FOREWORDS

The parasite resistance to artemisinins along the Thai-Cambodia border area in the last five years is an early warning to us that we are losing the most optimal weapons fighting the parasites. Vietnam shares a border line with Cambodia, where *P. falciparum* is proven highly resistant to chloroquine, mefloquine, quinin and reduced responsive to various currently used drugs, including artesunate. Confronted with the warning signs, the WHO has recommended world countries to switch to the artemisinin combination therapies (ACTs). The dihydroartemisin plus piperaquine combination, which was listed into the essential antimalarial drugs since 2007 in Vietnam, has been used for 5 years until resistance appears in some Southern, Central of Western highland provinces.

Additionally, chloroquine (CQ) has long been used in Vietnam as a multi-purpose drug such as prophylaxis and treatment for both *P. falciparum* and *P. vivax* malaria for almost 60 years. Although there haven't been any reports of CQ resistance by *P. vivax* in the Central – West highland areas; many studies in the Southeast Asian countries have found resistance of the parasite to the drug at different levels. Therefore, it is necessary to evaluate the efficacy of the antimalarial drugs to contribute to the data accomplishment and to propose malaria treatment regimens that fit into the current situation and base as fundamentals for designing the national dug policy in the future. The study was conducted with objectives:

1. To evaluate the drug efficacy of dihydroartemisinine-piperaquine in the treatment of uncomplicated *Plasmodium falciparum* patients;
2. To evaluate the drug efficacy of chloroquine phosphate in treatment of *Plasmodium vivax* patients.

THE NEW, SCIENTIFIC AND PRACTICAL CONTRIBUTIONS

With the general objective of this study is to assess the therapeutic efficacy and safety of dihydroartemisinin plus piperaquine (DHA-PPQ)

and chloroquine (CQ)_for the treatment of uncomplicated falciparum and vivax malaria, respectively in Central highland areas of Vietnam. This thesis contributed some new, scientific, and practical aspects:

- Measurement of the clinical and parasitological efficacy of DHA-PPQ and CQ by the adequate clinical and parasitological response (ACPR), early treatment failure (ETF), and late clinical or parasitological failure (LCF, LPF) in the treatment for uncomplicated falciparum and vivax malaria, then formulate recommendations and to enable the Ministry of Health to make informed decisions about whether the current national antimalarial treatment guidelines should be updated;
- Applying of advanced techniques in differentiate recrudescence from new infection by PCR analysis with polymorphism of molecular markers or CQ resistant determination by measure of chloroquine plus desethylchloroquine metabolite;
- Detection of DHA-PPQ resistant *P. falciparum* in the Phu Thien district - Gia Lai sentinel site without Vietnam-Cambodia cross border;
- The role of the spleen is very obvious in parasite clearing intervention, so that it is necessary to supplement this criterion into "exclusive criteria" of the proposals to assess the *P. falciparum* and *P. vivax* drug efficacy *in vivo* test when conducting the research in the field or evaluating the effectiveness at the hospital treatment system.

STRUCTURE OF THESIS

The thesis has totally 127 pages (not include references and annex parts). With foreword (2 pages), medical literature review (32 pages), subjects and methods (26 pages), results (29 pages), discusions (35 pages), conclusions and recommendations (2 pages). Total figures (8 images and 10 figures), 34 tables. The references included 118 (19 Vietnamese and 99 English references), and other 10 annexes.

Chapter 1. GENERAL MEDICAL LITERATURE REVIEW

1.1. Global malaria and antimalarial resistance

Malaria remains a major cause of morbidity and death in endemic areas. The most severe form of malaria, which is responsible for the great majority of malaria-related deaths, is associated with infection due to the species *P. falciparum*. Of the five *Plasmodium* species that infect man, *P. falciparum* has the multi-drug resistance. To date, parasite resistance has been documented in three of the five malaria species known to affect humans *P. falciparum*, *P. vivax* and *P. malariae*.

Efficacious antimalarial medicines are critical to malaria control, and continuous monitoring of their efficacy is needed to inform treatment policies in malaria endemic countries as resistance to antimalarial drugs is a major public health problem. The emergence of *P. falciparum* resistance to artemisinin is an urgent health concern, threatening the sustainability of the ongoing global effort to reduce the burden of malaria.

1.1.1. Antimalarial resistance emergence by *P. falciparum* and *P. vivax*

The development of resistance can be considered to occur in two phases. In the first phase, an initial genetic event produces a resistant mutant; the new genetic trait gives the parasite a survival advantage against the drug. In the second phase, the resistant parasites are selected for and begin to multiply, eventually resulting in a parasite population that is no longer susceptible to treatment. They are considered to occur randomly, independently of the drug. These events are characterized by gene mutations or changes in the number of copies of genes that determine the drug's target in parasite (Valderramos *et al*., 2010). In Africa, the advent of CQ resistance was not linked to the appearance of a new mutation there but to the slow, gradual spread of CQ-resistant parasites from South East Asia, which finally arrived in East Africa in 1978 (Sá *et al*., 2009). In contrast, resistance to antifolate and atovaquone arises more frequently (Vinayak *et al*., 2010), it was shown in microsatellite marker

studies that *P. falciparum* resistant to CQ or highly resistant to pyrimethamine both originated in South East Asia and subsequently spread to Africa (Roper *et al*., 2004). The emergence of resistance to mefloquine arose rapidly on the Cambodia-Thailand border in the 1980s.

Because of the perniciously increase in resistance of *P. falciparum* to drugs such as CQ, quinine, and mefloquine, new agents have had to be developed. Historically, malaria was treated by drug monotherapy, most notably with CQ, which was the standard treatment for more than 60 years and CQ resistance developed and is now highly prevalent in nearly all endemic regions. Resistance has been reported to most antimalarial drugs except for ACTs. The WHO has recommended that artemisinin combination treatment (ACTs) should be regarded as the "policy standard" for treatment of falciparum malaria. In developing ACTs regimens the aim is to achieve rapid schizontocidal activity by means of the selected artemisinins together with a different mechanism of action and longer half-life partner agent in delaying resistance.

