Research Article ISSN 2639-9458

Microbiology & Infectious Diseases

Antimalarial Treatment Study in South-Western Nigeria

Akanni E. Olufemi[1], Jerry T. Thornthwaite[2*], Ayankunle A. Ademola[3] and Alli O.A.T[1]

[1]*Medical Laboratory Science Department, Ladoke Akintola University of Technology, College of Health Sciences, P.M.B. 4400, Osogbo. Osun State. Nigeria.*

[2]*Cancer Research Institute of West Tennessee, 114 East Main Street, Henderson, TN 38340 USA.*

[3]*Pharmacology & Therapeutics Department, Ladoke Akintola University of Technology, College of Health Sciences, P.M.B. 4400, Osogbo. Osun State. Nigeria.*

***Correspondence:**
Dr. Jerry T. Thornthwaite, Cancer Research Institute of West Tennessee, 114 East Main Street, Henderson, TN 38340 USA, Tel: 731-608-2123.

Received: 28 February 2019; **Accepted:** 18 March 2019

Citation: Akanni E. Olufemi, Jerry T. Thornthwaite, Ayankunle A. Ademola, et al. Antimalarial Treatment Study in South-Western Nigeria. Microbiol Infect Dis. 2019; 3(1): 1-7.

ABSTRACT

Development of antimalarial treatment capable of providing a permanent cure for malaria has been a herculean task for drug researchers. A trial of a novel, proprietary blend formulation (Triantimal™) was conducted in Osogbo, Nigeria following the exciting report from previous clinical trials with malaria infected babies, children, and adults over 10 years in Haiti. There were 127 children, ages 2-15, who were positive for Plasmodium falciparum (P. falciparum) parasites, whose parents gave consent to participate in the study. Enrolled subjects were screened for malaria parasites, treated with Triantimal™ for 16 consecutive days and serums (n=112) and buffy coats (n=31) were collected on days 0, 5, 10, 16, 30, and 60. Of the 127 cases, 15 patients were lost to follow-up with 11 failures, three from one family suggesting non-compliance. No recurrences occurred within 30-60 days by being parasite free at 30-60 days and revealed an 86.2% no recurrence after 720 days. A recent new children study (n=51) also showed a 90.2% cure rate at 60 days with only one non-complaint patient. An adult study (n=21) showed a 100% cure rate at 60 days without any non-complaint patients. These data show for the first time a real possibility for a cure of malaria in Nigeria. The one-time, low dose, fast acting, extended treatment minimizes the ability of the parasites to develop resistance. Obtaining the serums and buffy coats, will allow for the study of humoral and (or) cell-mediated immunological mechanism(s) of permanent immunity.

Keywords

Triantimal™, Artemisinin, Malaria infection, Immunity, *Plasmodium falciparum*.

Introduction

Malaria is a disease of global public health importance creates social and economic burden in Nigeria and many of the world's poorest countries. In heavily affected countries, malaria alone accounts for as much as 40% of public health expenditure, 30% to 50% of hospital admissions, and up to 60% of outpatient visits [1]. Approximately 250 million episodes with more than a million deaths occur annually, especially in infants, young children, and pregnant women [2]. Malaria is spread from person to person by the bite of mosquitoes infected with Plasmodium. Among the different species, *P. falciparum* is the most common cause of malaria worldwide and it is responsible for the majority of deaths [2]. The World Health Organization (WHO) recommends Artemisinin-based Combination Therapy (ACTs) for treating uncomplicated malaria. The ACTs combine an artemisinin-derivative (short acting drugs which are very effective) with another longer lasting drug to reduce the risk of developing resistance. Artemisinin derivatives have been reported to produce more rapid relief of symptoms and faster clearance of parasites from the blood than other antimalarial drugs [3-5]. Artemisinin was recommended for at least seven days when taken as monotherapy, because of its short half-life [4,6]. Artemisinin derivatives may be administered be administered for shorter durations when combined with any other recommended antimalarials [3,7].

Artemisinin and its derivatives are known to reduce the development of gametocytes, the sexually reproductive form of the malaria parasite, and consequently the carriage of gametocytes in the peripheral blood [8,9]. This reduction in gametocytes has the potential to reduce the post-treatment transmission of malaria [3]. Artemisinin is generally reported as being safe and well tolerated [3,10].

