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Pattern of Parasitological Responses in Patients Treated With Artemether-Lumefantrine Combination for Uncomplicated Malaria in Elele, South-South Nigeria

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Abstract
Background: *Malaria ranks among the foremost health issues facing the world, especially in tropical countries like sub- Saharan Africa were it is still endemic and a no one killer disease. In Nigeria its endemicity keeps threatening established health care delivery services. Malaria has impacted negatively on the Nigerian economy with over a hundred billion naira lost to malaria annually as loss in man-hours and cost of treatment. The malaria parasites stay in the blood causing anaemia and lots of other complications to man. Tackling this malaria parasitaemia is a mandatory tool required for its control and prevention.* ***Purpose:*** *The present study evaluated the pattern of parasitological responses in patients treated with Artemether-Lumefantrine combination (ALC) in the treatment of uncomplicated malaria in Elele, a rural malaria endemic area in Rivers State Nigeria.* ***Method:*** *This study was done at the Madonna University Teaching Hospital (MUTH) and it's out station, Our Saviors Hospital Elele Rivers State Nigeria which is in a high malaria endemic area. Before patient recruitment commenced, ethical certificate was obtained from the MUTH ethical committee. It is a cross-sectional descriptive study in which the 100 participants were recruited longitudinally following adequate counseling and consent. Criteria for inclusion in the study were presence of symptoms and signs of malaria with body temperature of ≥ 37.5 ^{o}C, having a positive malaria parasite test and have not ingested any antimalarial in the past 2 weeks. Such patient should be at least 15 years of age and resident close to the study area to boost compliance. They were effectively counseled on the need for adequate compliance to the three day medication, Arthemeter-lumafantrine combination (ALC). In the study, plasmodial parasitological responses in these malaria patients on treatment with ALC were assessed. Laboratory investigation to confirm parasitaemia employed two methods including Immunoassay examination (Rapid test), which entails the detection of malaria antibodies in a human blood or serum and microscopic examination, which entails looking for the parasite in a drop of blood under the microscope (Slide test). Parasite clearance was determined in the patients taking the above regimen on days 0, 2, 4, 6, 8, 10, and 14. The data gotten was analyzed by tallying the responses in fives with the total number of each response calculated to give the frequencies using SPSS version 16.0 and Microsoft excel packages.*
Results: *The degree of parasitaemia among the participating patients on presentation (day 0) showed that the patients with mild (+) plasmodial parasitaemia were 88 (88%); the patients with moderate (++) plasmodial parasitaemia were 10 (10%) while those with severe (+++) plasmodial parasitaemia were 2 (2%). The parasite clearance was such that by day 8, there was no detectable parasite in those with mild (+) parasitaemia on presentatiion. But, 3 patients, (3%) who had moderate (++) parasitaemia on day 0, still had detectable malaria parasites (+) on day 8; while 1 of the patients, (1%) who had severe (+++) on presentation, still had detectable malaria parasites (+). By day 10, only 1 patient each of moderate (++) and severe (+++) parasitaemia on presentation, still had detectable malaria parasites (+). No parasite was detected in any patient by day 14. By day 14, there was effective clinical and parasitological clearance in the 100 patients studied.* ***Conclusion:*** *Malaria infection impacts negatively in the life of people leaving in its endemic areas. All hands must be on deck to help protect the current WHO recommended Artemether-Lumefantrine Combinations from parasite resistance.*

Key words: *Malaria, Artemether-Lumefantrine, Parasitological response, Symptom clearance.*

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I. Introduction

Malaria is one of the most life-threatening infectious diseases in the world especially in Sub Saharan Africa (Pollyanna et al, 2016). A key millennium development goal was to halve, halt, or reverse the scourge of malaria by **2015.** Till date, malaria infection is yet to be halved, although a major reduction in incidence of malaria has been achieved (World Malaria Report 2014). During the life cycle of malaria parasites, the human host receives most of the harm from the invading parasites during the blood stage of the infection. These illnesses are known to results from the host responses to the activities of the plasmodium parasites in the blood and the increased destruction of both infected and uninfected erythrocytes (Nicholas, 2017). Such malaria parasite's cellular destructions are either from the hosts' immune response or the administered pharmacological agents (Katrien et al, 2016; Pollyanna et al, 2016).