Indeed, CQ has been the first-line therapy for vivax malaria since 1946 and resistance by *P. vivax* was unknown until 1989 in Papua New Guinea, subsequent reports affirmed that finding, and CQ-resistant *P. vivax* was reported from Indonesia, Myanmar, India, Guyana, South America, Thailand, Philippines,…and emerging resistance to CQ by *P. vivax* threatens the health of the millions of people routinely exposed to the risk of infection with this agent.

1.1.2. ACTs and DHA-PPQ efficacy data from clinical trials

Current ACTs therapy with artemisinin derivatives rapidly decrease the parasite biomass, while the presence of a second antimalarial with a different mechanism of action reduces the probability of the emergence of resistant strains. DHA-PPQ is one of five specific ACTs have been recommended over time by the WHO. This DHA-PPQ as an ACTs option for the "first-line treatment of uncomplicated *P. falciparum* malaria

worldwide. The standard treatment regimen is a highly efficacious and safe treatment. In several studies the DHA-PPQ has resulted in high cure rates with 42 day or 63 day follow up (> 95%) with excellent tolerability in the treatment of adults and children with *P. falciparum* malaria (Karunajeewa *et al.,* 2004; Tangpukdee *et al.*, 2005; Karema *et al.*, 2006; Hasugian *et al.,* 2007).

The efficacy data are available from studies conducted from 2005-2008 from 14 clinical trials on DHA-PPQ combinations. Total of 2.636 patients were exposed to DHA-PPQ for the treatment of multidrug-resistant uncomplicated *P. falciparum* malaria, the efficacy of DHA-PPQ was excellent, with the overall cure rates of 97-98% in China, Cambodia, Myanmar, Laos PDR, Thailand and Vietnam. In the comparative studies, the efficacy of DHA-PPQ was as good as mefloquine + artesunate and it was better than artesunate + amodiaquine (Jannsens B *et al*, 2007; Adam I *et al*, 2010). Some studies in Africa showed that high cure rate low incidence of new infections (D'Alessandro U *et al.*, 2010).

1.2. Antimalarial resistance in Vietnam

Similar to other countries in the Mekong Subregion, Vietnam's antimalarial resistance has emerged to all classes of antimalarial drugs except ACTs. Hence, DHA-PPQ have been deployed effectively as first-line treatment for *P. falciparum* in line with WHO, and this DHA-PPQ proved to be a highly effective antimalarial drug for the treatment of *P.falciparum* malaria and suitable for use in many endemic areas of Vietnam, ACPR from 94.7 - 100% (Tran Tinh Hien *et al.*, 2004; Ta Thi Tinh *et al.,* 2012*;* Bùi Quang Phuc *et al.*, 2013) in Binh Phuoc, Dak Nong, Ninh Thuan, Gia Lai, Quang Tri from 2005-2013, but some recent data showed that positive asexual form of *P. falciparum* at D_3 in 17 - 30% as an indirect clinical indicator for resistance (Ta Thi Tinh *et al.*, 2012; Bui Quang Phuc *et al.,* 2013).

Chapter 2. SUBJECTS AND METHODS

2.1. Locations and timing of study

The study was conducted in multi-centers in malarial hyperendemic areas at: Phu Thien district (Gialai province), Thuan Bac district (Ninh Thuan province), and Huong Hoa district (Quang Tri province) from the years 2011 to the end of 2012.

2.2. Subjects and materials

2.2.1. The uncomplicated *P. falcipparum* malaria patient's group

Inclusion criteria

- Age between 6 months to under 70 years old;
- Mono-infection with *P. falciparum* detected by light microscopy;
- Parasitaemia of 1.000 - 100.000 asexual forms/µl blood;
- Presence of axillary temperature ≥ 37.5°C or history of fever (past 24h);
- Ability to swallow oral medication;
- Ability and willingness to comply with the study protocol for the duration of the study and to comply with the study visit schedule;
- Informed consent from the patient or parents in the case of children;
- Not yet take any antimalarial drugs.

Exclusion criteria

- Presence of general danger signs in children aged under 5 years or signs of severe falciparum malaria;
- Mixed or mono-infection with another *Plasmodium* species;
- Presence of severe malnutrition, febrile conditions due to diseases other than malaria (acute lower respiratory tract infection, severe diarrhoea) or other known underlying chronic or severe diseases, severely vomitting, or psychological disorders;
- History of hypersensitivity reactions or contraindications to any of the medicine(s) being tested;
- A positive pregnancy test or breastfeeding women;

2.2.2. The *P. vivax* malaria patient's group

Inclusion criteria

- Age over 6 months to < 70 year old;
- Mono-infection with *P. vivax* detected by light microscopy;
- Parasitaemia of asexual forms ≥ 250/µl blood;
- Presence of axillary temperature ≥ 37.5°C or history of fever (past 48h);
- Ability to swallow oral medication;
- Ability and willingness to comply with the study protocol for the duration of the study and to comply with the study visit schedule;
- Informed consent from the patient or parents in the case of children;
- Not yet take any antimalarial drugs.

Exclusion criteria

- Under 6 months or ≥ 70 year olds;
- A positive pregnancy test or breastfeeding women;
- Presence of general danger signs in children aged under 5 years or signs of severe vivax malaria;
- Presence of severe malnutrition, febrile conditions due to diseases other than malaria (acute lower respiratory tract infection, severe diarrhoea) or other known underlying chronic or severe diseases, severely vomitting, or psychological disorders;
- Mixed or mono-infection with another *Plasmodium* species.

