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Research Article

Pyronaridine-Artesunate versus Artemether-Lumefantrine: *Plasmodium falciparum* Clearance in Uncomplicated Malaria in Paediatrics in Mbita sub-county, Kenya

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Abstract: Malaria is still a major public health concern which leads to over 400,000 deaths globally especially in the pediatric population under five years. In Kenya, artemether – lumefantrine is the first-line drug recommended for the treatment of uncomplicated falciparum malaria. Although it has shown good efficacy and high cure rates, drug tolerability, patient non-compliance, administration with fat-rich foods for optimal absorption, and the emerging threat of artemisinin resistance are its major throwbacks. Thus, the need for improving the existing artemisinin-based therapies with novel partner drugs like pyronaridine to overcome these limitations. This study was a component of phase III single- blind randomized non-inferiority study in Mbita, Kenya that included patients of ≤ 12 years with a bodyweight of ≥ 5 kg and microscopically confirmed *Plasmodium falciparum* malaria. Eligible children were randomized (1:1) to receive either pyronaridine-artesunate or artemether-lumefantrine orally for three days according to their bodyweights and followed for 42 days for therapeutic responses. Of 155 patients, 81 received pyronaridine-artesunate while 74 received artemether-lumefantrine. A higher proportion of aparasitemic patients in pyronaridine-artesunate cohort was registered compared with artemether-lumefantrine (96.3% versus 95.8%) on day 3. Time to parasite clearance significantly differed between the treatment arms ($p<0.05$). Despite the significant difference in the proportions of parasitemic patients on days 28 and 42 ($p< 0.05$), there was no significant differences in day 28 and 42 ACPR without PCR corrections in both study comparators ($p>0.05$). This present study showed that pyronaridine-artesunate was efficacious as artemether-lumefantrine in the treatment of uncomplicated falciparum malaria.

Keywords: Uncomplicated falciparum malaria, Artemisnin –based combination therapies, Artemisinin resistance, Artemether-lumefantrine, Pyronaridine-artesunate, Pediatrics.

INTRODUCTION

Plasmodium falciparum malaria has an enormous public health impact worldwide resulting in approximately 228 million cases and 405,000 malaria-related mortalities annually in children below five years old. African children bear the heaviest burden accounting for 94%, that is, 272,000 of these deaths [1]. In Kenya, falciparum malaria is not only responsible for 3.5 million malaria cases but also 10,700 deaths are reported yearly particularly from Western Kenya [2]. The World Health Organization recommends the use of artemisinin-based combination therapies (ACTs) treatment of falciparum malaria in endemic African countries. However, the emerging threat of artemisinin resistance-an early sign of treatment failure- reported from South East Asia, Greater Mekong Region and the Kenyan Coast manifested as slower parasite clearance time in patients receiving artemisinin monotherapy or ACTs may jeopardize the malaria eradication efforts [3-6]. Artemisinin resistance is evident as the persistence of the *Plasmodium* strains in the peripheral blood circulation or detectable parasitemia on day 3 and the occurrence of recrudescence on days 28 or 42 after therapy. Decreased efficacy of artemisinins places greater selective pressure on partner drugs to which resistance is also increasing. This is alarming because it suggests that artemisinins may no longer be able to fulfil their key role in combination therapy of reducing the parasite biomass and protecting the partner drug [7, 8]. Judging from the history of anti-malarial drugs, this resistance is bound to spread to Africa which can be potentially catastrophic to pediatric health [4, 9-11].

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Artemether-lumefantrine is the first oral fixed-dose ACT to meet the World Health Organization prequalification criteria for efficacy, safety and tolerability profiles in adults, infants and children for the treatment of uncomplicated *Plasmodium falciparum* malaria and mixed infections including those caused by *Plasmodium vivax* [12-14]. In Kenya, it has been the first-line treatment for uncomplicated falciparum malaria since 2006. Despite its excellent cure rates of over 95% and good efficacy, patients non-compliance, tolerability, necessity of rich-fat foods for its optimal absorption, greater risk of the occurrence of recurrent infections soon after therapy plus the emerging threats of artemisinin resistance are its major setbacks [15, 12, 16]. Thus, the pressing need to develop and adopt novel ACTs like pyronaridine- artesunate which allow for single dose regimens, is more effective, safer and cheaper to avert these limitations.

Pyramax® is a fixed-dose combination of pyronaridine and artesunate developed by Medicine for Malaria Venture and Shin Poong Pharmaceutical Company for the treatment of uncomplicated *Plasmodium falciparum* as well as blood stage *Plasmodium vivax* infections. It is a once-daily dose taken for three days indicated for adults and children over 20kg (Pyramax tablets) and in children and infants between 5 and 20 kg (Pyramax granules) [5, 6]. It is a blood schizonticidal antimalarial drug whose rationale for development was the providence of rapid reduction of the parasite density from the bloodstream with the three-day regimen thereby leading to increment of patient' compliance as well as the reduction of the recrudescence risk. This is only possible because of the longer systemic half-life of pyronaridine of thirteen days which eliminates the residual malaria parasites [17-19].