Anti-malarial drug treatment results in the destruction of asexual forms of the malaria parasite and subsequent clearance. This appears to be by first order processes. Principally, the Spleen and other organs remove damaged malaria parasites in circulating erythrocytes from the circulation. During acute malaria, this Spleenic clearance function become maximal (Nicholas, 2017). Despite the repeated and continuous plasmodial parasite exposure to humans in endemic areas, immunity to malaria disease is slow and short-lived. Unfortunately, these parasites have long evolved an orchestrated machinery to evade the immune system. This is based on a range of genetic changes that include allelic variation, biomolecular exposure of proteins, and intracellular replications. All of these features increase the probability of survival of the plasmodium in both mosquitoes and the vertebrate hosts (Pollyanna et al, 2016).

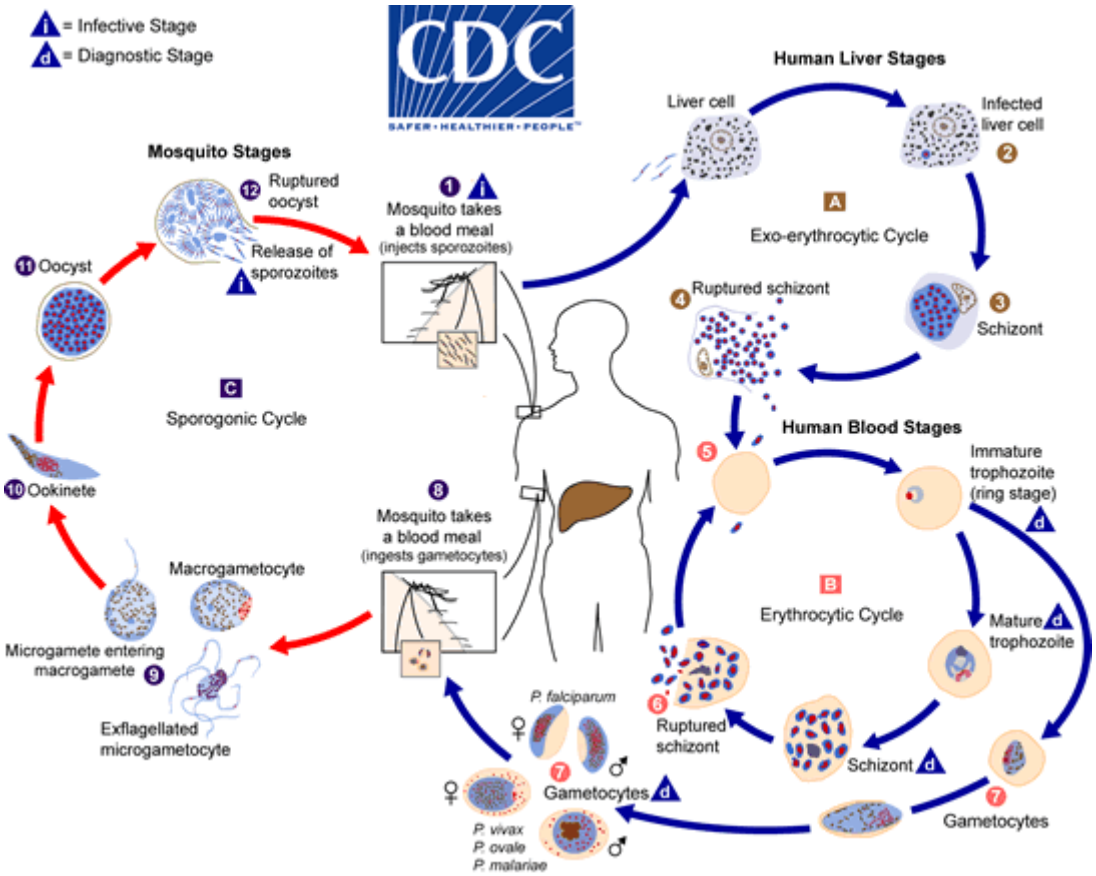
Use of artemisinin or any of its derivatives in the treatment for malaria is associated with variable decline in malaria parasitaemia as with other antimalarials. The rate of parasite reduction may exceed that associated with the antimalarial schizoticidals alone suggesting that these drugs are capable of inducing rapid changes in the circulating ring forms of the parasite. These infected erythrocytes allow recognition by the host-defense system (Chotivanich et al, 2000; White NJ, 1997). But till date, little or no work has been done on the pattern of parasitological response in patients treated with Artemether-Lumefantrine combination for uncomplicated malaria especially in rural malaria endemic areas like Elele in Nigeria, creating a research gap that need to be exploited. Also the variability of this decline in plasmodial parasitaemia needs to be explored regarding the use Arthemeter-Lumefantrine.