2.2.3. Antimalarial drugs to be tested in clinical trials

- Arterakine tablet (40mg dihydroartemisinin plus 320mg of piperaquin phosphate). Dosage regimen as followed:

Dosage by		**Dosage in 3 days regimen**			
Age group	**Body weight**	***0h***	***8thh***	***24thh***	***48thh***
< 3 years	< 15 kg	½	½	½	½
3 - < 8 years	15 - < 24kg	1	1	1	1
8 - < 15 years	25 - < 34kg	1 ½	1 ½	1 ½	1 ½
≥ 15 years	≥ 35kg	2	2	2	2

- CQ 250mg tablet (150 mg base) with 3-day regimen as followed by day 1 (10mg base/kg bw), day 2 (10mg base/kg), and day 3 (5mg base/kg).

2.2.4. In code in patient's clinical trials

- QTAK as Quang Tri arterakin, QTCQ as Quang Tri chloroquin ;
- NTAK as Ninh Thuan arterakin, NTCQ as Ninh Thuan chloroquin;
- GLAK as Gia Lai arterakin, GLCQ as Gia Lai chloroquin.

2.3. Study methods

2.3.1. Study design: Non-randomised controlled study design.

2.3.2. Sample size

Classical statistical methods are recommended for determining sample size, on the basis of an expected proportion of treatment failures, desired confidence interval (95%) and precision (10%).

In the DHA-PPQ regimen versus P. falciparum

In the case of a medicine with an expected failure rate of 20%, a confidence interval of 95% and a precision level of 10%, a minimum of 61 patients should be enrolled.

In the CQ regimen versus P. vivax

In the case of a medicine with an expected failure rate of 10%, a confidence interval of 95% and a precision level of 10%, a minimum of 35 patients should be enrolled

Estimated population proportion (p), confidece interval 95%										
d	0,05	0,10	0,15	0,20	0,25	0,30	0,35	0,40	0,45	0,50
0,05	73	138	196	246	288	323	350	369	380	384
0,10	18	**35**	49	**61**	72	81	87	92	95	96

2.4. Study techniques

- Clinical evaluation and Hackett classification of spleenomegaly;
- Body temperature, body weight taking, nutrition condition evaluation;
- Urine analysis for cheking antimalarial components;
- Microscopic slide checking and parasite counting;
- Molecular markers analysis and genotyping of malaria parasites
- Measure of chloroquine and desethylchloroquine.

2.5. Clinical and laboratory assessment procedures

Studies of directly observed treatment for uncomplicated malaria are prospective evaluations of clinical and parasitological responses on days 0, 1, 2, 3, 7, 14, 21 and 28 (with CQ) and 35, 42 (with DHA-PPQ). The day the patient is enrolled and receives the first dose of medicine is day 0.

Timing for follow up	**D_0**	**D_1**	**D_2**	**D_{3-6}**	**D_7**	**D_{14}**	**D_{21}**	**D_{28}**	**D_{35}**	**D_{42}**	**Other day**
Standard procedures											
1. Clinical evaluation	x	x	x	x	x	x	x	x	x	x	(x)
2. Body temperature	x	x	x	x	x	x	x	x	x	x	(x)
3. Slide checking	x	x	x	x	x	x	x	x	x	x	(x)
4. Urine test	x										
5. Blood analysis: - Haemoglobin - Haematocrite - PCR	 x x x				 x	 x	 x	 x	 x	 x	 (x)
6. Parasite genotyping	x			x	x	x	x	x	x	x	(x)
7. Drug analysis	x				x			x			Post D_7
Patients treatment											
1. DHA-PPQ	x	x	x								
2. Chloroquine	x	x	x								
2. Rescue drugs		(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)

2.6. Loss to follow up

- Loss to follow up occurs when despite all reasonable efforts, an enrolled patient does not attend the scheduled visits;
- Patients who are lost to follow up but who subsequently return to the study site before day 28/42 will not be turned away and will be encouraged to return for check up visits.

2.7. Patient discontinuation or protocol violation

- Study patients who meet any of the following criteria will be classified as withdrawn:
  - Withdrawal of consent of a patient at any time;
  - Failure to complete treatment, due to persistent vomiting of the treatment, or failure to attend the scheduled visits during the first 3 days or serious adverse events necessitating termination of treatment before the full course is completed.
- Enrolment violation: severe malaria on D_0 or voluntary protocol violation or involuntary protocol violation occurrence during follow-up of concomitant disease, or detection of mono-infection with another malaria species during follow-up.

2.8. Classification of responses to treatment outcomes

Early Treatment Failure (ETF)
- Danger signs or severe malaria on day 1, 2 or 3, in the presence of parasitaemia; - Parasitaemia on day 2 higher than on day 0, irrespective of axillary temperature; - Parasitaemia on day 3 with axillary temperature ≥ 37.5°C; and - Parasitaemia on day 3 ≥ 25% of count on day 0.
Late Clinical Failure (LCF)
- Danger signs or severe malaria in the presence of parasitaemia on any day between day 4 and day 28 (day 42) in patients who did not previously meet any of the criteria of ETF; and - Presence of parasitaemia on any day between day 4 and day 28 (day 42) with axillary temperature ≥ 37.5°C in patients who did not previously meet any of the criteria of ETF.
Late Parasitological Failure (LPF)
- Presence of parasitaemia on any day between day 7 and day 28 (day 42) with axillary temperature < 37.5 °C in patients who did not previously meet any of the criteria of ETF or LCF.
Adequate Clinical and Parasitological Response (ACPR)
- Absence of parasitaemia on day 28 (day 42), irrespective of axillary temperature, in patients who did not previously meet any of the criteria of ETF, LCF, LPF.

2.9. Adverse events and safety profiles

- An adverse event (AE): any unfavorable and unintended sign including an abnormal laboratory finding, symptom or disease associated with the use of a medical treatment, that occurs during the course of the study;
- Serious AE as any untoward medical occurrence that results in death, is life threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability.

2.10. Data analysis and study end-points

- Data in patient were entered on WHO-Ringwald Pascal software version 7.1. Results should be expressed as the proportion of ACPR (or proportion of ETF, LPF or LCF) before and after adjustment by PCR;
- Parasite clearance time (PCT), fever clearance time (FCT), and the proportion of patients who are parasitemic on day 3.