Findings from *in-vitro* studies on pyronaridine monotherapy in the early 1990s demonstrated that it was efficacious against the multidrug resistant *Plasmodium falciparum* [20]. Likewise, reports from the subsequent phase II and III clinical trials across African and South East Asian nations showed that pyronaridine-artesunate have good efficacy, safety and tolerability profiles in children and adult patients suffering from acute f*alciparum* and *vivax* malaria. Its polymerase chain reaction corrected cure rates on days 28 and 42 was greater than 95% and 93% respectively [18, 19, 6]. The availability of a granule formulation for children, the absence of a major effect of food intake on bioavailability, a longer shelf life and post-treatment prophylactic effect, and projected low price lend support to pyronaridine-artesunate becoming a powerful new arrow in the quiver for the fight against malaria. It may not only fulfil the need for new, easy to administer, pediatric ACTs, but might also be a valuable alternative in areas where resistance against commonly used ACTs is rising [17]. Therefore, the primary aim of this present study was to determine the efficacy of pyronaridine-artesunate versus artemether-lumefantrine in parasite clearance time of *Plasmodium falciparum* in children between six months and twelve years old with uncomplicated falciparum malaria in Mbita Sub county in Kenya.

MATERIALS AND METHODS

Study Area

This study was carried out at the St. Jude's Clinic of the International Centre of Insect Physiology and Ecology (ICIPE) in Mbita, Kenya between November, 2016 and May, 2017. Mbita's location along the shores of Lake Victoria provides favourable ecological conditions that supports mosquito breeding, thus its moderate malaria transmission which occurs throughout the year. Transmission peaks in June following long rains and more steadily between September and February [21, 22].

Study Population

Eligible children of either sex aged between six months to twelve years with a bodyweight of at least five kilograms suffering and diagnosed with microscopically confirmed uncomplicated *falciparum* malaria were included in the study. The inclusion criteria were patients having microscopically confirmed *Plasmodium falciparum* monoinfection with asexual parasite count of 1,000- 200,000 parasites /µl and were living within 10 Km radius from the study site. Conversely, children with clinical signs and symptoms of severe malaria, mixed *Plasmodium* infections, anaemia with haemoglobin level of <6 g/dL, known history of hypersensitivity, allergic or adverse reactions to artesunate, artemether-lumefantrine or other artemisinins, evidence of severe malnutrition, or those with concomitant febrile conditions were excluded from the study.

Study design and Treatment

This present study was part of the of phase III single- blind randomized non-inferiority clinical trial in Western Kenya that was comparing pyronaridine-artesunate and artemether-lumefantrine in the treatment of pediatric uncomplicated falciparum malaria. After patients had been screened and enrolled into the study, they were randomly allocated to receive either pyronaridine-artesunate or artemether-lumefantrine orally for three days (days 0, 1, and 2) according to their body weights under direct supervision at the study clinic. The study drugs were administered with foods such as *mandazis* or milk as recommended for optimal absorption of artemether-lumefantrine. *Mandazis* were also provided for the evening dose of artemether-lumefantrine. When the child vomited within 30 minutes following the administration of the first drug dose, the same drug was re-administered. Nevertheless, vomiting after repeat dosing or any subsequent dose of pyronaridine-artesunate or artemether-lumefantrine led to exclusion from the study and the child received rescue treatment according to the local guidelines. New

participants were enrolled into the study on a daily basis after they had satisfied the inclusion criteria. Likewise, follow-up sampling was done according to schedule on days 1, 2, 3, 7, 14, 28 and 42.

Study Interventions

Pyronaridine-artesunate was supplied by the Shin Poong Pharmaceutical Company, Seoul, South Korea in two formulations: tablet form which constituted of 180mg of pyronaridine-tetraphosphate and 60 mg of artesunate per tablet for children > 20kg. The granule formulations were provided in sachet form and they consisted of 60 mg of pyronaridine-tetraphosphate and 20mg of artesunate per sachet for children < 20 kg. It was given once as a daily course as follows; for tablets: children weighing between 20– <24 kg and 24– < 45 kg were administered with one and two tablets respectively. For granules, one sachet was given to children with a body weight between ≥5– <8 kg, two sachets for those between 8-15kg, and three sachets for study participants who weighed between 15– <20 kg three sachets. Artemether-lumefantrine was supplied by Novartis Company, Basel, Switzerland in tablet formulations containing 20mg of artemether and 120 mg of lumefantrine per tablet. It was given twice daily as follows; for children with a bodyweight of ≥ 5– < 15 kg they were given one tablet, two tablets for those weighing 15- < 25 kg, three tablets for those with a bodyweight of 25-< 35 kg, and four tablets for study participants of ≥ 35 kg. The first (morning) dose of each day was provided under the supervision of a clinical staff while the second (evening) dose was provided to parents or caretakers to administer at home. The evening doses with replacements were given to the parents or guardians responsible for the administration of the antimalarial medications at home with proper and clear verbal instructions on when and how to take the medication. For children who were unable to swallow the drug, the tablet was crushed and drug suspension prepared by adding 50ml of water.

