clearance time in Binh Phuoc was 1.84 ± 0.65 days longer than in Ninh Thuan was 1.27 ± 0.45 days .

- The effective treatment of chloroquine by *in vivo* before distinguishing relapse and re-infection by PCR at two study sites with ACPR rate was 98.9%; while in Ninh Thuan ACPR rate was 97.8% and LCF/LPF rate was 2.2%. In Binh Phuoc ACPR rate was 100.0%.

2. The sensitivity of *Plasmodium falciparum* malaria with dihydroartemisinine, piperaquine and chloroquine by *in vitro* techniques in Binh Phuoc 2010

- Dihydroartemisinin at 32.0 nmol/L inhibit to form schizonts reached 95.24%. EC_{50}, EC_{90} and EC_{99} respectively 3.1 nmol/L, 21.8 nmol/L and 105.6 nmol /L.
- Piperaquin EC_{50}, EC_{90} and EC_{99}, respectively 49.2 nmol/L, 222 nmol/L and 758.3 nmol/L.
- Chloroquin at 640 nmol/L inhibit to form schizonts reached 100%. EC_{50}, EC_{90} and EC_{99} respectively 74.9 nmol/L, 312.3 nmol/L and 1000.2 nmol/L.

RECOMMENDATIONS

1. Monitoring frequently efficacy of dihydroartemisinine, piperaquine and chloroquine to have early detection of drug sensitivity and giving policies to provide proper antimalarial drugs.

2. Research and complete assessment process relapse and new infection with malaria caused by *P. vivax* by using molecular biology techniques (PCR).

INTRODUCTION

There are now facing to *P. falciparum* resistance and reduce efficacy of some antimalarial drugs such as artemisinin group in Southeast Asia [46]. In Vietnam, the first case was early failure treatment with Arterakine for *P. falciparum* in Dak Nhau, Bu Dang, Binh Phuoc in 2009[45] and then to Phu Thien, Gia Lai in 2010 [46].

Meanwhile, malaria caused by *P. vivax* in Vietnam has tended to increase in recent years, due to difficulty and long-term therapy for *P. vivax*. Recently, the National malaria control program, chloroquine is still effective antimalarials fast and highly effective treatment for *P. vivax*. However, it was announced on the phenomenon that *P. vivax* is resistance to chloroquine in some countries in Southeast Asia and around the world [59], [115]. In Vietnam, up to now there is not any reports of *P. vivax* resistance with chloroquine.

Base on above information, the study "**The efficacy of dihydroartemisinine - piperaquine and chloroquine on malaria treatment in some malaria areas of Vietnam (2010-2012)**" was carried out with the aimed:

1. To Determine the efficacy of dihydroartemisinine-piperaquine in the treatment of malaria caused by uncomplicated Plasmodium falciparum and chloroquine in the treatment of malaria caused by Plasmodium vivax at some sites of Ninh Thuan and Binh Phuoc.

2. To Assess the sensitivity of Plasmodium falciparum to dihydroartemisinine, piperaquine and chloroquine in Binh Phuoc by vitro technique

SUMMARY THE NEW COMMENTS OF THE THESIS

Scientific: The study results *in vivo* showed that combination dihydroartemisinine-piperaquine and chloroquine are used in National Malarial Control Program in some malaria areas of Vietnam is still effective.

Reality: The results of the study is the scientific basis to give some advises for the National Malarial Control Programs about antimalarial drugs policy simultaneously, provide materials on teaching graduate and postgraduate.

New contributions of the thesis:

- It is the first time in Vietnam, we have determined sensitivity decrease on *in vitro* studies of antimalarial drugs (dihydroartemisinine, piperaquine) for *P. falciparum* in Binh Phuoc.
- The study results *in vivo* showed that therapeutic efficacy of the combination dihydroartemisinine - piperaquine started showing signs of reduced susceptibility to malaria *P. falciparum* with the rate parasite positive day 3 > 10% in Binh Phuoc.

The structure of the dissertation: Apart from the introduction has 2 pages, conclusion has 2 pages and recommendation has 1 page. The dissertation has four chapters with 134 pages, in which 43 tables and 34 figures.

Chapter 1: Background: 35 pages

Chapter 2: Objectives and methods: 26 pages

Chapter 3: Study results: 39 pages

Chapter 4: Discussion: 29 pages

The articles related to the dissertation: 1page

References 142 references, in which 58 Vietnamese documents, 84 documents in English. Appendix

the assessing method is micro test technique but in our research EC_{50}, EC_{90} and EC_{99} has increased almost three times compared with the study in 2001, however, compared the study in 2008 by these authors with our researching results of EC_{50}, EC_{90} and EC_{99} increased only 1,5 times. it shows that the drug-sensitive *P. falciparum* has increased rapidly.

CONCLUSION

1. The effective treatment of dihydroastemisinine- piperaquine in treatment malarial caused by uncomplicated *Plasmodium falciparum* and chloroquine in treatment malarial caused by *Plasmodium vivax* in Ninh Thuan and Binh Phuoc

1.1. The effective treatment of dihydroastemisinine - piperaquine in treatment malarial caused by uncomplicated Plasmodium falciparum in Ninh Thuan and Binh Phuoc.

