapine-based HAART (NVP-arm; n=32) and HIV-non-infected children (control-arm; n=40). Both groups of patients were treated with AL after microscopic confirmation of *P. falciparum* and were actively monitored for 28 days for efficacy and safety. Primary outcome was Adequate Clinical and Parasitological Response (ACPR) after treatment with AL by day 28.

Results: Day 28 ACPR was lower in the NVP-arm (90%) compared to the control-arm (100%) of the study. In the NVP- arm of the study, 5% of cases had early treatment failure and 5% had late parasitological failure. The cumulative risk of developing recurrent malaria in NVP-arm was not statistically significantly higher than in the control-arm (P= 0.07). The reported potential adverse reactions to AL were mild and included cough, pyrexia, anorexia, and abdominal pain. The cumulative risk of developing cough, pyrexia, anorexia and abdominal pain between NVP-arm compared to the control-arm was not statistically significant (Hazard ratio [HR], 0.51, 0.79 and 0.37 [95% confidence interval {CI}, 0.04–5.26, 0.07-9.32, and 0.04- 3.56]; P= 1.000, 1.000, and 0.637) respectively.

Conclusion: Treatment of uncomplicated malaria with AL was safe and effective after 28 days of follow-up in HIV- infected children on NVP. Nevirapine based HAART may, however, lead to delayed parasite clearance in children treated with AL.

1. Introduction

Malaria remains a major public health problem in Nigeria despite the giant strides taken by the government to control the disease. Malaria, in Nigeria, accounts for more deaths in children than in any other place in the world. It was estimated that the number of deaths accrued to malaria was 409,000 in 2019 compared to 411,000 reported globally in 2018; Nigeria being responsible for nearly 23% of the total death[1].

Artemether-lumefantrine is the first-line treatment for uncomplicated malaria in Nigeria. Artemether is short acting and responsible for the early clearance of malaria parasite in the system while the lumefantrine in the fixed-dose combination is long acting and responsible for the delayed clearance of malaria parasite and prevention of recrudescence[2,3]. Lumefantrine ensures efficacy of the combined antimalaria to adequately treat malaria. Artemether and lumefantrine are both substrates for CYP450 enzyme. The drugs are rapidly metabolized into their respective active metabolites, dihydroartemisinin (DHA) and desbutyl-lumefantrine (DBL)[4]. The DHA is formed from artemether through CYP450-mediated demethylation[5].

Antiretroviral agents for HIV/AIDS treatments have demonstrated varied effects on malaria prevention and treatments[6,7]. The overall effects have been linked to the treatment type and CYP450 enzyme mediated drug-drug interaction. Nevirapine-based antiretroviral therapy is a common treatment for HIV/AIDS in children in Nigeria with potential for interaction with artemether-lumefantrine[8]. This drug has the potential to impact negatively on the treatment outcome of malaria in this age group[9].

The efficacy and tolerability of AL have been established in diverse populations[10-13]. However, continuous efficacy check is mandatory for early detection of resistance to antimalarial treatment.

Since children living with HIV/AIDS are at a higher risk of increased morbidity and mortality due to inadequate malaria treatment, it is important to monitor the efficacy of malaria treatment because of possible therapeutic failure that may result from an interaction between the antimalarial and antiretroviral drugs. This study aims to compare the responses to artemether/lumefantrine (AL) treatment among HIV-infected children on nevirapine based highly active antiretroviral therapy (HAART) and HIV-non-infected children that were positive for *Plasmodium falciparum* malaria parasites.

2. Methods

2.1 Study Setting and Ethical Approval

The study was conducted between March 2017 and December 2018 at four HIV centers; Lagos University Teaching Hospital, Ikorodu General Hospital, Ijede General Hospital and Massey Street Children Hospital, Lagos, Southwest Nigeria. The protocol of this study was approved by the Health Research Ethics Committee of the Lagos University Teaching Hospital. The study was assigned the approval number (ADM/DCST/HREC/1437). Informed consent was obtained from the legally authorized representative for all the children below 7 years and assent obtained from older children. This research was conducted according to Good Clinical Practice[14].