Flavonoids are a group of polyphenolic compounds, which are widely distributed throughout the plant kingdom. Over 3000 varieties of flavonoids have been identified [11]. The vast majority have low toxicity in mammals and some of them are widely used in medicine for maintenance of capillary integrity [12]. Flavonoids exhibit several biological effects such as anti-inflammatory, anti-hepatotoxic and anti-ulcer actions [13,14]. They also inhibit enzymes such as aldose reductase and xanthine oxidase. They are potent antioxidants and have free radical scavenging abilities. Many have anti-allergic, antiviral actions and some of them provide protection against cardiovascular mortality [15,16]. They have been shown to inhibit the growth of various cancer cell lines in vitro [17] and reduce tumour development in humans and animals [18,19]. This study is focused on administering Artemisinin in combination with bioflavonoids (this combination is labelled TriAntiMal™) to cure malaria and prevent its re-infection in the people of Nigeria. A similar formulation study was conducted in Haiti and was found that this, which cured malaria and prevented re-infection for the past twelve years (data not shown).

The objectives of this study include assessment of the antimalarial efficacy of TriAntiMal™ for children, and adults and provide a model system for the analyses of buffy coat DNA amplification and serum to determine why patients experience long-term immunity.

Materials and Methods

Study site

The study was conducted at the Primary Health Centre, Sabo area, Olorunda local Government, European–Union Prime project facility, Osogbo, Osun State. Nigeria. The study was approved by the UNIOSUN Health Research Committee with monitoring by designated representatives of the HREC committee. Children (n=127 less 15 being non-compliant), ages 2-15 years old, were recruited into the study to determine the overall curative rate of *P. falciparum* in the patients.

Study design

Patients were screened for fever using infrared thermometers and those having temperatures >37.5 C were selected. The selected patients were further checked by a Giemsa-stained thick blood smear. Those with a positive smear results will be assessed by study clinicians for the following inclusion criteria: Patient diagnosed to have malaria with parasitemia load of 2,000 - 100,000 parasites/µl; fever with axillary temperature greater or equal to 37.5 C; age 2–15 years; HIV screened negative; those who will be available to have their blood drawn as scheduled; willingness to comply with the daily oral medicine of 16 days.

Patient Exclusion criteria included the following: unwillingness to take the Malaria Formulation for 16 consecutive days; concomitant infection, i.e., malaria infected patient that has any other infection; treatment with any other anti-malaria drug within the one week of evaluation for this study; acute severe complicated malaria e.g. vomiting frequently that requires the administration of intravenous fluid, convulsion, severe anaemia with PCV <18%, clinical evidence of pulmonary edema, feature suggestive of renal failure, history of dark brown colour urine which is suggestive of severe red blood cell haemolysis; hyperparasitemia with >105 parasites/µl; Patient with temperature <37.5C; hyperpyrexia with temperature ≥ 40 C; low density Parasitemia:<$2x10^3$ parasite per micro litre; HIV screened positive; and inability to obtain parental consent.

All treatments were directly administered at the clinic and patients observed for 30 minutes and doses re-administered when vomiting occurred, but those with repeated vomiting on day 0 were excluded from the study.

Laboratory procedures

Blood smears were air dried, stained with a 2% Giemsa solution for 15 min, rinsed with water and re-air dried and viewed under the microscope using oil immersion lens. Parasite densities were calculated from thick smears as the number of asexual parasites per 200 leukocytes (or per 500 leukocytes if the parasite density was <10 parasites per 200 leukocytes), assuming a leukocyte count of $6x10^3$ leukocytes/µL. Smear findings were considered negative when microscopic examination of 100 high-power fields did not reveal parasites. Counts were performed by two WHO-certified microscopists and discrepant readings resolved by a third reader. Thin blood smears were performed to evaluate parasite species. Packed cell volume was measured from finger-prick blood samples using heparinised capillary tube. HIV screening tests were done by finger prick sampling for an accurate measurement using the WHO approved DETERMINE HIV strips. Five ml of venous blood were taken, and the serums extracted were sent to Dr. Thornthwaite's Institute for immunological testing.