- Fever clearance time in Ninh Thuan was 1.14 ± 0.67 days, in Binh Phuoc was 1.26 ± 0.44 days. Parasite clearance time in Ninh Thuan was 1.76 ± 0.50 days, in Binh Phuoc was 2.05 ± 0.96 days. Parasite clearance time at Binh Phuoc in 2012 was longer than in 2010, while in Ninh Thuan no difference between 2011 and 2012. The rate of malarial parasites was positive on day 3 was 0.0% in Ninh Thuan, Binh Phuoc was 11.3 % , which was 3.1 % in 2010 and 2012 was 20.0%.
- The effective treatment of dihydroartemisinine - piperaquine by *in vivo* remains high with malaria caused by uncomplicated *P. falciparum* with ACPR rate was 100.0 % in 2 study provinces: Ninh Thuan and Binh Phuoc, 2010-2012.

1.2. The effective treatment of chloroquine in treatment malarial caused by Plasmodium vivax in Ninh Thuan and Binh Phuoc .

- Fever clearance time at Binh Phuoc was 1.23 ± 0.43 days longer than in Ninh Thuan was 1.04 ± 0.21 days. Parasite

Fever clearance time in Binh Phuoc was 1.23 ± 0.43 days longer than in Ninh Thuan was 1.04 ± 0.21 days; with $p < 0.05$. The results of our study is similar to the results of the others such as: Hong Huynh Quang Trieu Nguyen Trung researched in Ninh Thuan 2009 fever clearance time is less than 2 days.

4.1.2.3. Parasite clearance time

Parasite clearance time in Binh Phuoc was 1.84 ± 0.65 days longer than in Ninh Thuan was 1.27 ± 0.45 days ($p < 0.05$). Results of our study is higher than the results of the study of the others such as: Do Tuan Anh, Do Van Nghia Ha Giang (2007-2008) and the equivalent results of Huynh Hong Quang, Trieu Nguyen Trung research in Ninh Thuan province in 2009 and 3 in Central Highlands (Dak Lak, Ninh Thuan and Quang Tri) in 2012

4.1.2.4. The effective treatment of chloroquine

The effective treatment of chloroquine by *in vivo* before distinguishing relapse or re-infection by PCR at two study sites with ACPR rate was 98.9 %. In which, in Ninh Thuan, ACPR rate was 97.8 % and LCF/LPF rate was 2.2% (1 case the parasites appeared on D28), while in Binh Phuoc, ACPR rate was 100.0%. This result is lower than the result of Hong Huynh Quang, Trieu Nguyen Trung in Ma Noi, Ninh Son, Ninh Thuan in 2009, the results showed ACPR rate was 85%, the treatment failure rate was 15 %.

4.2. Assessing the sensitivity of *P. falciparum* malaria with dihydroartemisinine, piperaquine and chloroquine by *in vitro* techniques in Binh Phuoc 2010.

Comparing our study results with studies of Ngo Viet Thanh, Tran Quoc Toan et al was also studied in Binh Phuoc 2001, 2008, we found that after two years with the same assessment method is *micro test* technique but EC_{50}, EC_{90} and EC_{99} of dihydroartemisinine has increased nearly doubled and piperaquine rose to nearly triple the 2008 study. After 10 years with the same

Chapter 1. BACKGROUND

1.1. Malaria Parasites

According to the World Health Organization (WHO) now there are 5 species of *Plasmodium spp*. human disease has been recognized of which the most serious is *P. falciparum*, the rest of which are *P. vivax , P. malariae , P. ovale* and *P. knowlesi* can cause milder disease and less often leads to death mortality (WHO, 2012)

1.2. Drug resistance of malarial parasite

1.2.1. Definition

Up to now, drug resistance has been the World Health Organization, 2010 officially recognized for 3 of the 5 types of parasite causing human disease. It is *P. falciparum, P. vivax, P. malariae*, in which the most significant is the *P. falciparum* which resistance a lot of antimalaria drugs and is the only species decreased sensitivity to ART and its derivatives.

9/2011, the WHO has given a definition of antimalaria drugs artemisinin as follows:

+ Suspected artemisinin resistance: the number of malaria cases treated with medication regimens coordination artemisinin malaria (ACTs) that the parasite rate remains positive day 3 > 10%.

+ Identify artemisinin resistance: treatment failure after taking artemisinin or its derivatives alone (monotherapy), where parasites still exist to D7 or D3 was positive at day and relapse within 28/42 days although adequate antimalaria drug levels in the blood.

1.2.2. Mechanisms of resistance of P. falciparum

1.2.2.1. Chloroquin drug resistance and drugs in the same group (Amodiaquin) due to genetic mutation.

1.2.2.2. Artemisinin and its derivatives resistance: actually unclear

1.2.3. *P. vivax* resistance.