Parasite multiplication in man/ Asexual Reproduction

The bite of a probing female anopheline mosquito causes the inoculation of sporozoites into man. These sporozoites quickly disappear from the blood, enters the liver hepatocytes to begin their **asexual multiplication** and subsequent release of merozoites every 8 hours before release back to the blood erythrocytes. Within the erythrocytes the ring stage trophozoites mature into schizonts, which rupture releasing these merozoites. Blood stage parasites are responsible for the clinical manifestations of the disease. Meanwhile, a sub-population of sporozoites form dormant liver stages called "Hypnozoites" which awaken weeks or months later to cause relapses of malaria in P. vivax and P. ovale malaria. These dormant stages may be the cause of malaria treatment failures, resistance or rightly put relapses (Nicholas, 2017; CDC, 2018). They may equally be responsible for the persistence of malaria parisitaemia even after malaria treatment.

Sexual stage development/Multiplication

Some parasites (merozoites) differentiate into **sexual erythrocytic stages** (gametocytes). The male (microgametocytes) and female (macrogametocytes), gametocytes, are ingested by a female Anopheline mosquito during a blood meal. The multiplication of the parasite in the mosquito is known as the sporogonic cycle. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes which later becomes motile and elongated (ookinetes). The ookinete invades the mosquito's midgut wall developing into oocysts. These oocysts grow, rupture, and release sporozoites ready for inoculation to a human and animal host during another blood meal. Sporozoites inoculation to a new human host perpetuates malaria life cycle (CDC, 2018).



(Adapted from: CDC Malaria About Malaria Biology. Life cycle. Health – Division of Parasitic Diseases and Malaria. Page last Updated Nov. 13, 2018).

Malaria Parasite Clearance

One important measure of antimalarial drug efficacy which is particularly important in the assessment of artemisinin resistance is the assessment of parasite clearance rates. The parasite clearance estimator (PCE) developed by Worldwide Antimalarial Resistance Network (WWARN) seemed to be an accurate and reliable method for the early detection of artemisinin resistance. This method employs multiple times blood sampling (6, 8, or 12 hours) a day at measured time points to estimate the rate of parasite clearance (Flegg et al, 2011; Toure et al, 2018).

II. Method

Study location:

The study was done at Madonna University Teaching Hospital (MUTH), and its out station, Our Saviour Hospital (OSH), located along Owerri road, Elele. Elele is a densely populated rural suburb in Rivers State, South-South Nigeria. Malaria is endemic in Elele and most of our hospitals out patient's visits are due to malaria infection. It is a rural community with lots of bushes, forests, slumps, unkempt gutters with dirty waters, open wells and swamps etc. All these factors help to encourage mosquito breeding and consequently malaria transmission

Patient Recruitment:

Ethical certificate was applied for and obtained from the MUTH ethical committee. It is cross-sectional descriptive study in which the 100 participants were recruited following adequate counseling and consent obtained. Actually, a total of 120 patients were selected for the study but 20 of the selected candidates defaulted at some stage of the study leaving us with 100 who were studied in detail and adequately followed up. A semi – structured interview based questionnaire was used as a qualitative study instrument.

Inclusion criteria were: presence of symptoms and signs of malaria with body temperature of $\geq 37.5\ ^{o}C$ and having a positive malaria parasite test. Such patients were not to have ingested any antimalarial in the past 2 weeks, at least 15 years of age and resident close to the study area to boost compliance. Following recruitment, they were effectively counseled on how to take the medication and on the need for adequate compliance to the three day medication, Arthemeter-lumafantrine combination (ACL). All the patients received a 3 day, fixed dose

combination of Arthemether-Lumefantrinea (ALC) at start (0.0 hrs), 8 hrs, 12 hrs, 12 hrs, 12 hrs and 12 hrs later. The medication was well tolerated in all the patients. Contact addresses and phone numbers were politely gotten to enable follow up.

We assessed plasmodial parasite response/clearance in these malaria patients on treatment with Laboratory investigation to confirm parasitaemia. Two methods employed were Immunoassay examination (Rapid test), which entails the detection of malaria antibodies in a human blood or serum and Microscopic examination, which entails looking for the parasite in a drop anticoagulated blood under the microscope (Slide test). Parasite clearance was determined in the patients taking the above regimen on days 0, 2, 4, 6, 8, 10, and 14.