Sample collection schedule: HIV screening was done on day 0. Blood for haematocrit was obtained on days 0, 1, 2, 3, 7, 14, 30, and 60. Thin blood film for malaria parasite and haematocrit were obtained on days 0, 7, 14, 30, and 60. The serums for immunologic testing were sampled on days 0, 5, 10, 16, 30, and 60.

To understand the immune processes involved in long-term immunity, serums (n=112) and buffy coats (n=25) were from the original children at days 0, 5, 10, 16, 30, 60, and 730.

In this paper data will also be presented with new children (n=51) and adults (n=21). Serums were separated at days 0 and 60. The survival data from the new children was equivalent to the original study at 90.2% at Day 60; and the survival studies with the adults was 100% at Day 60.

All the above samplings were frozen at -70°C and shipped to the Cancer Research Institute on dry ice. Upon receiving them, the samples were thawed once and aliquoted into three equal parts and refrozen at -84°C. Studies are ongoing to present the results of the DNA buffy coat and serum analyses in future research reports.

Drug provision

The TriAntiMal™ formulations were supplied by Dr. Jerry T. Thornthwaite, Director, Cancer Research Institute of West Tennessee, 114 East Main Street, Henderson, TN, USA. Each

capsule contains a proprietary blend of 50 mg artemisinin (97%) and 50 mg antioxidants, bioflavonoids, synephrine, artemisinin, quercetin, curcuminoids, hesperetin, plus flavonoids (patent pending).

Drug administration

Using the TriAntiMal™ treatment designated for this study was the malaria medicine designate.

Handling of adverse effects

Symptoms and signs that were not part of presenting features were taking as adverse effects. Though artemisinin and bioflavonoids are known to be very safe, adequate medical personnel were available to take care of any side effects. There were no noticeable adverse side effects of the drug observed during or post treatment.

Confidentiality

Data was handled by the researchers and the names of each patient coded.

Alternative treatment

Dihydroartemisinin/piperaquine fixed antimalarial combinations were administered to patients who withdrew from the study before parasitemia was cleared or patients that fail on the study drug.

Ethical clearance

This was obtained from Ethical Committee, Osun State University in Osogbo, Nigeria.

Data analysis

All data were analysed statistically using standard deviations and the analysis of True Population Proportion Curve Rate at 95% confidence limits and p values determined.

Results

The original study of the malaria parasite-free children (n=101) is shown in Table 1 and Figure 1, while the original recurrent children (n=11) is presented in Table 2 and Figure 2.

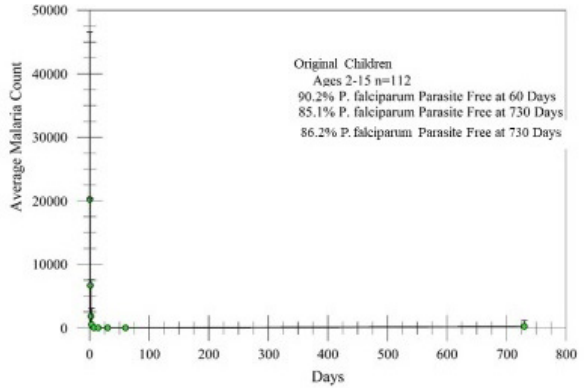

Figure 1: Original Children cured children at from 60 days (101) up to 730 days (n= 51) showing 90.2% parasite-free at 60 days and 86.2% at 730 days (average days over a two-week period of follow-up).

In Figure 3, the Original Children study (n=112), the ages ranged between 2.5 to 15 years old for an average age of 8.4 ±3.6 SD (n=101) for the cured children and 8.3 ± 2.4 (n=11) for the recurrent children. The average Malaria Parasite Load (MPL) at day zero was about 22,000 for both the cured and recurrent patients. The average age of the cured and recurrent patients was 8.4 ± 3.6 SD and 8.3 ± 2.4 SD, respectively. The patient groups were almost equally divided (Males 52.8 ± 5.8 SD n=112) between males and females.