1.2.3.1. *Resistance chloroquin*: is the gene mutated in Tyr976 of Pvmdr -1

1.2.4. The techniques for assessing drug resistant parasites

In vivo technique monitoring treatment efficacy is the most important method to determine the treatment regimen, development and assessment of the antimalarial drug national policy. *In vitro* technique evaluate sensitivity of malaria parasites to antimalarial drugs will provide early warning signs of drug resistant parasites and the need to strategic changes taking drugs on the site. Molecular biology techniques help to distinguish relapse or reinfection by the parasite genotypes identify.

1.2.5. The situation of malaria parasite resistance in Southeast Asia and the Greater Mekong Subregion (GMS)

According to WHO, Southeast Asia has 88% of the population live in areas where malaria edemic. Area Mekong Subregion appear and widely spread of parasite strains resistant to multiple drugs malaria. In addition to the drug resistant malaria classic regimen alone, the parasite was resistant to multiple drugs in combination regimens, even some types of ACTs combination also showed signs of reduced sensitivity.

1.2.5.1. Resistance monotherapy regimens

**** Resistance Chloroquin***

In Southeast Asia chloroquin resistant *P. falciparum* has a high rate, especially in the countries of the GMS

**** Artemisinine and its derivatives:***

Regional Thailand-Cambodian border where the first recorded evidence of *P. falciparum* resistance to ART and its derivatives

1.2.5.2. Reduced sensitivity to ACTs and derivatives

**** Artesunate - amodiaquin:*** At GMS treatment failure rates have been reported in Vietnam and Myanmar

**** Artesunate - mefloquine:*** Combining AS - MEF proved highly effective in most countries except from Cambodia and Thailand treatment has higher failure rates ≥ 10% 28 day follow-up

**** Dihydroartemisinine - piperaquine (Arterakine, CV artecan):*** Dihydroartemisinine - piperaquine drug is highly effective and is an

One of issues which we concerned in our study, it was the existence parasite on day 3. This is the difference with the study of the others. In our study, the percentage of parasite positive on day D3 was 0,0% in Ninh Thuan and 11.3 % in Binh Phuoc. Compared with studies of Ta Thi Tinh in 2009 at Dak Nhau - Binh Phuoc, our study (11.3%) is similar. According to WHO recommendations [134], the presence of parasites on the day 3 is regarded as an early warning sign of drug resistance (resistance doubts when patients were treated by ACT malarial parasite rate remains on $D_3 > 10\%$). This is becoming a concerning matter for the effective treatment of Arterakine in the future in Binh Phuoc in particular malaria and the key areas of the country in general.

4.1.1.3 The effective treatment of dihydroartemisinine - piperaquine(ACPR)

**** On the clinical with monitoring process during 28 days***

The study results showed that therapeutic efficacy of dihydroartemisinine-piperaquine by *vivo* before distinguishing relapse or re-infection by PCR in two provinces with ACPR rate was 99.2 %. In which, Ninh Thuan ACPR rate was 100.0%, and in Binh Phuoc ACPR rate was 98.4 %, LCF/LPF rate was 1.6% (1 case appeared on the D_{28} in 2010). This result is similar to the result study of Tran Tinh Hien in a study at the Tropical diseases Hospital in Ho Chi Minh City was 98.3 %.

**** Results of treatment response (ACPR) after analyzing by PCR***

The results after analyzing the case of malarial parasites appear again on day 28 was a new infection (reinfection) and showed effective treatment by dihydroartemisine- piperaquine at 2 provinces after analyzing by PCR with ACPR rate was 100.0%.

4.1.2. The effective treatment of Chloroquine in treatment malarial caused by Plasmodium vivax in Ninh Thuan and Binh Phuoc

4.1.2.2. Fever clearance time

Table 3.35. Chloroquine concentration effect on P. falciparum isolates in the study sites (n = 42)

Effective concentration (nM/l)	Average	Confidence level 95%	
		Low concentration	Low concentration
EC_{50}	**74,88**	56,97	98,43
EC_{90}	**312,29**	196,16	497,18
EC_{99}	**1000,20**	480,64	2081,42

Chapter 4 DISCUSSION

4.1. Assessing the effective treatment of dihydroastemisinine-piperaquine in treatment malarial caused by uncomplicated *Plasmodium falciparum* and chloroquine in treatment malarial caused by *Plasmodium vivax* in Ninh Thuan and Binh Phuoc

4.1.1. The effective treatment of dihydroastemisinine - piperaquine in treatment malarial caused by uncomplicated Plasmodium falciparum in Ninh Thuan and Binh Phuoc

4.1.1.1 Fever clearance time

Fever clearance time of patients with in Ninh Thuan was 1.14 ± 0.67 days in Binh Phuoc was 1.26 ± 0.44 days. Our research results are similar to research results of Cam Vu Hong in Ninh Thuan year in 2006-2008, Le Ngoc Anh et al. at Highland Corps in 2006-2007, Nguyen The Phong, Nguyen Xuan Thanh, Michael Edstein et al. Thuan Bac district, Ninh Thuan 2006-2007.