For the Rapid test examination, the **SD BIOLINE** Malaria Plasmodium falciparum (P.f) / Plasmodium vivax (P.v) kit, a one step malaria anti-P.f / P.v test was employed. It is an immune-chromatographic (rapid test) intended for the qualitative detection of antibodies of all isotypes (IgG, IgM, IgA) specific to P.f and P.v in human serum, plasma or whole blood. The kit is used for in vitro diagnosis only and served as our initial screening test for the detection of malaria antibodies in human blood or serum samples (Standard Diagnostics Inc, 2005). The entire malaria positive specimens were then confirmed by microscopic examination. According to the World Health Organization (WHO), light microscopy examination is the "gold standard" and most reliable criterion for the detection of malaria parasitaemia (WHO, 2004; CDC and Malaria, 2017).

Microscopic examination was done using both thick and thin blood films. In the course of this study the thick blood film was preferred owing to its higher sensitivity and rapid detection of malaria parasites. Venous blood, anticoagulated using EDTA (Ethylene diamine tetra acetic acid) was used. Romanowsky stains using giemsa staining technique were employed. It is an alcohol–based Romanowsky stain that requires dilution in pH 7.1 – 7.2 buffered water before use.

Microscopic blood film examination was done using magnifications of 10x, 40x and 100x objective lens, starting with the 10x and 40x objective lens to identify specific areas of the film to be viewed under the 100x objective lens. We examined for malaria parasites and pigments. The appropriate approximate number of parasites seen on viewing was reported (trophozoites, schizonts, and gametocytes) using the following criteria:

A. 1 – 10 Parasites per 100 high power field…… +
B. 11 – 100 Parasites per 100 high power field……++
C. 1- 10 Parasites in every high power field……. +++
D. More than 10 in every high power field……. ++++ (Baker et al, 1998; Cheesbrough, 2005).

Statistical Analysis:

The data gotten was analyzed by tallying the responses in fives with the total number of each response calculated to give the frequencies using SPSS 16.0 full version and Microsoft excel packages.

III. Results

This study described the patterns of parasitological response in patients treated with Artemether-Lumefantrine Combination Therapy (ACT) for uncomplicated malaria in Elele, South-South Nigeria. The sociodemographic characteristics of the patients that participated in the study showed that a total of one hundred subjects participated As well as completed the study. Half of the patients (50%) were of age range 21-40. 22 of the patients (22%) were aged ≤ 20 years, while 15 of the patients (15%)were of age range 41-60 years. There were 40 male (40%) and 60 female (60%) patients that participated in the study. The mean and std. deviation for the male and female sexes were **1.60 ± 0.49.** Married patients were 52 (52%) while single patients were 38 (38%). 30% of the patients attained primary education while 42% attained secondary education. Only 28% of the patients had tertiary education status (as shown in table 1).

The degree of parasitaemia on day 0 (day of presentation) showed that 88 patients (88%) had mild (+) parasitaemia, while 10 patients (10%) had moderate (++) parasitaemia. Only 2 patients (2%) had severe parasitaemia (+++) (as shown in table 2).

The pattern of malaria parasite clearance in the course of treatment was also studied. By day 8 of therapy, there was no detectable parasite on the 88 patients that presented with mild (+) parasitaemia on day 0. Still on day 8, there was detectable mild (+) parasitaemia in 3 patients that had moderate parasitaemia and 1 patient that had severe parasitaemia on day 0. However, by day 14 following therapy, there was no detectable malaria parasite in all the 100 patients studied. See also table 2 & 3 below. **There was effective parasitological response/clearance (ECR) by day 14.**

IV. Discussion

This out-patient study assessed the parasitological clearance/response in patients with uncomplicated malaria ACL.

From the results of the patient's biodata, those of age range 21-40 were in the majority. This may be a reflection of the fact that they represent a greater percentage of the work force age group, who can easily afford health care services. On the other hand, those of age > 80 years were in the great minority. People of ages more than 80 years represent a dependent age group in our society, most of who might start the study but may end up not completing the protocol. This compared with a study by Chukwu, et al of 2010 on the knowledge and practice of prevention of maternal to child transmission among HIV positive women in Nigeria. In that study, patients aged 25-29 were in majority. But on the contrary, those aged 30-34 and 40 years and above were in minority.