2.1.1 Study Population

The population studied are two groups of children 2 to 12 years infected with malaria. One group are HIV infected children being treated with nevirapine-based antiretroviral regimen for more than 2 months (n=32) and the other group are children without HIV serving as control (n=40).

2.2 Patients' Eligibility and Enrolment

All HIV infected patients accessing care at the HIV centers were routinely screened for falciparum malaria using rapid diagnostic test (RDT) kit SD Bioline® and subsequently confirmed through malaria microscopy, including those with fever and other symptoms of uncomplicated malaria such as muscle aches, headache, chills, body weakness, pallor, nausea, vomiting, and poor appetite. Patients were enrolled into the study only when they met the inclusion criteria which include: age 2 to 12 years; microscopic confirmation of *Plasmodium falciparum* malaria; body weight ≥6 kg; absence of severe malnutrition; hemoglobin concentration not less than 7g/dL; no history of associated comorbidity such as tuberculosis, liver or renal failure; no history of recent use of any drug that impact CYP450 enzymes; no prior malaria treatment in ≤28 days, no signs of complicated malaria such as multiple or repeated convulsions, hyper/hypo-glycaemia, macro-hemoglobinuria, extreme prostration and hyperpyrexia, and easy accessibility to the study centers.

2.2.1 Treatment, Sample and Procedure

Children positive for *Plasmodium falciparum* malaria; as examined using thick and thin Giemsa-stained blood smear through microscopy method done under the supervision of a WHO level one certified malaria microscopist were

enrolled for this study with the intent to treat. A standard 6-dose treatment of weight-based artemether-lumefantrine (Coartem® Dispersible 20 mg/120 mg, Novartis Pharma AG, Basel, Switzerland) was administered with milk in the clinic (initial dose) and at home (continued treatment), to enhance and control for lumefantrine absorption[15]. Active follow-up was instituted from day 0 (diagnosis) to day28. Blood sampling for laboratory assessment done on days 0 (pre-first dose), 3, 7, 14, 21, and 28. Venous blood was collected into vacutainer bottles containing Lithium-heparin for blood chemistry evaluation and EDTA for malaria parasite counting and hematological tests. A dry blood spot was made on Whatman filter paper for malaria parasite genotyping. Recurrent malaria was typified by parasite genotyping using *Plasmodium falciparum* merozoite surface protein 2 (PfMSP2) in a nested-PCR. Laboratory and clinical adverse drug reactions were monitored and documented.

2.2.2 Malaria Parasite Density Estimation

Malaria microscopy remains the primary endpoint measurement of the level of malaria infection that is routinely relied upon in epidemiological studies, intervention studies, and clinical trials, involving malaria parasite[16]. This is expressed as parasite density and is classically defined as the number of asexual forms of parasite relative to a blood volume in microliter[17]. In the four basic counting techniques using microscopy[18], White Blood Cells (WBCs) are relatively used in estimating *Plasmodium* parasitemia by counting the number of parasites against a predetermined number of WBCs on Giemsa-stained blood smears.

Due to the frequent lack of facilities in some malaria endemic countries to quantify WBCs, an assumed WBCs count of 8000/μL of blood has been accepted by World Health Organization as reasonably accurate to estimate malaria parasite densities[19]. Assumed WBCs count of blood may generate systematic errors which could produce incorrect conclusions in patient management or during clinical research that uses malaria parasite counts as an end point[18,20].

Parasite densities for all participants were, therefore, calculated using assumed WBCs count of 5000/μL, 6000/μL, 8000/μL, and 10000/μL of blood. In addition, we used the WBCs reference values established for children < 5 years (9200/μL)[21]. Blood smears from each participant were stained with Giemsa stain for 30 minutes. Parasite density was estimated by counting the number of asexual parasites per 200 (per 1,000 for gametocytes) white blood cells (WBC) on a thick smear. All thick blood smears were independently read by two experienced Technologists. A smear was declared negative if no asexual parasites were seen after examining 200 high-power fields. An additional reading was performed for discordant results. An average of the parasite counts from the two technologists was taken for each sample.