4.1.1.2 Parasite clearance time

Parasite clearance time of patients in Binh Phuoc was 2.05 ± 0.96 days longer than in Ninh Thuan was 1.76 ± 0.50 days. our study results are similar to the study results of Bui Quang Phuc, Ta Thi Tinh et al 2012 in Binh Thuan, Ninh Thuan, Dak Lak on parasite clearance time (1.9 days compared 1.7 days).

ACT WHO recommended use. In Vietnam the efficacy of dihydroartemisinine – piperaquine is still high. However, the decline began to appear sensitivity of *P. falciparum* with ART (WHO, 2010)

1.3. The studies used Dihydroartemisinine - Piperaquine and Chloroquine in the treatment of malaria

According to WHO, 2010: Arterakine is safe, highly effective. Around the world, study by Denis et al was conducted in Thailand on children with malaria caused by *P. falciparum*. Research by Nicola Gargano and colleagues in India in 2012 the results showed a very high cure rate > 95 % [99], [133]. The study of Hong Huynh Quang, Trieu Nguyen Trung, Nguyen Van Chuong et al studied the efficacy of chloroquin for treatment of *P. vivax* malaria in 3 provinces Central - Central Highlands in 2012, the results showed that ACPR was 100 %, no cases of treatment failure [41]. Therefore, proving that the treatment effect of chloroquine remain sustainable and maintain high levels of malaria caused by *P. vivax* in Vietnam.

Chapter 2. OBJECTIVES AND METHODS

2.1. Objectives, study sites, timing and duration of study

2.1.1.Objectives

Objects are all patients diagnosed with uncomplicated *P. falciparum* malaria or *P. vivax* malaria at the research sites.

2.1.1.1. Inclusion criteria:

** Treatment by Dihydroartemisinine – piperaquine*

- Age between 2 to 60 ages; mono - infection with *P.falciparum*
- Parasitaemia of 1000-200000 /µl asxual forms
- Presence of axillary temperature ≥ 37.5^0 C or a history of fever during the past 24 hours.
- Ability to swallow oral medication.
- No using antimalaria medication during 14 days.

- Ability and willingness to comply with the study protocol for the duration of study and comply with the study visit schedule. Informed consent from the patient or from a parent or guardian in the case of children.

** Treatment by Chloroquine*

- Age between 2 to 60 ages; mono - infection with with *P. vivax*
- Parasitaemia of 250/µl asxual forms
- Fever, axillary temperature ≥ 37.5^0C or a history of fever within 24 hours.
- Ability to swallow oral medication
- No using antimalaria medication during 14 days
- Ability and willingness to comply with the study protocol for the duration of study and comply with the study visit schedule. Informed consent from the patient or from a parent or guardian in the case of children.

** To assess the response of P. falciparum to chloroquine, piperaquine and dihydroartemisinine (in vitro)*

- Age between 5 to 60 ages; mono-infection with *P. falciparum*
- Parasitaemia of 2.000 - 80.000/µl asxual forms
- No using antimalaria medication during 14 days

2.1.1.2. Exclusion criteria

- Children aged under 2 years or adults aged over 60 years old.
- Presence of general danger signs or other signs of severe malaria
- Mixed or mono-infection with another *Plasmodium* species detected by microscopy
- Parasitaemia of < 1.000 or > 200.000/µl asxual forms for malaria caused by *P. falciparum* or < 250/µl asxual forms for *P. vivax*.
- Presence of severe malnutrition or the acute or chronic diseases
- Pregnant women, lactating woman
- Using antimalarial drugs or other antibiotics during 14 days.
- Unable to or unwilling to take part in study process.
- The patient vomited 2 more times after taking medicine.

There was one case appearing parasites on day 28 as following to the standard evaluation of clinical efficacy of WHO 2009 late clinical failure (LCF) accounting for 1.1%. ACPR for the whole of the case study was 98.9%. While in Ninh Thuan ACPR rate was 97.8%, LCF rate was 2.2%. In Binh Phuoc ACPR rate was 100.0%.

3.3. ASSESSING THE SENTITIVITY OF *Plasmodium falciparum* WITH DIHYROARTEMISININE, PIPERAQUINE AND CHLOROQUINE BY *IN VITRO* IN BINH PHUOC 2010

3.3.1. Assessing the sensitivity of Plasmodium falciparum with dihydroartemisinine

Table 3.31. Dihydroartemisinine concentration have effect on P. falciparum isolateting at the study sites (n = 42)

Effective concentration (nM/l)	Average	Confidence level 95%	
		Low concentration	High concentration
EC_{50}	3,15	2,28	4,34
EC_{90}	21,81	11,91	39,94
EC_{99}	105,65	39,60	281,86

3.3.2 Assessing the sensitivity of Plasmodium falciparum with piperaquine

Table 3.33. Piperaquine concentration effect on P. falciparum isolates in the study sites (n = 42)

Effective concentration (nM/l)	Average	Confidence level 95%	
		Low concentration	Low concentration
EC_{50}	**49,18**	36,85	65,64
EC_{90}	**221,98**	140,04	351,86
EC_{99}	**758,26**	372,67	1542,81

3.3.3. Assessing the sensitivity of Plasmodium falciparum to chloroquine

- Fever clearance time in Binh Phuoc was 0.43 ± 1.23 days longer in Ninh Thuan was 1.04 ± 0.21 days, with $p < 0.05$.