2.11. Ethical issues in clinical trials

- Approval by the official ethical and scientific committee. Study team members must be passed GCPs and do as SOPs in protocol;
- Informed consent when patients will be included in the study;
- Confidentiality: all information on patients will remain confidential;
- Health-care services: free health care throughout follow-up for any illness related to malaria will be provided to the study patients regardless of treatment outcome.

Chapter 3. RESULTS

3.1. DHA-PPQ efficacy in the treatment of falciparum malaria

Table 3.1. Baseline clinical characteristics of patients at D0

Study patient's profile (N = 206)	At the point start of study D_0		
	Quang Tri (n = 76)	Gia Lai (n = 65)	Ninh Thuan (n = 65)
Temperature & body weight			
Mean temperature in ^{0}C	38,22 ± 1,04	37,78 ± 1,2	38,16 ± 1,0
Mean weight in kg	41,5 ± 12,8	39,5 ± 15,1	40,5 ± 14,2
Fever day number before test	2,2 ± 1,6	2,6 ± 1,1	2,2 ± 1,2
Fever or history of fever			
Body temperature ≥ 37.5^{0}C	62 *(81,58)*	58 *(89,23)*	45 *(69,23)*
History of fever(past 48 hours)	8 *(10,53)*	7 *(10,77)*	12 *(18,46)*
Non fever & non-history of fever	6 *(7,89)*	0	*8 (12,31)*
Spleen status			
Enlarged spleen	42 *(55,26)*	8 *(12,31)*	46 *(70,77)*
Non-spleenomegaly	34 *(44,74)*	55 *(84,62)*	19 *(29,23)*
History of splenectomy	0	2 *(3,07)*	0

The mean temperature at D_0 were 38.22 ± 1.04^{0}C, 37.78 ± 1.2^{0}C, and 38.16 ± 1.0^{0}C in sentinel site of Quang tri, Gia Lai, and Ninh Thuan, respectively. Number fever days of patients at D_0 was around 1-4 days. Number of cases of enlarged spleen were 55.26%, 12.31% and 70.77% respectively in Quang Tri, Gia Lai and Ninh Thuan. These suitable for most of patients were living in hyperendemic malaria areas, hence they often get malaria and many "reinfection attack" lead to spleen parenchyma hypertrophy and fibrosis. Especially, in this trial, 2 cases with abnormal spleen status (one case of congenital hyposplenism syndrome and one of totally splenectomy due to accident.

Table 3.2. Laboratory findings and malaria parasite profile in patients

Patients' parameters	Quang Tri	Gia Lai	Ninh Thuan
Median parasite density			
- Asexual parasite/ μl	28.125 (12.121 - 49.862)	33.197 (16.520 - 76.268)	30.192 (18.415 - 52.202)
- Positive gametocyte cases	9 *(11,84%)*	11 *(16,92%)*	*4 (6,15%)*
Haematology parameters			
- Mean haemoglobine	9,9 (g/dL) (3,9 – 17,4)	11,8 (g/dL) (9,2 – 14,6)	11,3 (g/dL) (6,1 – 14,8)
- Mean haematocrite	40,12% (37,25 – 41,71)	39,58% (41,42 – 44,02)	41,17% (36,80 – 45,82)

The median malaria parasite density of asexual *P. falciparum* at three sentinel sites around 28.125-33.197/μl. Number of cases with positive gametocyte were low (6.15-16.92%) in Quang Tri, Gia Lai, and Ninh Thuan, respectively.

Bảng 3.3. Efficacy of DHA-PPQ *vs.* uncomplicated falciparum malaria

Treatment outcomes	Quang Tri		Gia Lai		Ninh Thuan	
	n	%	n	%	n	%
ETF	0	*0*	2	*3,71*	0	*0*
LCF	0	*0*	3	*4,62*	0	*0*
LPF	0	*0*	0	*0*	0	*0*
ACPR	69	***100***	55	***91.67***	46	***100***
Total of analysis	69		60		46	
Withdraw	2	*2,63*	0	*0*	2	*3,08*
Loss of follow (post D_7)	5	*6,58*	5	*7,69*	17	*26,15*
Total of study	**76**		**65**		**65**	

Followed up to the day 42 *in vivo* guidelines from WHO, efficacy analysis at each sentinel site showed that in Quang Tri and Ninh Thuan with ACPR or cure rate of 100%, but in Gia Lai sentinel site with ACPR at D_{42} only 91.67%, accompanied with ETF of 3.71%, LCF of 4.62%. In 2 cases of ETF, the case of 05GLAK with high parasite density at D_0

(99.857/µl), therefore till prolong to the day D_5 this case was completely parasite clearance. And the case of 65GLAK with parasite density of 49.673/µl, but till positive asexual *P. falciparum* forms after 3 days regimen and high body temperature. Both of cases are normal spleen parenchyma as above mentioned. Regarding to role of the spleen, when patients are infected with malaria, intra-erythrocytic parasite development results in remodeling of both infected and non-infected red blood cells (RBCs). The spleen filters these altered RBCs. This suggested that this splenic-pitting is responsible for a majority of the parasite clearance that is seen in patients. In fact, asplenic individuals living in falciparum endemic regions, exhibit more frequent fevers, higher parasitemias, and longer parasite clearance times following treatment with antimalarials such as artemisinin. Studies using *P. berghei*-infected mice, found that animals with full splenectomies had parasitemias double those found in intact and partially splenectomized mice. In other study in experiment animal, when assessed parasite clearance in DHA-treated intact and asplenic mice, it was found that the capacity to clear parasites was reduced in the asplenic population (Moore *et al.,* 2009).