Ethical Considerations

The study received ethical approval from the Kenya Medical Research Institute (KEMRI, Nairobi, Kenya) Ethical Steering Committee (NON -SSC protocol No 479). Besides, oral and written informed consent was obtained from each parent or the legal guardians of all study participants and when appropriate assent from pediatric patients who were old enough were also sought.

Sample Size Determination

For an assumed efficacy of 95%, a minimum sample size of 200, 100 study participants in each treatment arm, were needed to be included in the study assuming a 7% margin of error, 95% confidence interval and 91 % statistical power. When correcting for 10% lost to follow-up, 222 patients were to be included. However, 155 patients were finally enrolled into the study.

Laboratory Procedures

Collection of Blood Samples

Finger- prick capillary blood samples for the preparation of malaria blood smears and the determination of haemoglobin levels were collected using sterile and aseptic techniques from the study participants during screening, their visit on follow up days or any unscheduled visits.

Malaria Blood Smear Preparation and Examination

Thin and thick blood smears were prepared and examined according to the World Health Organization guidelines [23]. Thin blood smear was used for the identification and confirmation of the *Plasmodium* species while the thick blood smear was used for the quantification of the asexual stages of *Plasmodium falciparum.*

Quantification of *Plasmodium falciparum*

Parasitemia was determined from the thick smear by counting the number of the asexual stages of *Plasmodium falciparum* per microliter of blood against 200 leukocytes assuming the standard white blood cell count (WBC) of 8,000 leucocytes per microliter of blood.

Determination of Haemoglobin Levels

Finger prick capillary blood was used to measure haemoglobin level in grams per deciliter (g/dl) of blood using a portable Spectrophotometer and cuvettes (B-Hemoglobin HemoCue, Angelholm, Sweden) on days 0, 3, 7, and 28.

Follow Up Phase

Patients recruited for the study visited the research clinic for follow-up on day 1, 2, 3, 7, 14, 28 and 42 and anytime they fell ill (unscheduled visit). During each visit, general physical examination by the chief physician was done during screening, finger-prick blood samples for preparation, staining and microscopic screening of thick and thin blood smears and haemoglobin levels measurements were collected. On treatment days, sampling was done before treatment. Additionally, finger-prick blood samples were collected at homes of children who failed to attend the clinic on any of the follow up days.

Children who became parasitemic at any time point after day 3 received rescue-treatment with quinine and were excluded from further efficacy follow-up. Other reasons for the discontinuation of the study drug interventions on the study patients were cases of drug adverse effects, non-compliance to study protocol, loss of patients to follow up, or consent withdrawal. However, the study patients were monitored for safety for at least 28 days following the last dose of study medication. In case of any complications that required higher level of care than can be provided at St. Jude's clinic, they were referred to Suba district hospital located 1km from the study site.

DATA ANALYSIS

Data entry and analysis was performed using MS Excel worksheet and SPSS version 24 for windows. Descriptive statics like mean, range and percentages were also performed. Parasite clearance time was defined as the time from the first dose to the time in which the patient was aparasitemic followed by two consecutive *Plasmodium falciparum* free malaria blood smears in the subsequent follow up days. Parasite clearance time after treatment with pyronaridine-artesunate and artemether-lumefantrine was determined using Analysis of Variance (ANOVA) model one whereby the effects of age, sex and weight were tested. Furthermore, Pearson's correlation was used to test for any existence of strong relationship between parasitemia versus age, sex, and weight.

The efficacy outcomes of the study interventions were determined by comparing the proportions of patients who were parasitemic on days 28 and 42 following treatment with either pyronaridine-artesunate or artemether-lumefantrine. The reappearance of *Plasmodium falciparum* parasites was interpreted either as reinfection or recrudescence, hence, a treatment failure. The cure rate was defined as the percentage of patients where the asexual stages of *Plasmodium falciparum* were completely cleared from the peripheral blood and who were free of the *Plasmodium falciparum* after 28 and 42 days of follow-up. Independent t-test was used to compare both the proportions of parasitemic patients on days 28 and 42 and the day-28 and 42 cure rates or the Adequate Clinical and Parasitological Response (ACPR) between pyronaridine artesunate and artemether lumefantrine. Tukey High Significance Difference [HSD] was also used to compare the mean parasitemia during follow-up between the study interventions.