3.2.2.2. Parasite clearance time

Table 3.26. The percentage of patients remaining parasites after treatment with chloroquin and parasite clearance time (days)

Site / Day	Ning Thuan		Binh Phuoc	
	n	(%)	n	(%)
Cases	45	100,0	43	100,0
D_1	12	26,7	30	69,8
χ^2 , p	$\chi^2 = 16,37$; $p < 0,05$			
D_2	0	0,0	6	14,0
D_3	0	0,0	0	0,0
Parasite clearance time (day)	**1,27 ± 0,45**		**1,84 ± 0,65**	
t , p	t = 4,81; p < 0,05			

- Parasite clearance time caused by *P. vivax* in Binh Phuoc was 1.84 ± 0.65 days longer than Ninh Thuan was 1.27 ± 0.45 days, with $p < 0.05$.

3.2.2.3. Therapeutic efficacy of chloroquine on clinical

Table 3.29. Therapeutic efficacy of chloroquine on clinical

Classification of responses to treatment / Sites	ACPR		ETF		LCF/LPF	
	n	(%)	n	(%)	n	(%)
Ninh Thuan	44	97,8	0	0,0	1(D_{28})	2,2
Binh Phuoc	43	100,0	0	0,0	0	0,0
Total	87	98,9	0	0,0	1	1,1

- Patients were considered treatment failures early.

2.1.2. Study sites

2.1.2.1 In the field:

We conducted to monitor and follow-up efficacy of drugs during 28 days in accordance with WHO at two provinces: Ninh Thuan and Binh Phuoc where malaria is edemic and assessing the sensitivity of *Plasmodium falciparum* to chloroquine, piperaquine and dihydroartemisinine by *in vitro* technique in Binh Phuoc.

2.1.2.2 In the labo

Analysis of blood samples from patients with malaria parasites reappear from D_7 to D_{28} by PCR in the Department of Molecular Biology, National Institute of Malariology - Parasitology - Entomology to determine relapse and re-infection with *P. falciparum*.

2.1.3 Timing and duration of study: from 2010 to 2012

2.2 . Research Method

2.2.1. Study design

2.2.1.1 Research design of the study 1 (assessment of treatment effect): As an open clinical trial, no control (one -arm)

2.2.1.2 Research design of the study 2 (response to antimalarial drugs *in vitro*): As a cross sectional and descriptive study, assessing the sensitivity of *P. falciparum* to the antimalarial drug dihydroartemisinin, piperaquin and chloroquin by *in vitro*

2.2.2 . The sample size

2.2.2.1 Assessing the effectiveness of Dihydroartemisinine - piperaquine on patient with malaria caused by P. falciparum.

The rate of parasite is still positive on D_3 when they are treated with dihydroartemisinine-piperaquine in Vietnam from previous studies from 10-20 % [NIMPE, 2008-2010], we also estimate the rate of positive 15% for drugs with 95% reliability and accuracy about 10%, the minimum sample size is about 50 patients. Because of the long follow-up during 28 days, we increased in 20 % to prevent the patient leave or withdraw from

the study. Thus, the number of patients is 60 persons for each, 120 patients for 2 sites.

2.2.2.2 Assessing the effectiveness of chloroquine on patient with malaria caused by P. vivax

The rate of treatment failure in Vietnam with chloroquine from previous studies is from 0.05 to 0.1 [NIMPE, 2004-2006], 10% was chosen as the treatment failure rate estimate for drugs. With 95% reliability and accuracy estimated at 10%, refer to the table is the minimum sample size of 35 patients selected for the study. We added 20% to prevent the patient from leaving or withdrawing the study during the 28 days. Thus, the number of patients was 42 persons for each, 84 patients for 2 sites.

The rate of the population, confidence level 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**[a]	**35**[a]	**49**[a]	61	72	81	87	92	95	96

2.2.2.3 Research on antimalarial drug response in vitro

The sample size assess the response of *P. falciparum* to the antimalarial drug dihydroartemisinine, piperaquine and chloroquine *in vitro* and also from patients' blood samples collected from the monitoring effect in 2010. The total of blood samples was 42 samples for studies on antimalarial drug response *in vitro*.

2.2.3. Sampling method: Sampling intentionally included all patients with fever at the study sites, which fully meet the sampling criteria.

2.2.4. Drugs and regimens used in the study

2.2.4.1 Drug dihydroartemisinine-piperaquine

Arterakine (dihydroartemisinine 40mg- 320mg piperaquine) by JSC Central 1 pharmaceutical manufacturing. Treatment regimen prescribed by the Ministry of Health in 2009, with 3 days of treatment, depending on the age group.