Table 3.4. Analysis of early treatment failure cases by *in vivo* test

Study's code	Parasite density		D_{appear}	Classified by *in vivo*
	Do	D_{appear}		
05GLAK	99.857	141 (*P.f*)	D_3	ETF (+ prolong → D_4)
65GLAK	49.673	36 (*P.f*)	D_3	ETF ($\geq 38.0^0$C)
29GLAK	45.245	108 (*P.f*)	D_{42}	LCF
48GLAK	11.544	72 (*P.f*)	D_{42}	LCF
62GLAK	40.320	81.030 (*P.f*)	D_{26}	LCF

In two cases of ETF, the case of 05GLAK with extremely high parasite density at D_0 (99.857/µl), therefore till prolong to the day D_5, and the case of 65GLAK with parasite density of 49.673/µl, but till positive

asexual *P. falciparum* forms after 3 days. The cases of LCF at the D_{26} or D_{42} must be done in PCR analysis.

Table 3.5. Discrimination of reinfection or recrudescence by PCR

Study's code	Parasite density		Classified by *in vivo*	Classified by PCR adjusted
	Do	$D_{failure}$		
GLAK29	45.245	108 (*P.f*)	LCF (D_{42})	Reinfection
GLAK48	11.544	72 (*P.f*)	LCF (D_{42})	Reinfection
GLAK62	40.320	81.030 (*P.f*)	LCF (D_{26})	Recrudesence

The result of adjusted-PCR showed that 2 cases of LCF were reinfection or new infection of *P. falciparum* (29GLAK and 48GLAK) at the day D_{42}. Specially, the case of 62GLAK was recrudescence on the D_{26}. This patient have parasite cleared after 3 days treatment of DHA-PPQ, then reappeared of *P. falciparum* alone at D_{26}. With the results of LCF in Gia Lai sentinel, after corrected-PCR, efficacy was changed increasing the ACPR rate from 91.67% to 95%, the LCF rate reducing from 4.62% to 1.29%, two cases of reinfection or new infection, and the rest 2 cases of ETF were 3.71%.

Table 3.6. PCR-adjusted and unadjusted treatment outcomes

Treatment outcomes	Before PCR-adjusted		After PCR-adjusted	
	n	%	n	%
ETF	2	*3,71*	2	*3,71*
LCF	3	*4,62*	1	*1,29*
LPF	0	*0*	0	*0*
ACPR	55	***91,67***	**57**	***95,0***
Total of analysis	60		60	
Withdraw	0	*0*	*0*	
Loss to follow up	5	*7,69*	5	*7,69*
Total of study	**65**		**65**	

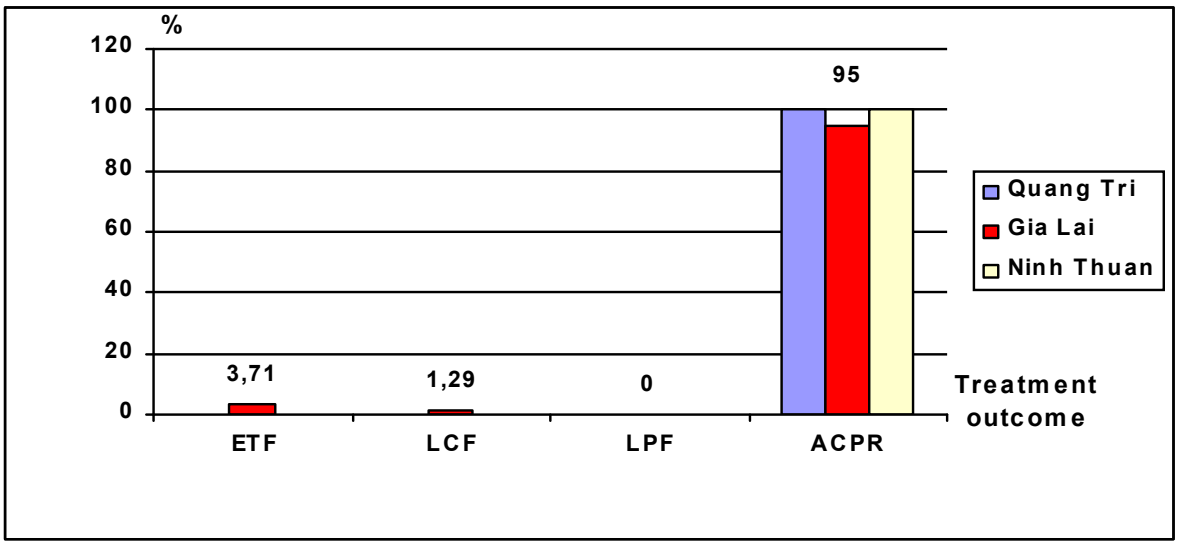

Figure 3.1. Efficacy of DHA-PPQ *versus P. falciparum* at 3 sentinel sites

With the results PCR corrected in discrimination of recrudescence and reinfection, DHA-PPQ efficacy was changed increasing the ACPR rate from 91.67% to 95%, the LCF rate reducing from 4.62% to 1.29%.

Table 3.7. Efficacy of DHA-PPQ in parasite and fever clearance

Analysis parameters	Quang Tri	Gia Lai	Ninh Thuan
Parasite density/µl at D_0	28.125 (12.121-49.862)	32.197 (16.520-76.268)	30.192 (18.415-52.202)
Parasite clearance time (PCT)	37.8h (35.6-39.8)	61.5h (36.2-79.6)	37.3h (34.6-38.6)
Body temperature at D_0	37.3 (36.0-40.1)	38,1 (36.2-40.5)	37.3 (35.6-39.4)
Fever clearance time (FCT)	25.7h (24.7-26.7)	30.7h (29.0-32.4)	27.3h (25.4-29.2)

The differences in the baseline median parasite density between the treatment groups are not significant at the day D_0. However, compared to Quang Tri and Ninh Thuan, Gia Lai sentinel showed that parasite clearance time (61.5h) much longer than Quang Tri (37.8h) and 37.3h (Ninh Thuan). In paralell with rapid PCT, the clinical symptomes would be improved better at Quang Tri and Ninh Thuan, but in Gia Lai seemed to

be longer in fever clearance time as some patients who are positive asexual parasite at 72 hours or more.