Levene's test for equality of variances was used to check for data normality and statistical significance was considered for $p< 0.05$. Furthermore, SPSS version 24 software was used for analysis purpose. All the data and the laboratory test results collected from the study participants and the entire study were kept confidential and were exclusively used for the intended research purposes.

RESULTS

Study Participants and Recruitment

Between November, 2016 and May, 2017, 887 children clinically suspected to be having febrile malaria were screened for eligibility. Of these, 539 (60.77%) tested negative for *Plasmodium falciparum*, 52 (5.86%) had mixed *Plasmodium* infections, 11 (1.24%) were positive for *Plasmodium malariae* and 1(0.11%) had taken antimalarial drugs in the past two weeks. Among 284 (32.02%) patients who had *Plasmodium falciparum* monoinfection, 112 had low parasite count below 1000 parasites/ µL of blood, 16 had high parasite count above 200,000 parasites/µL of blood and 1 had gametocytes only, thus were excluded from the study.

Study Flow Chart

Out of 155 children; 78 females and 77 males met the eligibility criteria and were enrolled in the study. Eighty one (81) of them were randomized to receive pyronaridine-artesunate while 74 received artemether-lumefantrine. One hundred and forty five (145) patients completed the 42-day follow up period successfully. During this period, 10 patients were excluded from the study: 4 were lost to follow up, 3 moved out of the study area, and the remaining 3 withdrew consent (Figure-1).

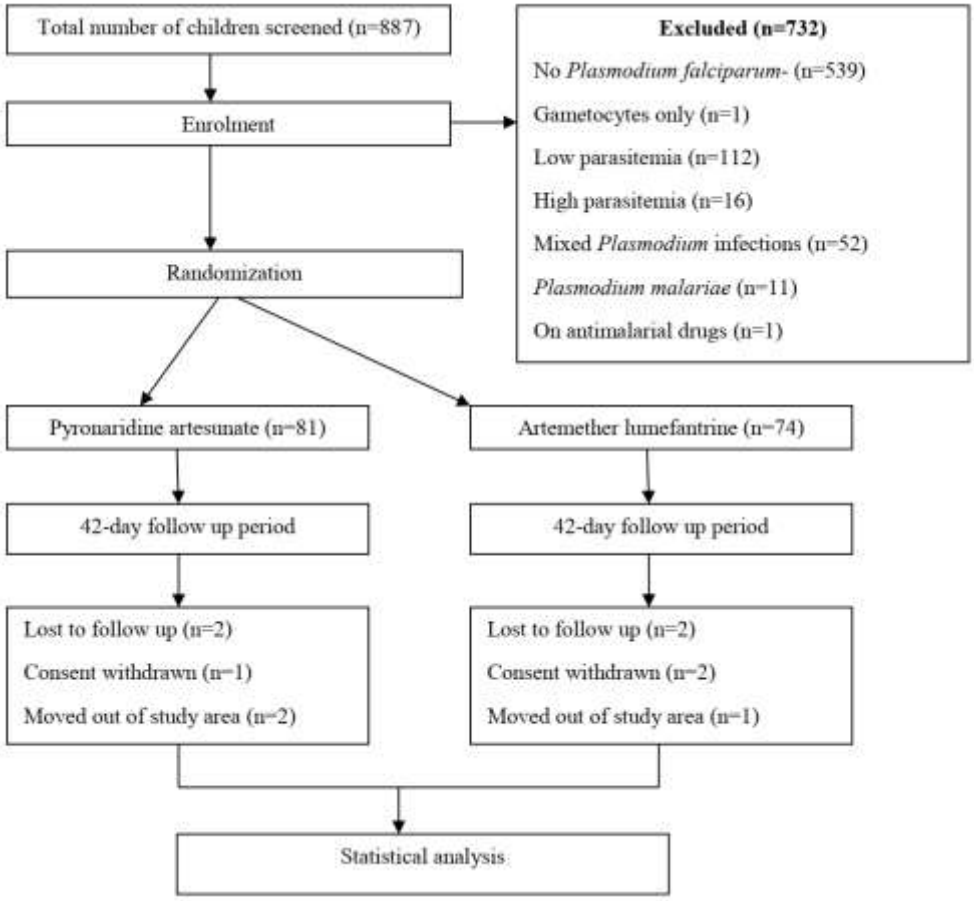

Fig-1: Pyronaridine Artesunate/Artemether-Lumefantrine Clinical Trial Flow Chart

Characteristics of Study Population

The mean age of the study participants was 6.9 years in the pyronaridine artesunate group and 6.7 years in the artemether lumefantrine group. However, majority of the study participants were in the age group of 6 - 12 years. Moreover, the mean weight of the patients was 22.6 kg and 23.0 kg in the pyronaridine artesunate and artemether lumefantrine cohorts respectively. The average parasite density in the pyronaridine artesunate and the artemether lumefantrine treatment arms were 51,131 and 47,190 *Plasmodium falciparum parasites*/μL of blood respectively. The mean haemoglobin levels were 11.65 g/dl in the pyronaridine artesunate cohort and 11.67 g/dl in the artemether lumefantrine cohort. Additionally, the number of males in the pyronaridine artesunate group (54.3%) was higher when compared with those in the artemether lumefantrine group which consisted of 43.2%. Conversely with the number of females in the pyronaridine artesunate and artemether lumefantrine treatments were 45.7% and 56.8% respectively (Table-1).