Classification of responses in treating / Site		**ACPR before PCR**		**Re-infection cases**		**Result of PCR analysis**	**ACPR after PCR**	
		n	**(%)**	**n**	**(%)**		**n**	**(%)**
Ninh Thuan	2011	30	100,0	0	0,0	0	30	100,0
	2012	33	100,0	0	0,0	0	33	100,0
Binh Phuoc	2010	31	96,9	1(D_{28})	3,1	Re-infecion	30	100,0
	2012	30	100,0	0	0,0	0	30	100,0
Total		124	99,2	1(D_{28})	0,8	Re-infecion	123	100,0

Appearing malaria parasites cases (LCF) after determining again by PCR analysis give the results of a new infection. ACPR rate was 100.0% in Ninh Thuan, Binh Phuoc was 100.0%. ACPR rate for 2 study sites was 100.0%.

3.2.2. The efficacy of Chloroquine

3.2.2.1. Fever clearance time

Table 3.23. The percentage of patients have still fever after treatment with chloroquine and fever clearance time (days)

Site / Date	**Ninh Thuan**		**Binh Phuoc**	
	n	**(%)**	**n**	**(%)**
Cases	45	100,0	43	100,0
D_1	1	2,2	10	23,3
χ^2 **, p**	$\chi^2 = 8,89$; $p < 0,05$			
D_2	1	2,2	0	0,0
D_3	0	0,0	0	0,0
Fever clearance time (day)	**1,04 ± 0,21**		**1,23 ± 0,43**	
t , p	$t = 2,64$; $p < 0,05$			

1.04 days longer than markedly compared to 2010 was 1.69 ± 0.74 days with $p < 0.05$. In Ninh Thuan, there was not any patient with parasites on blood on day D_3 but in Binh Phuoc was still 11.3% of patients positive for parasites (7 patients), up to the day D_4 100.0% patients clear parasites on blood smear tests. In the patient group studied in Binh Phuoc, the parasite rate positive on day D_3 was 3.1% in 2010 lower than in 2012 20.0%, with $p < 0.05$

3.2.1.3. Therapeutic efficacy of DHA - PIP on clinical

Table 3.21. Therapeutic efficacy of DHA - PIP on clinical

Kinds of respond / Sites		ACPR		ETF		LCF/LPF	
		n	(%)	n	(%)	n	(%)
Ninh Thuan	2011	30	100,0	0	0,0	0	0,0
	2012	33	100,0	0	0,0	0	0,0
Binh Phuoc	2010	31	96,9	0	0,0	1	3,1
	2012	30	100,0	0	0,0	0	0,0
Total		124	99,2	0	0,0	0	0,8

There was one case of the malarial parasites appeared on day D_{28} in Binh Phuoc in 2010, according to the evaluation criteria on the clinical efficacy of WHO 2009, late clinical failure (LCF) accounting for 0.8%. ACPR rate was 99.2%. In Ninh Thuan ACPR rate was 100.0%. Meanwhile, in Binh Phuoc ACPR rate was 98.4%, and LCF rate was 1.6%.

Table 3:22. Therapeutic efficacy of DHA- PIP after PCR analysis

2.2.4.2 Chloroquine tablets

Chloroquine phosphate tablets 250mg containing 150mg chloroquin base. Drug company Central Pharmaceutical Joint Stock 2. Treatment regimen prescribed by the Ministry of Health in 2009, the total dose is 25mg chloroquine base/kg body weight for 3 days. Primaquine is used after D_{28}

2.2.5. The process of conducting research and monitoring:

2.2.5.1 Process evaluation of treatment efficacy of dihydroartemisinine-piperaquine and chloroquine on clinical: was conducted in monitoring with the 28 days of WHO, 2009

Table 2.1. Abstract mornitoring process from D_0 - D_{28}

Day / Indicator	D_0	D_1	D_2	D_3	D_7	D_{14}	D_{21}	D_{28}	On any day
Clinical examination	x	x	x	x	x	x	x	x	x
Measure axillary temperature	x	x	x	x	x	x	x	x	x
Parasite test	x	x	x	x	x	x	x	x	x
Urine test	x								
Making medical records	x								
Signed commitment	x								
PCR samples	x				x	x	x	x	x
Treatment	x	x	x						x
Monitoring side effects	x	x	x	x	x	x	x	x	x

2.2.5.2. Process distinguish relapse, reinfection by PCR: The study was conducted according to the method of Snounou G. et al, 1999 [114]

2.2.5.3. Process evaluation on antimalarial drug response in vitro: The study was conducted according to the technical process *in vitro* micro-test (Mark III) WHO [126].

2.3. The techniques used in the study

2.3.1. Technical tests for malaria parasites in stained blood: According to routine techniques of National Institute of Malaria - Parasitology and Entomology

2.3.2. Technical distinguish relapse and re- infection by PCR

2.3.2.1 DNA separation technique:

DNA was isolated by using Chelex-100 according to the specifications of Plowe C.V. et al 1995 and QIAamp DNA blood mini kits according to the manufacturer's directions QIAgenes .