2.3 Outcome measures

An adequate clinical and parasitological response in patients at 28 days after antimalarial treatment was the primary objective of the study. The classification of treatment outcome was based on WHO guidelines[22]. Adequate clinical and parasitological response (ACPR) was defined as the absence of parasitemia by day28 after initial treatment with AL, irrespective of axillary temperature, and not meeting any previous criteria for early treatment failure (ETF), late clinical failure (LCF), or late parasitological failure (LPF). The secondary outcome was the clinical and laboratory adverse events.

2.4 Statistical Analysis

Data were evaluated based on intention-to-treat analysis including all eligible patients in nevirapine and control groups. Continuous data were expressed as mean with standard deviation or median with interquartile range (IQR) and the statistical significance was tested between the nevirapine group and the control group using student's t-test statistic or Mann-Whitney test for continuous variable, respectively. Categorical variables were expressed as frequency (percentage) and the statistically significant associations between variables were tested using the Chi-square test or Fisher exact test. Kaplan-Meier survival curve was used to determine cumulative success in parasite clearance against days and a log-rank test was used to determine the equality of survival distributions for the different groups. The adverse reaction was assessed based on the cumulative risks of the first occurrence of individual adverse events following the initiation of artemether/lumefantrine and was estimated using the Kaplan-Meier product limit formula. The risks of individual adverse events between treatment arms were compared using Cox proportional hazard models. A two-tailed P-value of <0.05 was considered statistically significant.

3. Results

Out of a total of 1,032 children screened for falciparum malaria with rapid diagnostic test kit, 72 (6.98%) children

were positive and were recruited for this study; consisting of 32 children in the nevirapine group and 40 children in the control group. A total of 52 children were included in the final analysis, consisting of 20 children in the nevirapine group and 32 children in the control group. The reasons for the attrition were summarized in Table 2.

3.1 Baseline Characteristics of the Study Participants: The median (Inter-quartile range, IQR) age of the study participants was 7.50 (6.25-9.00) years in the control group and 9.50 (8.00-12.00) years in the nevirapine group (P= 0.0015). The median (IQR) weight for the control and nevirapine groups were 24.50 (21.25 – 29.00) kg and 24.00 (22.00 – 29.00) kg, respectively. The control group and the nevirapine group consist of 16 (50%) and 12 (60%) females, respectively.

Table 1: Baseline characteristics of the demographics, laboratory parameters and antimalarial doses for all the participants with uncomplicated malaria

Baseline Characteristics	Control group (n=32) [a]	Nevirapine group (n=20) [a]	*P*
Age (years)	7.25 (6.25 – 9.00)	9.50 (8.00 – 12.00)	0.0015 [b]
Weight (kg)	24.50 (21.25 – 29.00)	24.00 (22.00 – 29.00)	0.6597 [b]
Gender, No. (%)			
Female	16 (50.00)	12 (60.00)	0.573[c]
Male	16 (50.00)	8 (40.00)	
HB (g/dL)	10.67±0.78	9.37±0.27	0.193[d]
AST (U/L)	9.09±0.93	7.98±0.85	0.407[d]
ALT (U/L)	5.27±0.75	4.52±0.41	0.454[d]
ALP (U/L)	25.13±1.02	25.08±0.52	0.974[d]
Urea (mg/dL)	25.19±1.42	23.59±0.98	0.418[d]
Creatinine (umol/L)	2.29±0.68	1.26±0.05	0.244[d]
Parasite Density	21478 (7774-310080)	20907 (8378-41512)	0.915[b]
CD4 count (cells/uL)	N/A	456 (320-750)	
Artemether dose (mg)	285.00 ±73.09	288.00±60.31	0.879[d]
Lumefantrine dose (mg)	1710±438.53	1728±361.89	0.879[d]