Table-1: Baseline Characteristics of Study Participants on Day 0

Characteristic	Pyronaridine-artesunate [N=81]	Artemether-lumefantrine [n=74]
Sex	N%	N%
Male	54.3 [44/81]	43.2 [32/74]
Female	45.7 [37/81]	56.8 [42/74]
Mean Weight [Kg]	22.60 [10.00-44.00]	23.00 [8.50-45.00]
5-<15	13	15
15-<25	38	27
≥ 25	30	32
Mean Age [Years]	6.9 [1-12]	6.7 [0.9-12]
<1	0	1
1-<6	38	36
6-≤12	43	37
Mean Parasitemia [μL-1]	51,131 [2,040-174,560]	47,190 [1,920-154,560]
Mean Haemoglobin levels [g/dl]	11.65 [6.2-15.90]	11.67 [6.3-16.40]

Study Outcomes

Parasite Clearance Time

Both pyronaridine-artesunate and artemether-lumefantrine rapidly cleared *Plasmodium falciparum* completely from the patients' blood stream after 72 hours: on day 1, 56.3% and 48.6% of the study participants in the pyronaridine-artesunate and artemether-lumefantrine cohorts respectively were aparasitemic. Accordingly, a higher percentage of patients who were *Plasmodium falciparum* free was registered on day 2 with pyronaridine-artesunate and artemether-lumefantrine groups having 91.3% and 88.9% respectively. Moreover, on day 3, parasitemia was completely cleared in more than 90% of the study participants in both treatment groups with pyronaridine-artesunate group possessing a slightly higher percentage of 96.3% than those of the artemether lumefantrine group who had 95.8% (Figure-2).

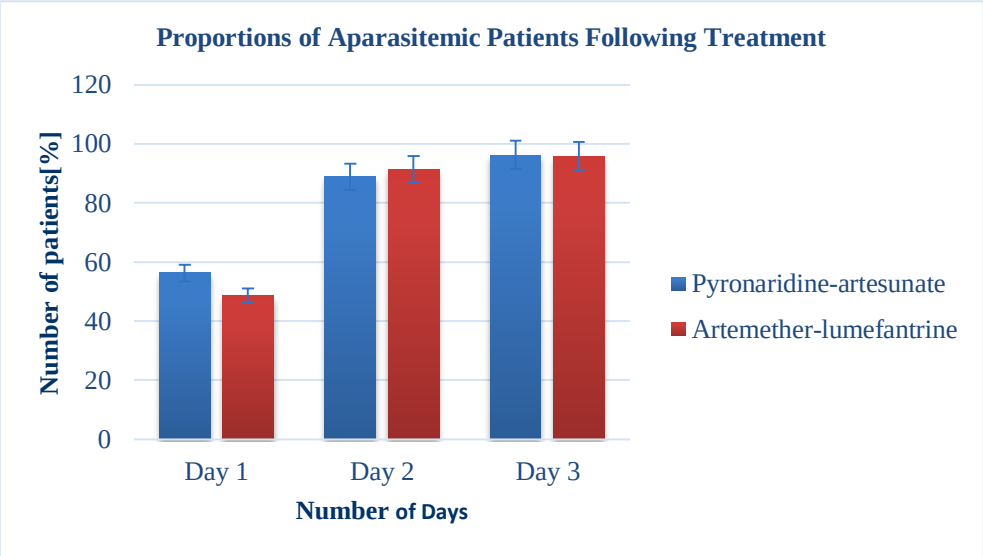

Fig-2: Proportions of Aparasitemic Patients Following Treatment with the Study Interventions