2.2.3.2. PCR technique to distinguish relapse and re- infection of *P. falciprum* by analysing 3 locus genotyped MSP1, MSP2 and GLURP

* Nest1 reaction: in the locus MSP1, MSP2 and use innovation GLURP: M1-OF/M1-OR, M2-OF/M2-OR, G-OF/G-OR.

* Nest2 reaction: cloning genes for specific genotypes at 3 loci using the following primer pair: the M1 genotype K1 - 2KF / 2KR - M1, M1 - MAD20 genotype 2MF/ M1 - 2MR, genotype RO33 M1 - 2RF / M1 - 2RR , genotype M2 - FCF FC / FCR - M2, M2 - genotype IC ICF/M2 - ICR, variant alleles of the locus GLURP G- FN/OR G - by method of Snounou G. et al 1999

2.3.3. Microphone technique test assess antimalarial drug response by in vitro: Technical *in vitro* micro - test (Mark III) of WHO (Rev CTD/MAL/97.20.2.2001). Techniques to detect malaria parasite by Giemsa method

2.4. The study variables

- Information of the patient before treatment.
- Fever clearance time, parasite clearance time
- The relationship between the number of parasites with age, sex, body temperature
- Relation between the number of parasites and temperatures D_0
- Parasite density changes from D_0-D_3, temperature changes from D_0-D_3
- Relation between temperature and parasite density D_0

0.05. Fever clearance time in Ninh Thuan was 1.14 ± 0.67 days shorter in Binh Phuoc was 1.26 ± 0.44 days, with p > 0.05

3.2.1.2 . Parasite clearance time

Table 3:18. The percentage of patients have still fever after treatment and parasite clearance time (days)

Site, Year / Day		Ninh Thuan			Binh Phuoc		
		2011	2012	Total	2010	2012	Total
Case		30	33	63	32	30	62
D_1	n	21	25	46	18	24	42
	(%)	70,0	75,8	73,0	56,3	80,0	67,7
χ^2 , **p**		χ^2 = 0,26; p > 0,05			χ^2 = 3,99; p < 0,05		
D_2	**n**	0	2	2	3	13	16
	(%)	0,0	6,1	3,2	9,4	43,3	25,8
χ^2 , **p**					χ^2= 9,33; p < 0,05		
D_3	**n**	0	0	0	1	6	7
	(%)	0,0	0,0	0,0	3,1	20,0	11,3
χ^2 , **p**					χ^2 = 4,40; p < 0,05		
Fever clearance time (day)		**1,70±0,47**	**1,82±0,53**	**1,76±0,50**	**1,69±0,74**	**2,43±1,04**	**2,05±0,96**
t , p		t = 0,94; p > 0,05			t = 3,27; p < 0,05		

Parasite clearance time caused by *P. falciparum* in Binh Phuoc was 2.05 ± 0.96 days longer than in Ninh Thuan was 1.76 ± 0.50 days (p < 0.05). In Ninh Thuan, parasite clearance time in 2012 was 1.82 ± 0.53 days longer than in 2011 was 1.70±0.47 days (p > 0.05) while in Binh Phuoc parasite clearance time in 2012 was 2.43 ±

CHLOROQUINE IN TREATMENT MALARIAL CAUSED BY *P. vivax* IN NINH THUAN AND BINH PHUOC.

3.2.1. The efficacy of dihydroartemisinine-piperaquine (DHA-PIP)

3.2.1.1. Fever clearance time

Table 3:15. The percentage of patients have still fever after treatment with DHA-PIP and fever clearance time (days)

Site, Year / Day		Ninh thuan			Binh Phuoc		
		2011	2012	Total	2010	2012	Total
Case		**30**	**33**	**63**	**32**	**30**	**62**
D_1	**n**	10	5	15	8	8	16
	(%)	33,3	15,2	23,8	25,0	26,7	25,8
χ^2 , p		χ^2 = 2,86; p > 0,05			χ^2 = 0,02; p > 0,05		
D_2	**n**	1	1	2	0	0	0
	(%)	3,3	3,0	3,2	0,0	0,0	0,0
χ^2 , p		χ^2 = 0,005; p > 0,05					
D_3	**n**	0	0	0	0	0	0
	(%)	0,0	0,0	0,0	0,0	0,0	0,0
Fever clearance time (day)		**1,37±0,56**	**0,94±0,70**	**1,14±0,67**	**1,25±0,44**	**1,27±0,45**	**1,26±0,44**
t , p		t = 2,65; p < 0,05			t = 0,15; p > 0,05		

Fever clearance time in Ninh Thuan was 1.37 ± 0.56 in 2011 longer than in 2012 0.94 ± 0.70 days with p < 0.05. Fever clearance time in Binh Phuoc in 2012 was longer than in 2010 (1.27 ± 0.45 days compare with 1.25 ± 0.44 days) with p >

- Percentage inhibited schizonts at each drug concentration compared to the evidence. Mean EC_{50} , EC_{90} , EC_{99}

2.4.1. In vivo studies: Classification results of treatment based on the results of clinical assessment and treatment of parasites of malaria according to WHO criteria (2009)

2.5.2. In vitro studies: Identify effective drug inhibiting 50%, 90% and 99% of parasite growth (EC_{50}, EC_{90}, EC_{99})

2.5. Analyzing data

Research data is processed and analyzed in specialized software for evaluating the validity of WHO clinical and SPSS 19.0 software and the probit Wernsdorfer (1995)

2.6. Ethical consideration

The study is approved by the Ethics committee of the National Institute of Malaria - Parasitology and Entomology.