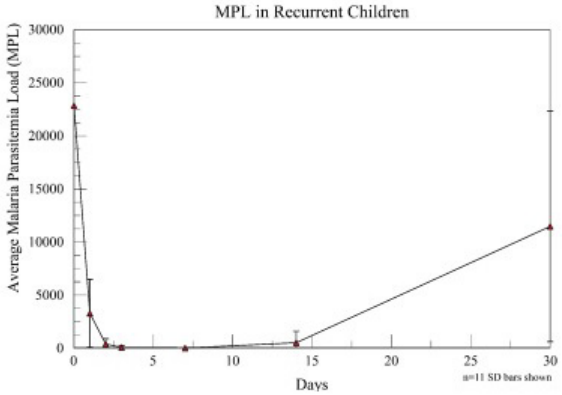

Figure 2: Average Malaria Parasite Load (MPL) for Recurrent Children in the original group (n=11). SD bars shown.

Statistical Data from the Children Treated with the TriAntiMal™

Average Malaria Parasite Load MPL) at Day 0 Cured: 20,224 ± 26339 SD Recurrent: 22,851 ± 24,060

Average Age of the Patients: Cured: 8.4 ± 3.6 Recurrent: 8.3 ± 2.4

Percentage of Males – Cured: 55.4%

Cure Rate – 90.2%

The True Population Proportion Cure Rate is between 0.847 and 0.957 at a 95% confidence interval for 112 children total with 101 being cured

$$\hat{p} - Z_{\alpha/2}\sqrt{\frac{\hat{p}\ x\ \hat{q})}{n}} \le p \le \hat{p} + Z_{\alpha/2}\sqrt{\frac{\hat{p}\ x\ \hat{q})}{n}}$$

$$0.902 - 1.96\sqrt{\frac{(0.902)(0.098)}{112}} \le p \le 0.902 + 1.96\sqrt{\frac{(0.902)(0.098)}{112}}$$

$$0.847 \le p \le 0.957$$

$Z_{\alpha/2} = 1.96$ for 95% confidence interval for $n \ge 30$ p = Proportion of Cured Patients q = Portion of Patients with recurrent Malaria

p = Cure Rate n = Total Number of Patients Cure rate will tend to go toward the higher value and the range will narrow for n >> 1,000

Figure 3: The True Proportional Cure Rate (TPCR) at a 95% confidence interval is between 0.847 and 0.957 for all 112 children in the original group with 101 MPL free at 60 days. At 730 Days (Figure 1), the TPCR experimentally fell within the range at 0.851. The average ± SD MPL at Day 0 and age for cured (MPL = 0 at Day 60) and Recurrent Children are shown.

In Figure 3, The True Population Proportion Cure Rate is between 0.847 and 0.957 at a 95% confidence interval for the original 112 children. This means that there is a 95% confidence in a much larger study, and the cure rate would fall between these values. As the number of patients increase, the range should become narrower and the average cure rate would move toward the higher value. This range remained within these limits at Day 730 ± 2 weeks where 51 patients were retested as available and at random after consent was granted in writing with a brief history of no previous recurrence was reported. The cure rate in the Original Children's study was 90.2% and decreased 4.0% by Day 730 (n=51).