Although, both the study drugs rapidly decreased *Plasmodium falciparum* density to 0 on day 3, there was a significant difference on the parasite clearance time between the treatment within the days with pyronaridine artesunate and artemether lumefantrine having the p-values of (F= 97.728, P=0.00) and (F= 37.740, P=0.00) respectively. There was also a significant difference in mean parasitemia levels on day 0 in comparison with day 2 and 3 in both pyronaridine artesunate and artemether lumefantrine cohorts; p= 0.000. In the pyronaridine artesunate group; the mean parasite density between day 0 to day 1, day 0 to day 2, and day 0 to day 3 were 47,533 parasites/ µL of blood at 95% CI, 40,132-54,933, 49,231 parasites/µL of blood at 95% CI, 41,805-56,656 and 49,249 parasites/ µL of blood at 95% CI, 41,858-56,635 respectively. More, so, there were no significant difference in the mean parasite density between day 1 to day 2 (mean parasitemia of 1, 698, p=0.997 at 95% CI, -5,764-9,159) and day 2 to day 3 (mean parasitemia of 16, p=1.000 at 95% CI, -7,433-7,465). On the contrary, the mean parasite density in the artemether lumefantrine cohort between day 0 to day 1, day 0 to day 2 and day 0 to day 3 were 45,283 parasites/ µL of blood at 95|% CI, 33,891-56,676, 47,161 parasites/ µL of blood at 95% CI, 35,769-58,552 and 47,187 parasite/ µL of blood at 95% CI, 35,755-58,620 respectively. Furthermore, there was no significant difference of mean parasitemia between day 1 to day 2 (mean parasite density of 1,877, p=1.000 at 95% CI, -9,573-13,347) and day 2 to day 3 (mean parasite density of 27, p=1.000 at 95% CI, -11,484-11,537).

Effects of age, sex and weight on parasite clearance time

The results show that the p-values of age, sex and weight were F=0.797, P=0.375; F=0.00, P=0.987 and F=0.544, P=0.461 respectively indicating that these parameters had no effect on the parasite clearance time of pyronaridine artesunate and artemether lumefantrine. More so, there was a significant strong correlation of age with weight (r=0.862, P=0.00) which signified the importance of dosing the study population correctly in respect to their age and body weight. On the other hand, the weak, non-significant negative correlation of *Plasmodium falciparum* count with age (r=0.022, P=0.445) probably show that any child is vulnerable to acute falciparum malaria irrespective of their age.

Parasitemic patients after treatment with either Pyronaridine-artesunate or Artemether-lumefantrine

Days 3 and 7

The findings from this present study showed that there was no cases of early treatment failure and late clinical failure observed on days1, 2, 3, 7 and 14 in both the treatment groups. Early treatment failure(ETF) refers to the development of danger signs of

complicated malaria on days 1, 2, or 3 in the presence of parasitemia; parasitemia being higher than day 2 count regardless of the axillary temperature; parasitemia on day 3 with axillary body temperature of ≥37.5^0 C; parasitemia on day 3≥ 25% count of day 0. On the other hand, late clinical failure is the development of danger signs of severe malaria after day 3 in the presence of parasitemia without previously meeting any criteria for ETF; presence of parasitemia and axillary temperature ≥37.5^0 C or history of fever on any day from day 4 to 28, without previously meeting any of the criteria of ETF.

Day 28 and Day 42

Six (6) patients from the pyronaridine artesunate group; 3 males and 3 females aged between 3 - 12 years old and 11 from the artemether lumefantrine cohort; 6 females and 5 males ranging from 2 to 12 years of age were still parasitemic, hence exhibiting late parasitological failure. This is the presence of parasitemia on any day from day 7 to day 28 and axillary body temperature of <37.5^0 C, without previously meeting any of the early treatment failure and late clinical failure. The proportion of patients who was still parasitemic after treatment differed significantly (p=0.036) with pyronaridine artesunate (6 of 77 [7.79%] versus artemether lumefantrine, 11 of 71, [15.49%]) (Table-2). All these patients were discontinued from the study and were treated with dihydroartemisinin-piperaquine. They were, however, monitored for safety for the rest of follow up period. Also, there was a significant difference in mean parasite density of day0 to day 28 between the two study interventions; pyronaridine artesunate-mean density-44,186 parasite/ µL, p=0.000 at 95% CI,36,641-51731.3 versus artemether lumefantrine-mean parasite density-44,998,p=0.00 at 95% CI, -30,250-53,745.The mean parasitemia between day 3 –day 28 did not differ between the comparator drugs with pyronaridine artesunate and artemether lumefantrine having mean parasite density of -5,060,p= 0.46 at 95% -12,629-2,508 and mean parasite density of -5,189,p=0.889 at 95% CI,-17,051-6,673 respectively.