[a]Values are Mean ±SD or Median (Interquartile range)
[b]Reported *p* values calculated using the Mann-Whitney test for continuous variable
[c]Reported *p* values calculated using Chi-squared test for categorical variable
[d]Reported *p* values calculated using unpaired t-test for continuous variable
N/A refers to not applicable

3.2 Treatment outcome of uncomplicated malarial episodes treated with artemether/lumefantrine

Standardized WHO[23] treatment outcomes after 28 days of follow-up and the cumulative risk of recurrent malaria on or before day 28 were presented in Table 2 and Figure 1. Adequate Clinical and Parasitological Response (ACPR) was recorded in 100% (95% CI= 90 - 100) at day 28 in the control group compared to 90% (95% CI= 70.0 - 100) recorded in the nevirapine group. Early treatment failure in the form of delayed parasite clearance was observed in the 5.0% (95% CI =0.0-5.0) of the nevirapine group. PCR analysis of the recurrent malarial episode in 5.0% (95% CI =0.0-5.0) showed a case of recrudescence. Kaplan-Meier survival curve for cumulative success in parasite clearance against day for the control and nevirapine groups is as presented in Fig. 1. Log Rank test for equality of survival distributions for the different groups shows no statistically significant differences between the two curves ($p = 0.07$).

Table 2: Malaria treatment outcome for 28 days

	AL		Nevirapine +AL	
End point for Day 28	Number	Proportion (95%CI)	Number	Proportion (95%CI)
Adequate Clinical and Parasitological Response (ACPR)	32	1.0(0.9-1.0)	18	0.90(0.14-0.20)
Early Treatment Failure (ETF)	0	0.0(0.0-0.03)	1	0.05(0.00-0.05)
Late Clinical Failure (LCF)	0	0.0(0.0-0.03)	0	0.00(0.00-0.03)
Late Parasitological Failure (LPF)	0	0.0(0.0-0.03)	1	0.05(0.00-0.05)
Total analysis	32		20	
Withdrawal	3		3	
LFU*	5		9	
Total	40		32	