Dr. Akanni
Dr. Thornthwaite

TABLE 1

MALARIA PARASITEMIA LOAD (MPL) Children treated for Malaria

LAB NO.	Age	Sex	Day 0	Day 1	Day 2	Day 3	Day 7	Day 14	Day 30	Day 60	Day 730*
PF/001	13	f	16484	12320	1240	0	0	0	0	0	0
PF/005	7	f	12760	6240	440	0	0	0	0	0	0
PF/007	9	f	2040	80	0	0	0	0	0	0	0
PF/008	10	f	12160	1800	120	0	0	0	0	0	0
PF/009	13	f	2480	0	0	0	0	0	0	0	0
PF/010	3	f	3560	880	0	0	0	0	0	0	0
PF/019	13	f	89040	20480	5640	1160	0	0	0	0	0
PF/020	3	f	47760						0		
PF/022	6	f	13200	3640	960	0	0	0	0	0	
PF/023	11	f	15280	4480	880	0	0	0	0	0	0
PF/026	11	f	9760	1120	280	0	0	0	0	0	0
PF/027	4	f	10960	2240	160	0	0	0	0	0	0
PF/029	11	f	2040	160	0	0	0	0	0	0	0
PF/036	5	f	4200	360	0	0	0	0	0		
PF/037	6	f	3640	200	0	0	0	0	0	0	0
PF/038	6	f	3640	840	0	0	0	0	0		
PF/041	7	f	3680	1960	120	0	0	0	0		
PF/042	11	f	2920	160	0	0	0	0	0		
PF/051	7	f	2420	80	0	0	0	0	0	0	0
PF/052	4	f	2800	160	0	0	0	0	0	0	0
PF/053	4	f	2040	0	0	0	0	0	0		
PF/057	12	f	21760	2760	80	0	0	0	0	0	0
PF/058	10	f	5120	120	0	0	0	0	0		
PF/060	3	f	62840		4840	1360	0	0	0		
PF/064	13	f	4360	320	0	0	0	0	0		
PF/072	3	f	2760	80	0	0	0	0	0		
PF/075	5	f	2240	480	0	0	0		0		
PF/078		f	74400	14680		360	0	0	0	0	0
PF/082	14	f	2360	320	0	0	0	0	0	0	0
PF/083	15	f	3240	560	0	0	0	0	0	0	0
PF/084	6	f	77640	15520	2120	0	0	0	0	0	0
PF/087	15	f	27600	8560	1160	0	0	0	0		
PF/088	4	f	3320	280	0	0	0	0	0	0	0
PF/089	8	f	84360	25280	1240	0	0	0	0	0	0
PF/094	10	f	98240	72680	45160	22280	0	0	0		0
PF/098	7	f	18320	5240	2240	160	0	0	0		
PF/100	12	f	9480	2440	160	0	0	0	0	0	0
Pf005	9	f	15200	5480	1680	0	0	0	0	0	
Pf009	12	f	2840	120	0	0	0	0	0	0	0
Pf012	13	f	4480	1680	560	0	0	0	0	0	
Pf015	15	f	4360	760	0	0	0	0	0		
Pf016	12	f	32640	10840	4880	1840	0	0	0	0	
Pf017	12	f	46480	15480	2360	0	0	0	0	0	0
Pf019	11	f	3360	480	0	0	0	0	0	0	
Pf021	7	f	22480	4240	0	0	0	0	0	0	
Pf029	11	f	5280	2120	0	0	0	0	0	0	0

*At 730 Days ± 14 days, the MPL = 0 for 86.2% (n=51) of the patients

MALARIA PARASITEMIA LOAD (MPL) Children treated for Malaria

LAB NO.	Age	Sex	Day 0	Day 1	Day 2	Day 3	Day 7	Day 14	Day 30	Day 60	Day 730*
PF/002	6	m	8800	3440	520	0	0	0	0	0	
PF/003	8	m	31868	6720	0	0	0	0	0	0	
PF/004	4	m	5120	760	0	0	0	0	0	0	
PF/006	4	m	3480	160	0	0	0	0	0	0	
PF/012	7	m	7120	2160	180	0	0	0	0	0	1680
PF/015	2.5	m	24084	4800	1640	0	0	0	0	0	0
PF/017	5	m	2400	440	0	0	0	0	0		
PF/021	7	m	18560	6720	1680	240	0	0	0	0	
PF/024	9.5	m	2120	240	0	0	0	0	0	0	0
PF/025	8	m	69040	38240	4360	1280	0	0	0	0	
PF/028	7	m	3480						0		
PF/031	15	m	2160	0	0	0	0	0	0	0	
PF/033	4	m	10320	2880	0	0	0	0	0	0	0
PF/034	8	m	52480	12240	4880	200	0	0	0		0
PF/035	8	m	22720	3360	160	0	0	0	0	0	0
PF/039	14	m	2240	120	0	0	0	0	0		0
PF/040	7	m	48360	2240	200	0	0	0	0	0	2880
PF/046	3	m	14720	2880	0	0	0	0	0	0	0
PF/047	5	m	2440	160	0	0	0	0	0	0	
PF/048	11	m	6480	1160	280	714	0	0	0	0	0
PF/049	7	m	2720	200	0	0	0	0	0	0	
PF/050	6	m	12440	3440	80	0	0	0	0	0	0
PF/055	9	m	3040	160	0	0	0	0	0	0	
PF/056	10	m	12480	2240	160	0	0	0	0		0
PF/059	4	m	15480	7440	2480	0	0	0	0	0	4640
PF/063	4	m	2880	100	0	0	0	0	0	0	
PF/066	6	m	4840	160	0	0	0	0	0	0	0
PF/067	12	m	29200	5440	880	160	0	0	0		
PF/069	10	m	4680	200	0	0	0		0		
PF/074	11	m	3880	440	0	0	0	0	0	0	0
PF/076	10	m	2480	160	0	0	0	0	0	0	
PF/077	15	m	8320	480	0	0	0	0	0	0	6280
PF/079	15	m	48880	4240	1760	0	0	0	0	0	0
PF/080	2	m	62440	16840	2920	160	0	0	0	0	
PF/081	3	m	3640	280	0	0	0	0	0	0	760
PF/085	15	m	4360	160	0	0	0	0	0	0	0
PF/086	11	m	12480	2240	200	0	0	0	0	0	0
PF/090	4	m	48440	13560	2880	0	0	0	0	0	0
PF/091	9	m	2960	240	80	0	0	0	0	0	0
PF/092	4	m	12880	4880	2000	1160	280	0	0	0	1120
PF/096	7	m	18480	3040	120	0	0	0	0	0	1880
PF/099	5	m	99160	66280	6480	1360	280	0	0	0	
Pf001	14	m	3480	440	0	0	0	0	0	0	
Pf002	9	m	32640	22120	3240	280	0	0	0	0	
Pf003	5.5	m	72360	56200	24680	10360	0	0	0	0	0
Pf007	13	m	10480	2360	160	0	0	0	0	0	
Mean	**8.36**		**19650**	**6649**	**1740**	**510**	**5.83**	**0**	**0**	**0**	**377.2**
±SD	**3.69**		**25408**	**13855**	**5735**	**2550**	**40.2**	**0**	**0**	**0**	**2901**