On day 42, 8 patients from the pyronaridine artesunate cohort; 6 males and 2 females between 5 - 11 years and 5 from the artemether lumefantrine cohort; 4 males and a female between 2 - 9 years were found to be still harboring the *Plasmodium falciparum* after treatment with the intervention drugs. However, the proportion of parasitemic patients were similar between the treatment groups with 8 of 68 [8.82%] in pyronaridine artesunate compared to 5 of 58 [8.62%] in the artemether lumefantrine cohort (Table-2). Besides, there was a significant difference in mean parasitemia between day 0-day 42 in both pyronaridine artesunate and artemether lumefantrine treatment arms {pyronaridine artesunate- mean parasite density -45,610, p=0.000 at 95% CI 38,079- 53,141 and AL-mean parasite density-41, 672, p=0.000 at 95% CI, 2,995-53,420.} However, there was no significant difference in mean parasitemia between days 28-42 in both the treatment groups. Pyronaridine artesunate-mean parasite density-1,423, p=0.999 at 95%, CI,-9,132-6,284 versus artemether lumefantrine mean parasite density- -325, p=1.000 at 95% CI,-12,490-11,840.

ACPR on Day 28 and Day 42

This present study showed that ACPR on day 28 without PCR corrections for pyronaridine artesunate was higher (92.21%) than that for artemether lumefantrine which was 84.51%. On the other hand, ACPR on day 42 for both pyronaridine artesunate and artemether lumefantrine were 91.18% and 91.38% respectively (Table-2). However, there was no statistical difference on day 28 and day 42 ACPRs in pyronaridine artesunate and artemether lumefantrine regimens (p=0.438).Therefore, both drugs were efficacious in treating uncomplicated falciparum malaria in children.

Table-2: Cure Rates during Follow Up

Treatment failure	Pyronaridine-artesunate	Artemether-lumefantrine
Age [Years]	< 1 1-<6 6-≤12 N[%]	< 1 1-<6 6-≤12 N[%]
ECF	0 0 0 0	0 0 0 0
LCF	0 0 0 0	0 0 0 0
LPF Day 28	0 2 4 6/77 [7.79]	0 2 9 11/71[15.49]
ACPR Day 28	71/77 [92.21]	60/71 [84.51]
LPF Day 42	0 1 7 8/68 [8.82]	0 2 3 5/58[8.62]
ACPR Day 42	62/68 [91.98]	53/58[91.38]

DISCUSSION

Study Participants and Recruitment

The higher percentage of patients who tested negative for *Plasmodium falciparum* (60.77%) in this present study implies that there is reduced rate of malaria cases in the study site. The lower numbers of malaria infections suggest that Kenya's efforts in the fight against malaria both at the national and county levels are yielding positive outcomes. These government interventions such as the mass distribution of insecticide-treated nets to expectant mothers in hospitals, indoor residual spraying, the availability of free but effective antimalarial drugs in the mission and public health facilities as well as the presence of frequent public health education forums on malaria

treatment, management, and control to the local community has greatly attributed to the reduced malaria morbidity and mortality [24]. Additionally, various research institutions have spear-headed an array of malaria-related studies not only on malaria vectors but also in clinical trials on ACTs to aid in understanding the dynamics of malaria transmission and the efficacy of the first line ACTs which are currently in use [22, 25, 26]. However, the downward trend of malaria in this region can be further reduced to zero through prompt diagnosis and treatment using World Health Organization recommended malarial drugs. These drugs ought to be in line with the national and global guidelines since the treatment with substandard medicines which are readily available in the market as over the counter drugs are more likely to deteriorate patient' health, undermine malaria control strategies and lead to huge economic costs [27]. With the average weight of the study participants being 22.60 kg in the pyronaridine artesunate cohort and 23.00 kg in the artemether lumefantrine group, it is inevitable that artemether-lumefantrine total drug course of 12 tablets is higher compared to pyronaridine-artesunate of 3 tablets for three days. This suggests that patient's compliance to complete treatment regimen is likely greater in those taking pyronaridine-artesunate.

Parasite clearance time of pyronaridine-artesunate and artemether-lumefantrine

Over 90% of patients from both treatment groups were *Plasmodium falciparum* free on day 3 suggesting high parasite clearance rate. Subsequently, this leads to rapid resolution of the clinical manifestations, thus the recovery of the study participants from acute *falciparum* malaria. This suggests that the *Plasmodium falciparum* from the study area are still sensitive to artemisinin thus absence of the possible threat of artemisinin resistance emergence. Likewise, the fast parasite clearance rate demonstrated by the two study interventions is in line with established reports on the intrinsic potency and stage-specificity of artemisinins on clearing both the asexual and sexual stages of *Plasmodium* parasite [28, 29, 9]. Artemether and artesunate, for instance, rapidly metabolizes to their active ingredient, dihydroartemisinin, which is also absorbed rapidly into the bloodstream culminating in rapid decline in the parasite density hence fast clearance of the parasite biomass [12]. Besides, their relatively shorter half-lives of between two to three hours necessitates their combination with a slowly acting partner drugs, lumefantrine and pyronaridine whose half-life are four and thirteen days respectively. When patients are compliant to finish the total drug course of artemether-lumefantrine and pyronaridine-artesunate, there is the successive accumulation of the drugs which not only eliminates the residual parasites but also prevents the probable risk of the occurrence of reinfection or recrudescence [17-29].