The result showed a high female population in the studied group. There were 40 male (40%) and 60 female (60%) patients who participated in the study. The mean and std. deviation for the male and female sexes were **1.60 ± 0.49.** The high female population in this our study is consistent with an Ivorean study of malaria parasite clearance following ACT treatment were the study population consisted of 44.4% males and 55.6% females in the AL group. In the AS + AQ group, 40.3% of patients were males (Toure et al, 2018). This may depict the fact that females represent a highly vulnerable group especially when pregnant and most women enjoy the support of their spouses especially when engaged in cordial relationships in their marriage. A WHO study reported that males utilize health care services less than females. In the same report from Papua New Guinea, adolescents (10-19 years old) and adults (20-40 years old) women were more likely than men of same ages to walk long distances to obtain treatment for malaria (Muller, et al 1998; WHO, 2007). On the contrary, in Ethiopia, women were found to be using community health services for malaria less frequently than men. This may probably be due to their workload giving them little time to attend to their personal health needs and that of their children (Ghebreyesus et al, 2000; WHO, 2007).

Married people (especially married women) have easier access to health care services than single women as most of them receive financial and other support from their partners (husbands). This agreed with the finding in the study, Chukwu, et al 2010, which identified married women as having better and easier access to health care facilities and services than single women.

From this study also, those with high educational status (tertiary level) were in minority when compared with those with lower educational status (i.e. primary & secondary education). In the study mentioned above (Chukwu, et al 2010), higher educational status was found to have a direct relationship with prevention of mother to child transmission of HIV/AIDS and highlighted that both malaria and HIV/AIDS are diseases of serious Public Health concerns. This may mean that those with higher educational status may practice malaria preventive measures than their counterparts with lower educational status.

From the study, there was a decrease in the malaria parasite load in the course of treatment. By day 8, there was no detectable malaria parasite in all patients with mild (+) parasitaemia on day 0. However on the same day, malaria parasites, mild (+) was detected in three (3) of those who had moderate parasitaemia (++) on day 0 and in one (1) of those with severe parasitaemia on day 0. However, those with severe parasitaemia did not show other symptoms and signs of severe malaria and there was no organ dysfunction. From the study we concluded that, by the 8th day, there was Effective Parasitological Response/Clearance (EPR), in all the subjects with day 0 mild (+) parasitaemia. **By day 14, there was effective parasitological clearance (EPR) in the 100 patients studied, 100%; p <0.05 which was significant (whether mild, moderate or severe parasitaemia on day 0);** See table 3 below. However, our results are consistent with those of a Ghanaian study in which malaria patients were treated with Artemether-Lumefantrine combination and other ACT regimen, the proportion of subjects with Adequate Clinical and Parasitological Response were, 97.1% (100/103) and 98.2% (107/109) on day 14, and 94.2% (97/103) and 95.3% (102/107) on day 28 in the Artemether-Lumefantrine regimen and Artemether-Amodiaquine groups, respectively (Adjei, et al 2008). In another study in Mali, the 14 day per protocol cure (ACPR) rates before adjusting for cases of re-infection was 98.7% (228 of 231) for subjects receiving artemether-lumefantrine. When adjusted for cases of re-infection on the 14- day cure rate for the regimen was 100% (Issaka, et al 2008). It is also worthy of note that according to WHO, detectable parasitaemia does not mean malaria disease in a malaria endemic area (WHO, 2004). In the Ivorean study above, out of 120 patients (57 in the AS + AQ group and 63 in the AL group) who were randomized among 298 patients screened. The median parasite clearance time was 30 hours (IQR, 24–36 hours), for each ACT. The median parasite clearance rate had a slope half-life of 2.36 hours (IQR, 1.85–2.88 hours) and 2.23 hours (IQR, 1.74–2.63 hours) for AS + AQ and AL, respectively. They concluded that, Patients treated with AS + AQ and AL had cleared parasites rapidly. ACTs are still efficacious in Bouaké, Côte d'Ivoire, but continued efficacy monitoring of ACTs is needed.

Conclusions from our study: We hereby concluded that 88% of malaria patients may likely present with mild (+) parasitaemia; 10% will have moderate parasitaemia while only 2% will have severe malaria parasitaemia in a malaria endemic area like Elele, Rivers State.