*Lost to follow-up

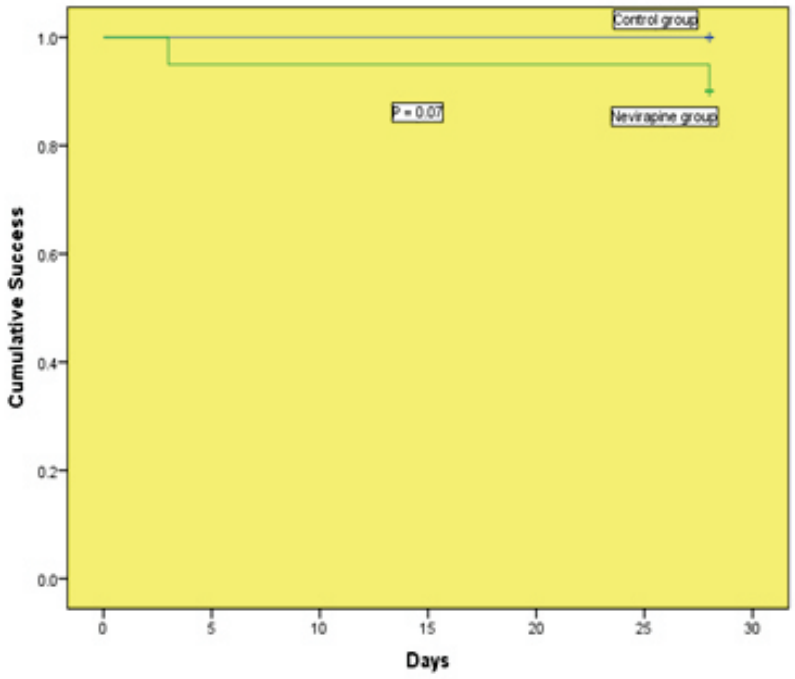

Fig. 1. Kaplan-Meier survival curve

3.3 Risks for clinical adverse effects of AL for all the participants

Among the list of pre-determined clinical adverse effects documented for AL use in children, four of them (cough, pyrexia, anorexia, and abdominal pain) were cumulatively recorded in 16 (30.8%) of the participants. The severity of all the adverse effects was mild and resolved within seven days following initiation of AL therapy. Adverse effects due to cough, pyrexia, anorexia, and abdominal pain occurred in 11 (34.4%) of the control group compared to 5 (25.0%) of the nevirapine group. The risk of developing each of the adverse effects reported and the hazard ratio between the groups was recorded in Table 4. The Cox proportional hazard model shows no statistically significant difference in the risks for these adverse effects between the groups at different time intervals.

Table 3: Risk of common adverse effects to AL after days 1 to 7 of antimalarial therapy initiation

		Control	Nevirapine		
Days	ADR	Risk*		HR† (95% CI)	*p* value
1-7	Cough	9.38%	5.00%	0.51 (0.04-5.26)	1.000
	Pyrexia	6.25%	5.00%	0.79 (0.07-9.32)	1.000
	Anorexia	6.25%	10.00%	1.67 (0.22-12.89)	0.634
	Abdominal pain	12.50%	5.00%	0.37 (0.04-3.56)	0.637

* Cumulative risk during the time interval of interest estimated using the Kaplan-Meier product limit formula
†HR = hazard ratio for Nevirapine vs. control, generated using Cox proportional hazard models adjusted for repeated measures in the same patient

3.4 Comparison of the Risks for Laboratory Adverse events

Laboratory parameters measured include hemoglobin (Hb), Liver function (AST, ALT, and ALP), and Kidney function (urea and creatinine clearance) were measured and recorded for all the participants at each visit. The mean values of the parameters at each visit were compared for both the control nevirapine groups. The mean Hb values for the control groups did not statistically significantly differ from those of the nevirapine groups from day 0 to day 28 of treatment (Table 4).

Table 4: Changes in the hemoglobin levels for all the participants before and after initiating the antimalarial (AL) drug

Parameter (Normal)	Days	Control	Nevirapine	*p* value [a]
HB (>7)	D0	10.67±0.78	9.37±0.27	0.1925
	D3	10.53±0.67	9.26±0.27	0.135
	D7	11.6±1.27	10.05±0.58	0.4994
	D14	11.15±0.37	9.55±0.44	0.0584
	D21	11.83±1.39	9.3±0.01	0.4856
	D28	9.43±0.68	10.21±0.31	0.2472

[a] Reported *p* values calculated using unpaired t-test for continuous variable; HB: Hemoglobin

4. Discussion

Artemether/lumefantrine showed a high efficacy against uncomplicated falciparum malaria in both treatment groups in this study. Despite the endemicity of malaria in the study locations, recurrent malaria was not common. In this study, adequate clinical and parasitological response (ACPR) was recorded in 100% of the patients in the control group treated with AL only, at day 28. This under-scored the efficacy reports of AL in the treatment of uncomplicated malaria across all ages as reported in previous studies[11-13]. ACPR was recorded in only 90% of the participants in the nevirapine group. However, this was lower than the rate recorded in adult population of similar design in Tanzania[22]. Comparing the two arms of this study, the 95% confidence intervals of the ACPR from both arms falls within the 90% minimum efficacy threshold defined by the WHO[23]. There was a recorded case of early treatment failure in the form of delayed parasite clearance representing 5% of the participants in the test group and none was recorded among

the participants in the control group. Further monitoring of the participants showed no parasite presence in the blood samples on day 7 after antimalarial therapy initiation. Although this may be a proxy indication of resistance to AL in the test group as suggested by the WHO[24]. The WHO has defined an increased parasite clearance time with ≥ 10% of cases with detectable *P. falciparum* parasites on day 3 as a suspected artemisinin resistance[24], yet the threshold was not met in this study.