The proportions of patients who were aparasitemic on Days 1, 2 and 3 after treatment with pyronaridine-artesunate was 56.3%, 91.3%, and 96.3 % respectively while those in the artemether-lumefantrine treatment arm was 48.6%, 88.9% and 95.8% respectively. These findings suggest that time to parasite clearance was faster in the pyronaridine-artesunate cohort than in the artemether-lumefantrine one. Therefore, they are congruent with the earlier reports that reported that median time to parasite clearance was shorter in the pyronaridine-artesunate a than in artemether- lumefantrine [30, 31]. The only difference is that the latter two studies are precise in determining parasite clearance time since study participants were hospitalized and blood samples taken after every 8 hours for parasitological assessments. This was not possible in the present study since it was done in an outpatient clinic. Likewise, these studies differed in study design and location. Conversely, cases of patients with detectable parasitemia after 72 hours following treatment with ACTs have been reported in Western Cambodia and the Great Mekong Region [32, 33, 6].

Parasitemic patients after treatment with either pyronaridine artesunate or artemether lumefantrine

All the study participants demonstrated satisfactory initial therapeutic response to study interventions. Malaria infections arise due to host immune response to the *Plasmodium* parasites as well as the higher destruction of both the infected and the uninfected red blood cells by the spleen. Also, rapid malaria parasite clearance leading to resolution of clinical symptoms and the ultimate patient recovery may result from the following mechanisms: host immune response, effects of the antimalarial treatment regimens as well as sequestration. In this case, the presence of the ring-stage malaria parasites in the peripheral blood stimulates the synthesis of the pro-inflammatory cytokines which then causes illness forcing an individual to seek treatment. Besides, the active ingredients of the drugs kill and ultimately removes the circulatory ring –stages of *Plasmodium* species. This, in turn, prevents cytoadherence and the pathological sequences of sequestration. Furthermore, the spleen removes the infected erythrocytes plus the malaria parasites which have been destroyed by the drugs from the circulation. This leads to successive accumulation of the drug after the completion of the full dose that is sufficient for the elimination of residual parasites and possibly prevention of new infections [16].

The absence of early treatment failures in both treatment regimens suggest that both ACTs have high therapeutic efficacy in treating cases of uncomplicated falciparum malaria. In contrast, cases of the emergence of artemisinin resistance is suspected in a region when ≥ 10% of patients treated with ACTs have detectable *Plasmodium* parasites after 72 hours [8]. These findings

contrast reports from a study by Kayentao and colleagues [30]. The 92.21% and 84.51% cure rates of pyronaridine-artesunate and artemether-lumefantrine respectively on day 28 showed high therapeutic efficacy of both study drugs. This is similar to reports documented from preliminary therapeutic efficacy studies of other forms of ACTs in children and adults from African countries and other parts of the world [17, 31, 34].

The day 42 ACPR of 91.18% and 91.38% with pyronaridine artesunate and artemether lumefantrine respectively was in contrast to the findings from earlier studies that were lower [6, 31, 34]. The differences in these cure rates may be explained by the longer half-lives of the partner-drugs which probably increases the risk of resistant selection. Other factors that may also influence the efficacy of the drugs include the immune and nutritional status of the patients, inherent parasite susceptibility and the initial parasite density. Individuals from malaria endemic region are more susceptible to reinfections than those from low malaria transmission settings. Notably, immunity plays a critical role in slowing multiplication of parasite and in accelerating parasite clearance following malaria treatment. It arises from the development of B-cell memory cells to malaria. People who have been exposed to multiple malaria infections in their earlier years tend to acquire premunition which enable them to tolerate higher levels of *Plasmodium* parasite density without exhibiting any clinical signs and symptoms than the non-immune individuals [33, 16].

CONCLUSIONS

From the findings of this present study, it can be concluded that: parasite clearance time was faster in pyronaridine-artesunate than artemether –lumefantrine with pyronaridine-artesunate having higher percentage of aparasitemic patients on day 3 (96.3%) than the latter (95.8%). Moreover, both pyronaridine-artesunate and artemether-lumefantrine are efficacious in the treatment of uncomplicated falciparum malaria thus high cure rates of over 80% registered on day 28 and 42.

RECOMMENDATIONS

Pyronaridine-artesunate is non-inferior to artemether lumefantrine in the treatment of uncomplicated falciparum malaria. As a result, pyronaridine-artesunate is a promising novel ACT which can be used as first –line treatment of acute malaria in cases of artemisinin resistance. However, there is need to regularly monitor parasite susceptibility on the currently used ACTs particularly in malarious regions to ascertain their effectiveness since their adoption. Additionally, performance of molecular analyses to distinguish recrudescent from reinfections on parasitemia detected on days 28 and 42 is vital.

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