The cure rate of this regimen (artemether-lumefantrine) is high in Elele, Rivers State of Nigeria. The **effective parasitological clearance (EPR) in the 100 patients studied** was **100% (n = $^{100}/_{100}$) on day 14 of therapy**. Hence artemether-lumefantrine combination is 100% efficacious in the treatment of uncomplicated malaria in Elele, Rivers State of Nigeria.

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Conflict of interests: There was no conflict of interests.

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FIGURE LEGEND PAGE:

Sociodemographic Characteristics		N=100	Percentage (%)
Age (in years)	≤20	22	22
	21-40	50	50
	41-60	15	15
	61-80	9	9
	>80	4	4
Sex	Male	40	40
	Female	60	60
Marital Status	Single	38	38
	Married	52	52
Educational Status	Primary	30	30
	Secondary	42	42
	Tertiary	28	28

Table 1: Shows the sociodemographic characteristics of the patients:

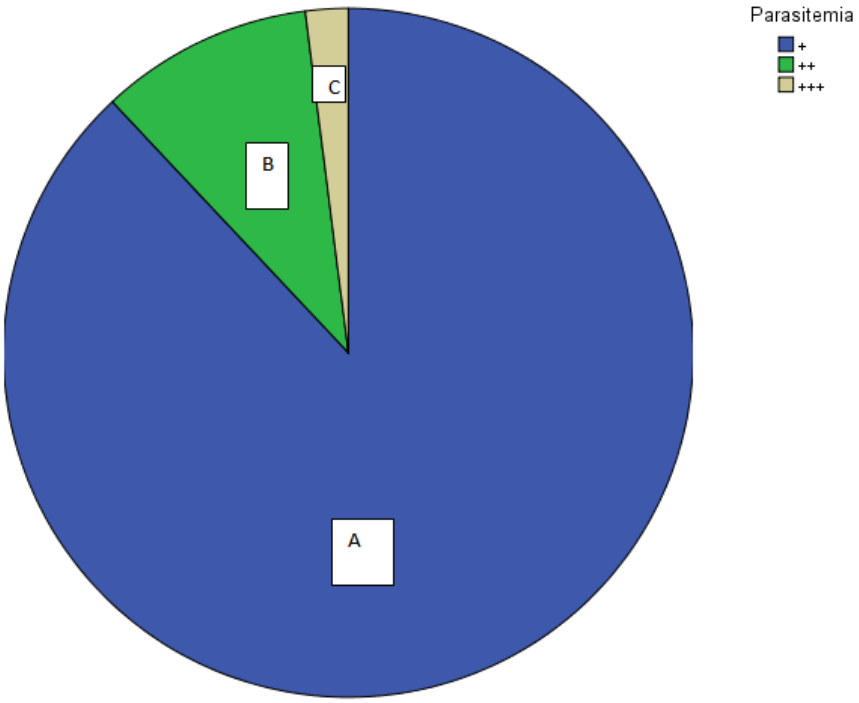

Figure 1: Showing the degree of parasitaemia among the patients on day O:

KEY:
A (blue) = mild (+) parasitaemia, 88 patients (88%)
B (green) = moderate (++) parasitaemia. 10 patients (10%)
C (grey) = severe (+++) parasitaemia. 2 patients (2%)

	Parasitaemia	DAYS TREATMENT & FOLLOW UP						
		Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 14
No of patients with MP	Mild	88	80	62	40	0	0	0
No of patients with MP	Moderate	10	10	9	5	3	1	0
No of patients with MP	Severe	2	2	2	2	1	1	0

Table 2. Shows degree of parasitemia and parasite clearance time:

Parasitaemia	Days of treatment						
	0	2	4	6	8	10	14
+ (n=88)	88 (100.0)	80 (90.9) (p <0.05)	62 (70.5)	40 (45.5)	0 (0.0)	0 (0.0)	0 (0.0)
++ (n=10)	10 (100.0)	10 (100.0)	9 (90.0)(MP+)	5 (50.0)(MP+)	3 (30.0)(MP+)	1 (10.0) (MP+) (p >0.05)	0 (0.0)
+++ (n=2)	2 (100.0)	2 (100.0)	2 (100.0)	2 (100.0)	1 (50.0)(MP++)	1 (50.0)(MP+) (p<0.05)	0 (0.0)

Table 3. Shows degree of parasitemia and parasite clearance time with p values:

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