Chapter 3. STUDY RESULTS

3.1. GENERAL CHARACTERISTICS OF RESEARCH SUBJECTS

3.1.1. General characteristics of patients with malaria caused by *P. falciparum* in Ninh Thuan and Binh Phuoc

Total number of eligible patients enrolled in the study in two provinces was 125, of which had 63 patients in Ninh Thuan, Binh Phuoc had 62 patients. Male/Female rate overall in the two provinces was 92/33 (73.6% and 26.4%). The average age was 23.1 overall. The group ≥ 15-60 years old had the highest rate 90.3% and 54.0%, and then group from 5 - < 15 years was 9.7% and 39.7% and the lowest rate was group 2 - < 5 years old with percentage was 0% and 6.3% respectively in the two provinces: Binh Phuoc and Ninh Thuan.

Table 3.2. The temperature and density of parasites of patients before treatment

Site / T⁰, density of parasites			Ninh Thuan	Binh Phuoc	Total
Cases			63	62	125
Fever	< 39⁰C	n	42	32	74
		(%)	66,7	51,6	59,2
	≥ 39⁰C	n	21	30	51
		(%)	33,3	48,4	40,8
χ^2 , p			χ^2 = 2,93; p > 0,05		
Average temperature day D_0 ($\overline{X}$ ± SD)			38,56 ± 0,82	38,87 ± 0,86	38,71 ± 0,85
t, p			t = 2,097; p < 0,05		
Average density of parasites D_0 (Geometric mean, CI 95%)			**15.759** (10.860-22.390)	**16.677** (12.273- 23.302)	**16.208** (12.734- 20.381)
t, p			t = 0,11; p > 0,05		

The average temperature of patients on day D_0 in Binh Phuoc was 38.87^0 C ± 0.86 higher than in Ninh Thuan was 38.56^0C ± 0.82 (p<0.05). The average temperature of patients at two study sites on day D_0 was 38.71^0C±0.85. Average density of parasites/µl (*Geometric mean*) of patients on day 0 was 16208, of which average density of parasites/µl in Ninh Thuan was 15759 lower than in Binh Phuoc was 16677; p> 0,05.

3.1.2. General characteristics of patients with malaria caused by *P. vivax* in Ninh Thuan and Binh Phuoc

The total number of patients with malaria caused by *P. vivax* have enough standards for the research in two provinces: Ninh Thuan and Binh Phuoc was 88 cases, of which 45 cases in Ninh Thuan, Binh Phuoc had 43 cases. Male/Female rate overall in the two provinces was 55/33 (62.5% and 37.5%). The overall average age was 23.5. The group ≥ 15-60 ages accounted for the highest rate of 90.7% and 33.3%, and then the group from

5-< 15 years was 7.0% and 46.7% and lowest group 2 - < 5 years of age at the rate of 2.3% and 20.0% respectively in the two provinces Binh Phuoc and Ninh Thuan (p < 0.05)

Table 3.9. The temperature and density of parasites of patients before treatment

Site / T⁰, density of parasites			Ninh Thuan	Binh Phuoc	Total
Cases			45	43	88
Fever	< 39⁰C	n	39	24	63
		(%)	86,7	55,8	71,6
	≥ 39⁰C	n	6	19	25
		(%)	13,3	44,2	28,4%
χ^2 , p			χ^2 = 10,29; p < 0,05		
Average temperature day D_0 ($\overline{X}$ ± SD)			37,61 ± 0,88	38,64 ± 1,18	38,11 ± 1,16
t, p			t = 4,67; p < 0,05		
Average density of parasites D_0 (Geometric mean, CI 95%)			**2.382** (1.618-3.206)	**5.354** (4.137-7.696)	**3.538** (2.785-4.679)
t, p			t = 2,27; p < 0,05		

The average temperature of patients on day D_0 in Binh Phuoc was 38.64^0C±1.18 higher than the average temperature of patients on day D_0 in Ninh Thuan was 37.61^0C±0.88 (p<0,05). Average density of parasites/µl (*geometric mean*) of patients on day D_0 was 3538; of which the average density of parasites/µl in Binh Phuoc was 5354 higher than the average parasite density was 2382 in Ninh Thuan (p <0.05).

3.2. ASSESSING THE EFFECTIVE TREATMENT OF DIHYDROARTEMISININE - PIPERAQUINE IN TREATMENT MALARIAL CAUSED BY UNCOMPLICATED *P. faciparum* AND