Table 3.8. Proportion of positive asexual *P. falciparum* at ≥ D_3

Results in sentinel sites	**Proportion of parasitaemic on ≥ D_3**		
	Quang Tri	**Gia Lai**	**Ninh Thuan**
Cases with positive at D_3	0	10 *(15.38)*	0
Cases with positive at post-D_3	0	1 *(1.62)*	0
Total	0	11 *(17.0)*	0

Analysis of all cases of asexual parasite existence, the positivity rate of 17% at day 3 (72 hours) after treatment with DHA-PPQ in Gia Lai as an important indirect clinical indicator of treatment failure or resistance.

Table 3.9. Progression and asexual parasite clearance from D_0 to ≥ D_3

Pt's code		**Age**	**D_0**	**D_1**	**D_2**	**D_3**	**D_4**	**D_5**
1	GLAK05	29	99.857	36.666	4.890	282	80	0
2	GLAK14	32	48.875	2.130	165	48	0	0
3	GLAK 15	30	75.559	16.395	360	6	0	0
4	GLAK23	28	46.941	29.480	102	537	0	0
5	GLAK27	15	56.303	6.225	66	12	0	0
6	GLAK44	33	43.385	17.139	1.875	66	0	0
7	GLAK46	13	29.919	17.213	1.695	162	0	0
8	GLAK60	14	16.493	1.155	54	24	0	0
9	GLAK61	31	31.014	11.333	756	267	0	0
10	GLAK62	4	40.320	6.120	201	150	Recrudescence	
11	GLAK65	5	49.673	12.300	483	36	0	0
Mean parasite density D_0/µL			**44.121**	**9.633**	**358**	**66**	**8**	**0**

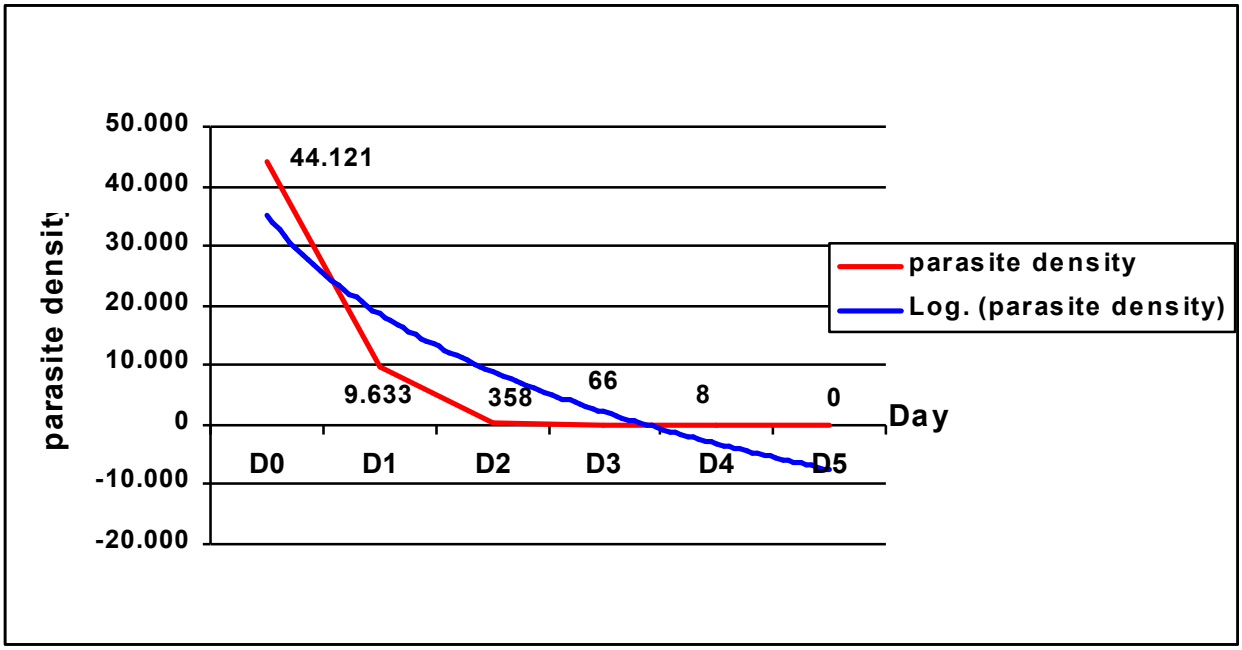

Figure 3.2. Progression of parasite clearance day by day (24h)

In total of 65 cases in Gia Lai sentinel site, there was 17% rate of positive parasite at day D_3 or D_4. This mean parasite clearance data by step every 24h from D_0 to D_5 in detail of 44.121/µL → 9.633/µL → 358/µL → 66/µL → 8/µL → 0/µL, respectively. This is currently the best available indicator here, need to be more analysis of pharmacokinetics.

Analysis of all cases of asexual parasite existence, the positivity rate of 17% at day 3 (72 hours) after treatment by DHA-PPQ regimen in Gia Lai as an important indirect clinical indicator of treatment failure or resistance, and the parasitemic on day 3 is currently the best available indicator used in routine monitoring to measure *P. falciparum* sensitivity to artemisinins.

With an increase in parasite clearance time (61.5h) and evidence 17% of cases with parasites detectable on day 3 after treatment with DHA-PPQ. To make sure exact resistance, need to be done more pharmacokinetic and molecular marker analysis in the next time.