Table 1: Malaria Parasite Free Children showing the Male and Female comparisons. Mean ± SD are shown for the average MPL, Sex, and Age for up to 730 days.

Lab No.	Age	Sex	Day 0	Day 1	Day 2	Day 3	Day 7	Day 14	Day 30	Result
PF/011	5	f	55120	4240			0	2880	2400	Failed
PF/065	7	f	50920	10480	1240	480	0	0	28960	Failed
pf018	8	f	16440	4560	520	0	0	0	8640	Failed
PF/025	12	f	2040	160	0	0	0	0	22160	Failed
PF/018	5	m	69920					2160	8040	Failed
PF/030	7	m	17040	3480	560	0	0	0	32160	Failed
PF/032	12	m	2760				0	0	4840	Failed
PF/043*	10	m	15560	3840	0	0	0	0	4360	Failed
PF/044*	7	m	12560	1480		0	0		6680	Failed
PF/045*	9	m	4360	720		0	0	0	3240	Failed
pf006	9	m	4640	520	0	0	0	0	4680	Failed
Mean	**8.3**		**22851**	**3276**	**387**	**60**	**0**	**504**	**11469**	
SD	**2.4**		**24060**	**3200**	**495**	**170**	**0**	**1076**	**10870**	

Table 2: MPL results for Children with Recurrent Malaria (n=11). All but one had a MPL=0 by day 3. Mean ±SD shown for MPL and Age.

Complete *P. falciparum* clearance by Day 2 or 3 occurred in all age groups and even in the recurrent patients as shown, for example, with the original children study (Figure 2 and Table 2).

Table 2 shows the results of the treatment for the 11 recurrent children. Interestingly, three of the recurrences were from the same family (PF/43-45). All failures had parasite content go to zero by Day 7. Eight were positive by Day 30. Our clinical standard of practice suggests that we should repeat with an adult dosage; however, these patients had already started treatment with the standard malaria drug protocols. There were 15 children who did not comply with the protocol in the original study and were dropped early within a few days. The later buffy coat group, which is included in the original group, only had one drop out.

In the New children group (n=51), there was only one non-compliant patient, while none of the adults (n=21) dropped the study. Apparently, the news of success of this treatment in the first trial of the children was accepted, and new children and adults enthusiastically adhered to the 16-day protocol. The Mean Parasite Load versus days after the start of treatment are shown for the New Children (Figure 4) and Adults (Figure 5) where the rapid clearance of the parasites can be seen. A summary of these studies is summarized in Table 3 for the children and adults.