Recrudescence, re-emergence of *falciparum* parasite with similar genotype, represented late parasitological failure was reported in 5% of the participants in the test group but none was recorded in the control group. Comparable magnitude of recrudescence has been reported in earlier study on the efficacy of AL in the treatment of uncomplicated malaria in under five children in Nigeria[11]. Recrudescence may have occurred in this study due to increased elimination clearance of lumefantrine in the presence of nevirapine in the system of the participants in the test group compared to those in the control group. Recrudescent infection may not be an indication of resistance to AL but due to the short half-lives of both artemether and lumefantrine as previously reported in Thailand before the emergence of resistance to the drugs[25]. We also reported no clinical failure in both arms of the study; therefore, no emergency or rescue treatment was warranted.

The results from this study showed that AL is safe for the treatment of uncomplicated malaria in HIV infected children on nevirapine based therapy and non-infected HIV children. The adverse reactions to AL antimalarial drug recorded were mild in severity and did not necessitate treatment discontinuation. Clinical adverse reaction recorded are cough, pyrexia, anorexia, and abdominal pain. The proportion of participants who experienced each of the adverse effects to AL were not statistically significantly differ between the control and nevirapine groups (Table 3). Artemether and Lumefantrine has been extensively studied in clinical trials primarily from Asia and Africa. A review of AL safety and tolerability in 27 RCTs for children from Africa and Papua New Guinea, adverse drug events recorded in the review were mainly cough and gastro-intestinal symptoms[25]. In a similar review of 1,869 patients of which 33% were children from Asia, gastro-intestinal symptoms (nausea, vomiting, and diarrhea), and neurological symptoms (headache, and dizziness) were the most commonly reported adverse events[26]. In this study, there was non-significant lower risk of abdominal pain, cough, and pyrexia due to AL in HIV-infected children treated with nevirapine compared to the control group (Table 3). However, there was non-significant higher risk of anorexia due to AL in HIV-infected children treated with nevirapine compared to the control. This is also in-line with the report of Katrak *et al*.[10], who found no statistically significant difference in the risk of development of adverse event to AL among HIV infected and HIV non-infected children.

Furthermore, we observed laboratory abnormalities. The commonest laboratory abnormalities in malaria studies were related to hematological derangement, especially anaemia[27]. In our study, due to ethical considerations, children with underlining severe anemia were excluded from enrolment. Parikh *et al.* [28], in their study reported less than 20 cases of grade 3 laboratory abnormalities which are hematological related abnormalities in the context of 366 episodes of malaria recorded in that study.

Protease inhibitor-based antiretroviral therapy compared nevirapine-based has been reported to increase the plasma lumefantrine level in children[7,28]. This may have far-reaching effects especially in the context of adverse drug events occurrence. Although, these drugs tend to potentiate the antimalarial effect of lumefantrine[6] by inhibition of CYP3A4[9] in one hand, these drugs have also been reported to possess intrinsic antimalarial effect especially Lopinavir/ritonavir[29,30]. In this study, nevirapine-based ART demonstrated a modulation of ADR, when compared to the control group.

The key limitation of this study lies in the limited number of subjects recruited for the study especially in those treated with nevirapine-based ART. Since, the study is hinged on intent to treat, positive microscopy for *falciparum* malaria was one of the major inclusion criteria. Unfortunately, access to self-treatment with AL without running adequate malaria test is common in Nigeria especially among parents or guardians of HIV-infected children. Therefore, this tend to limit the number of visitations to the hospital and possibility of being included in the study.

5. Conclusion

It is concluded that AL is generally safe and retains its efficacy against uncomplicated malaria in HIV-infected children treated with nevirapine-based ART. Nevirapine based HAART may, however, lead to delayed parasite clearance in children treated with AL.

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