Table 3.10. Proportions of adverse events in DHA-PPQ treated group

DHA-PPQ	Quang Tri	Gia Lai	Ninh Thuan	Time at occurrence
On drug tolerability	**n *(%)***	**n *(%)***	**n *(%)***	
Diarrhea	0	0	0	
Vomit after oral taken*	2 *(2.63)*	0	1 *(1.54)*	D_0 - D_1
*Temperature at vomitting				
≥ 37,5 - < 39^{0}C	0	0	0	
≥ 39.0^{0}C	2 *(2.63)*	0	1 *(1.54)*	
Adverse events	**n *(%)***	**n *(%)***	**n *(%)***	
Headache	6 *(7.89)*	3 *(4.62)*	4 *(6.16)*	D_0 - D_3
Dizziness	2 *(2.63)*	2 *(3.08)*	3 *(4.62)*	D_0 - D_2
Nausea	3 *(3.95)*	2 *(3.08)*	2 *(3.08)*	D_0 - D_1
Loss of appetite	4 *(5.26)*	2 *(3.08)*	3 *(4.62)*	D_0 - D_3
Mild abdomen pain	1 *(1.32)*	0	1 *(1.54)*	D_0 - D_2
Mouth dry	0	1 *(1.54)*	0	D_0 - D_3
Icth, urticaria	1 *(1.32)*	2 *(3.08)*	0	D_0 - D_3
Sleep troubles	1 *(1.32)*	2 *(3.08)*	0	D_0 - D_3

Using consecutive 3 day of DHA-PPQ therapy, some adverse events were observed. Several other mild side-effects including headache, dizziness, nausea, itching and rash. It is very difficult to differentiate these adverse events with malaria disease symptoms.

3.2. Chloroquine efficacy in the treatment of vivax malaria patients

Table 3.11. Baseline clinical characteristics of patients before trials

Patients' profile	Quang Tri (n = 56)	Gia Lai (n = 62)	Ninh Thuan (n = 47)
Body temperature & weight			
Mean body temperature (^{0}C)	38.51 ± 1.15	38.29 ± 1.20	38.29 ± 1.20
Mean weight in kg	42.8 ± 14.6	36.5 ± 18.2	39.5 ± 15.2
Fever day number before test	2.1 ± 1.1	3.1 ± 1.1	3.2 ± 1.4
Fever or history of fever			
Fever ≥ 37,5^{0}C	47 *(83.93)*	39 *(62.90)*	38 *(80.6)*
History of fever (past 48h)	9 *(16.07)*	23 *(37.10)*	9 *(8.96)*
Enlarged spleen status	19 *(33.93)*	16 *(25.81)*	27 *(40.30)*
I + II grade	17 *(30.36)*	12 *(19.35)*	24 *(35.82)*
III grade	2 *(3.57)*	4 *(6.46)*	3 *(4.48)*

Some baseline clinical and paraclinical characteristics of patient profile showed that most of patients were fever or history of fever during past 48hs before study enrollment, and mean body temperature at 3 sentinel sites around 38.51 ± 1.15^{0}C; 38.29 ± 1.2^{0}C and 38.29 ± 1.20^{0}C in Quang Tri, Gia Lai and Ninh Thuan, respectively. Because of living in hyperendemic malaria areas, the patients had enlarged spleen of 33.93%; 25.81% and 40.30% in Quang Tri, Gia Lai, and Ninh Thuan, respectively. Majority in spleenomegaly cases, grade I and II.

Table 3.12. The haematology and malaria parasite profile in patients

Patient's profile	At the point start of study D_0		
	Quang Tri	**Gia Lai**	**Ninh Thuan**
Median parasite density			
- Asexual parasite/µl	3.185 (748-52.154)	3.256 (200-33.121)	2.810 (250-37.750)
- Positive gametocyte cases	42 *(75.0%)*	50 *(80.65%)*	42 *(89.36%)*
- Gametocyte density/µl	102.7 (66.3-139)	101.5 (67.3-128)	108.4 (62.2-130)
Haematology parameter			
- Haemoglobine (g/dL)	9.4 (4.2-15.2)	11.5 (9.4-14.2)	10.3 (5,1-14.4)
- Haematocrite (%)	39.34 (36.21-42.72)	39.51 (40.41-42.12)	41.2 (37.9-44.85)

Median asexual form of *P. vivax* parasite were 3.185; 3.256 and 2.810/µl in Quang Tri, Gia Lai, and Ninh Thuan sentinel sites, respectively. Especially, number of vivax malaria cases had positive gametocyte of 75%, 80.65% and 89.36%. .

Table 3.13. The efficacy of CQ regimen vs. *P. vivax* malaria

Treatment outcomes	Quang Tri		Gia Lai		Ninh Thuan	
	n	%	n	%	n	%
ETF, LCF, LPF	0	0	0	0	0	0
ACPR	**54**	*100*	**52**	*100*	**44**	*100*
Total of analysis	54		52		44	
Withdraw	0	*0*	2	*3.23*	0	0
Loss to follow up	2	*3.57*	8	*12.90*	3	6.38
Total of study	**56**		**62**		**47**	