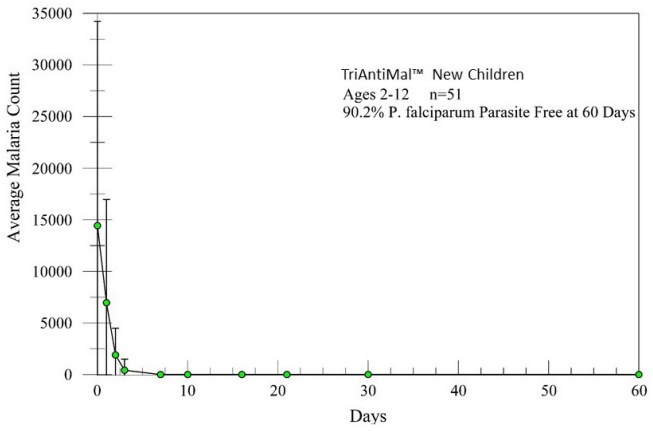

Figure 4: Average MPL count over 60 Days for the TriAntiMal™ treatment (n=51)for New Children. SD bars shown. MPL=0 was 90.2% at Day 60.

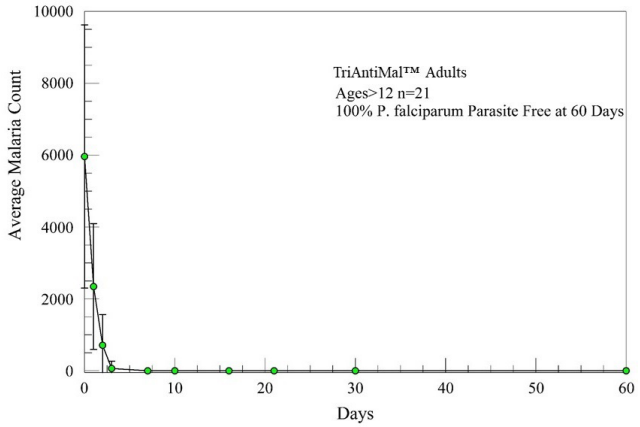

Figure 5: Average MPL count over 60 Days for the TriAntiMal™ treatment (n=21) for Adults. SD bars shown. MPL=0 was 100% at Day 60.

Discussion

The TriAntiMal™ therapy offers a combination treatment that uses a proprietary formulation of citrus bioflavonoids, artemisinin, curcuminoids with selected antioxidants to not only inhibit the enzymes in the intestines that break down artemisinin but also serve to strengthen the walls of blood vessels [20]. Many plants produce flavonoids that may be involved in the defense against plant-threatening factors, such as microbes and toxins [21]. Inflammation and oxidative stress are two major causes of various life-threatening diseases [22]. The antioxidant function for the bioflavonoids apparently does not inhibit the internalized oxidative function of the Artemisinin, while supplying an important role in minimizing oxidative stress in the children. Furthermore, the flavonoids casticin and chrysosplenol D from the Sweet Wormwood plant (Artemisia annua.), the major source of Artemisinin, inhibits inflammation in vitro and in vivo [23].

A pharmaceutical approach to purifying artemisinin from the Sweet Wormwood plant and making derivatives to make it more water soluble and more bioavailable may have decreased the antimalarial effectiveness of treatment compared to making a Sweet Wormwood tea containing both artemisinin and bioflavonoids. We have gone back to nature and added the bioflavonoids and other components back with the artemisinin to make an effective low dose treatment over 16 days.

Artesunate is recommended by the World Health Organization (WHO) in preference to quinidine for the treatment of severe malaria and has been used worldwide for many years. Common artesunate side effects include vomiting, nausea, pyrexia, visual acuity reduced, liver function tests being abnormal, jaundice, dehydration, diarrhea, and hepatic trauma. In the U.S., it is available only for treating patients hospitalized for severe malarial requiring intravenous treatment [24]. Delayed haemolytic anaemia related to artesunate has a strong indication for a drug-immune related mechanism [25,26]. Since haemolysis is commonly associated with this class of artemisinin derivative drug, safety issue may lead to life-threatening anaemia and is particularly concerning for regions of Africa where safe blood products are not readily available.