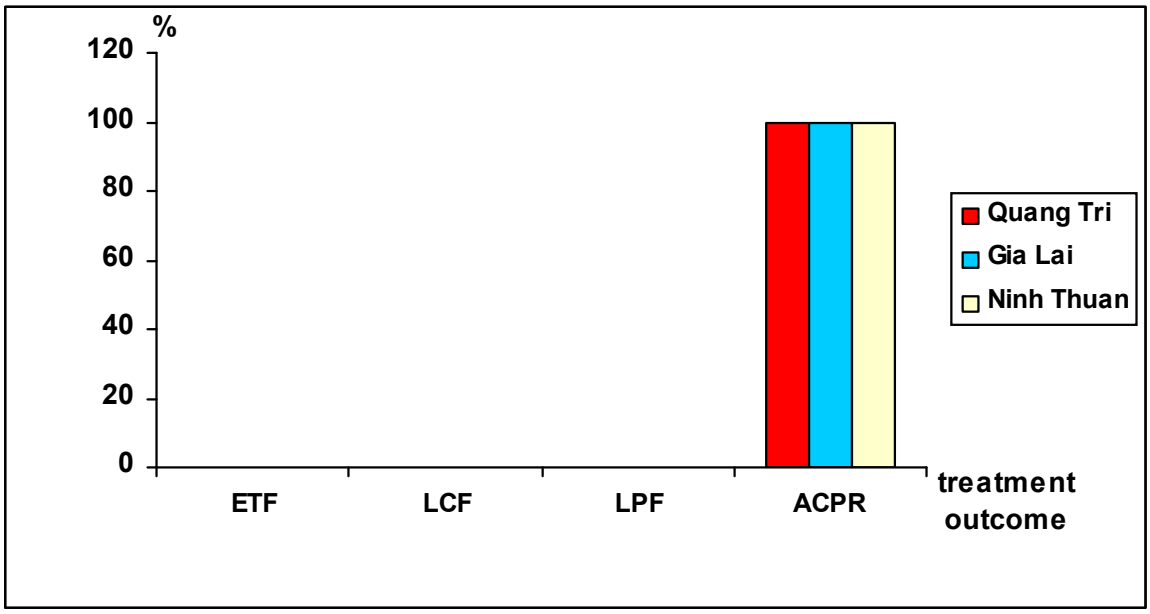

Figure 3.3. Efficacy of CQ regimen vs. *P. vivax* malaria

The data showed that the ACPR were 100% in three sentinel sites, none of cases had ETF, LCF, and LPF.

Table 3.14. Efficacy in parasite clearance and fever clearance of CQ

Analysis parameters	Quang Tri	Gia Lai	Ninh Thuan
Median parasite density/µl at D_0	3.185 (748-52.154)	3.256 (210-33.121)	2.810 (250-37.750)
Mean parasite clearance time (PCT)	39.26±7.68	31.22 ± 6.28	41.2 ± 4.69
Mean temperature at D_0	38.5 ± 1.5	37.5 ± 1.7	38.5 ± 1.0
Mean fever clearance time (FCT)	28.14 ± 10.16	25.16 ± 12.17	29.20 ± 13.2

Concerning parasite and fever clearance time, after 3 days of CQ therapy, PCT were 39.26 ± 7.68h; 31.22 ± 6.28h and 41.20 ± 4.69 h in sentinel of Quang Tri, Gia Lai and Ninh Thuận, respectively. Although mean body temperature of patients were 38.5 ± 1.5^{0}C, 37.5 ± 1.7^{0}C and 38.5 ± 1.0^{0}C, the FCT were about 48 hours. These does mean stable CQ-sensitive *P. vivax.*

However, with the literature of drug resistance all over the world, especially in the neighboring countries of the Mekong subregion, and with the fact that CQ has been used for over 60 years with various purposes, the warning signs of reduced susceptibility or resistance may occur in Vietnam and Central-Western highlands in particular in the near future.

Routine supervisions of the drug efficacy in some areas, especially in endemic areas with higher proportion of *P. vivax* of the parasite formula, is really necessary.

Table 3.15. Proportions of adverse events in CQ treated group

Symptoms	Quang Tri	Gia Lai	Ninh Thuan	Time
Headache, dizziness	0	2 *(3.22)*	1 *(2.13)*	D_1- D_3
Nausea	1 *(1.79)*	2 *(3.22)*	2 *(4.26)*	D_0 - D_2
Abdomen pain	2 *(3.58)*	0	0	D_1 - D_2
Itch, urticaria, rash	1 *(1.79)*	1 *(1.61)*	1 *(2.13)*	D_0 - D_2
Vision blurred	0	2 *(3.22)*	0	D_1 – D_3

Due to difficulties in recording the symptoms reported or exclusively relating to *P. vivax* infection, these studies showed that some mild and low proportion adverse events by every 12 hours interval examination in first 3 consecutive days, such as nausea, abdomen pain, itching, headache, dizziness, rash, or vision blurred.

CONCLUSIONS AND RECOMMENDATIONS

1. Conclusions

1.1. The efficacy of DHA-PPQ regimen to *P. falciparum* malaria

- Adequate clinical and parasitological responses (ACPR) in 3 sentinel sites were from 95-100%, including 100% of absolute cure rate in Quang Tri, Ninh Thuan; except for Gia Lai sentinel with the ACPR after corrected-PCR of 95%, LCF of 1.29% and ETF of 3.71%;
- PCT and progression was very quick, within 48 hours only; except for some cases in Gia Lai was positive asexual parasite in day 3 or ≥ 72 hours. FCT in parallel with PCT was just within 48 hours in Quang Tri & Ninh Thuan, but longer in Gia Lai.

1.2. The drug efficacy of chloroquine in treatment of *P. vivax malaria*

- The efficacy CQ was still maintains at absolutely high level to *P. vivax* malaria patients, and the ACPR were 100% in 3 sentinel sites;
- The chloroquin's PCT and FCT as well were quick, within 48 hours since CQ was given;
- Generally, good tolerance of DHA-PPQ and CQ was found in the treatment of uncomplicated *P. falciparum* and *P. vivax* malaria patients. Some side-effects occurred at mild level, with low proportion, no serious reactions that require no interventions with first aid or drug withdrawals, especially these symptoms have improved remarkably after drug withdrawals.

2. Recommendations

At present, the combined DHA-PPQ regimen is highly efficacious; however, the weakness include LCF (1.29%) and ETF (3.71%) in 2 patients with abnormal spleens, and high rate of parasite existence in D_3 day (17%) in DHA-PPQ regimen as an indicator of drug resistance. Therefore, it is recommended that:

- The role of the spleen is very obvious in parasite clearing intervention, so that it is necessary to supplement this criterion into "exclusive

criteria" of the proposals to assess the *P. falciparum* and *P. vivax* drug efficacy *in vivo* when conducting the research in the field or evaluating the effectiveness at the hospital treatment system;

- All the cases with parasite existence until D_3 after completing DHA-PPQ therapy, there should be more analyses of pharmacokinetics aspect, molecular markers to confirm the susceptibility/ resistance of *P. falciparum* in the treatment with DHA-PPQ more thoroughly;
- The CQ regimen as recommended by the MoH still remains effective; therefore, CQ is considered the first of choice for *P. vivax* malaria treatment, but need to monitoring of their efficacy routinely.