The reasoning for using low dose Triantimal™ is based on the authors' knowledge with treating cancer. Oncologists are rethinking chemotherapy and are beginning to see the beneficial effects of low dose chemotherapy given on a more frequent basis than using conventional chemotherapy approaches. Excellent antiangiogenic results are seen with low dose chemotherapy [27]. For example, Cyclophosphamide is considered one of the most successful chemotherapeutic drugs and is on the List of Essential Medicines published by WHO. The efficacy of low dose cyclophosphamide is primarily due to its ability to promote anti-tumour immunity, by selectively depleting regulatory T cells and enhancing Natural Killer Cell (NKC) effector T-cell function [28].

The mean short clearance times within 48-60 hr is in contrast with the relative long clearance times experienced with artesunate with their pharmaceutical partners, which were
60-90 hr [29,30].

Following the low dose chemotherapy model in cancer, we set out to treat malaria victims, with the worst form of malaria in Haiti, *P. falciparum*. We treated mainly children and adults involved with the SonLight Children's home (n=37), the children fed five days a week there (n=167) and among the members of at least seven Christian churches that we support with medical missions' clinical studies with at least three visits per year by our medical mission teams. While detailed data are not recorded formally, we do know that follow-up to treatment was well documented. We treated diagnosed malaria cases from babies (n=15), children (n>200) and adults (n>200), many of which would have a history of two or more recurrences a year despite taking malaria medicine. Everyone was given the malaria formulation over a 16-day period. Each day, babies, children and adults were given the Triantimal™ supplements in a Minister of Health approved study. Babies were treated with a half capsule continually mixed with milk and given daily to the babies. After a single 16-day treatment, no malaria victims ever got malaria again as monitored by the churches. Malaria resistance occurred even though these malaria victims being bitten 800-1,000 times a year (African estimates).

The reasoning for using a 16-day treatment was based on the daily low dose treatment which covered the IgM and IgG transition stage in developing what may be called an "in vivo immunization" in which the continuous destruction of the parasites to allows for the presentation of antigen for the humoral, cell mediated, or/and Defensin processes. Studies are being conducted to elucidate the mechanism(s) for the long-term immunity against the *P. falciparum* parasites.

The vast numbers of children, babies and adults have been cured with almost complete parasite clearance by Days 2-3, which

minimizes the opportunity for the development of artemisinin resistance and results in the long-term immunity. Therefore, the serum and buffy coat samples provide a sample base to discovery the mechanism(s) for long-term immunity. While the analysis of the serum and buffy coat samples are the subject of the next paper, our reasoning for a 16-day treatment is based, in part, on a possible IgM-IgG transition determination with a host of cell-mediated immunity determinants. A thorough examination of these parameters along with the DNA amplification results will help us understand why the people of Haiti and Nigeria are developing long-term immunity to malaria after a single treatment protocol. Also, comparisons with the few so-called failures may explain the immunologic cause for failure. The results from these analyses, will help us better understand the mechanisms for anti-parasite infection to possibly shorten the treatment time and determine what other parasite types are being killed during this treatment process. The TriAntiMal™ regiment is safe, efficacious, possibly one-time ACT treatment that may warrant the treatment of all people living in malaria infested countries, regardless if they have active malaria in the blood or not, since the parasite is endemic in the possibly the entire population.

Acknowledgments

This research was funded by the Cancer Research Institute of West Tennessee with support from Henry Respess, the Shumard Family Fund, and the Carter Foundation. This work began 12 years ago in collaboration between Dr. & Mrs. Thornthwaite with Roberta Edwards, Dr. Wildert Charles and the Ministry of Health in Haiti, in treating babies, children and adults for *P. falciparum*. We thank Professor Alebiosu, Chairman of the UNIOSUN HREC Committee, and the College of Health Sciences at the Osun State University in Osogbo, Nigeria and the Ladoke Akintola University of Technology, College of Health Sciences, Osogbo, Nigeria. Special thanks to the doctors, nurses and staff who were successful in maintaining compliance to the protocol of the study presented herein. We thank Dr. Pat Evans and Seth Thibado for their statistical analyses and graphics, and Dr. Tony Kirk and Bonita Thornthwaite for their careful proof reading of this manuscript. We thank Demetria Kalodimos for her careful documentation of our research journey. This study is dedicated to the memory of Roberta Edwards.

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