PREFACE

The using of artemisinin (ART) and its derivations in 70's has changed completely malarial condition in Vietnam. However, recently researches indicate that the efficacy of artesunat (one of the derivations of ART) which is used in Thailand - Cambodia border has been decreased critically. To struggle with this problem, the National Malarial Control Program used Arterakin as a kind of ACT. Therefore, evaluating efficacy of Arterakin as well as monitoring the choloroquin resistance of *P. falciparum* are indispensable. Thus, the main objectives of this study are:

1. Evaluat efficacy of Arterakin on uncomplicated P. falciparum malaria in Quangtri and Daknong provinces.

2. Evaluate the resistance of chloroquine from specimens of blood that are subdivided from P. falciparum malaria (in first objective) by using PCR technique.

SUMMARY OF RESULTS

1/ The resistance of chloroquin of *P. Falciparum* has no effect on the efficacy of Arterakin on treatment.

2/ The efficacy of Arterakin on treatment is still high but its efficacy begins to decrease the sensitivity with *P. Falciparum*.

3/ The rate of chloroquin resistance was high in Quang Tri and Dak Nong provinces meanwhile the recently reports show that the chloroquin has a tendency to increase its sensitivity.

STRUCTURE OF THE THESIS

The thesis has totally 115 pages (not include references and annex): Preface (2 pages), Background (31 pages), Subject and Method of Study (25 pages), Results (31 pages), Discussion (23 pages), Conclusion(2 pages) and Petition (1 page) and other minor contents.

CHAPTER I

BACKGROUND

1.1. MALARIA PARASITE AND MALARIAL DRUG RESISTANCE

1.1.1. Overview of malaria parasites

There are four regular types of malaria parasite that infected humans:

P. malariae (Laveran, 1881)

P. vivax (Grassi & Feletti, 1890)

P. falciparum (Welch, 1897)

P. ovale (Stephen, 1922)

P. knowlesi (was found in monkey's body 1931 and in human's body 2004)

1.1.2. Definitions related to drug resistance

The resistance of a malaria parasite to a drug which is previously effective can be given as below:

"The ability to survive and multiply of a malaria parasite when the patient is treated and absorbed a standard dose or a higher dose limits specified on the suffering of the body". (WHO 1976)

The sensitivity of a drug can be determined by performing in vivo test (28 days) *"The density of malaria parasite in blood reduces by 75% after 48 hours of treatment, it is totally disappear around day 6 or day 7 and after 28 days from the end of the treatment process, there are no malaria parasite detected".* Afterwards, Bruce-Chwatt and partners made some additions to this statement:

- A drug that can resists malaria parasites must have the ability to stay inside malaria parasites and infected red blood cells in a long time.

"Treatment failure" is a definition that is used widely in researching the efficacy of malarial drugs and it can be determined as below:

- Treatment failure is the condition that a drug loses its ability to destroy malaria parasites in blood and appearing more severe symptoms despite adequate treatment dose prescribed.

1.1.3. Drug resistances of malaria parasites and other factors that affect the drug resistances

1.1.3.1. P. falciparum's resistance

• Resists chloroquine and drugs in the same group (amodiaquine, PQP)

Mutation in gene *Pfcrt* that controls the operation of drug clearance process reduces the concentration of drug inside malaria parasites, especially, the mutation at point 76 transforming Lysine to Threonine is the most important one.

• Resists antifolate group:

Studies of hereditary in Central America and Thailand indicate that the mutation occurs at point *Ser108Asn* in gene *Pdhfr*. This genetic mutation determines pyrimethamine resistance.

For sufdadoxine, the mutation which occurs at point 437 and 540 in gene *Pfdhps* determines sulfamides resistance of malaria parasites.

• Resists drug combination pyrimethamine-sulfadoxine:

Pyrimethamine-sulfadoxine resistance mechanism is the inhibition of the biosynthesis of folic acid caused by the mutation in gene *Pfdhfr* and *Pfdhps*.

• Resists amino-alcohol:

The resistance to MEF and LUM related directly to the mutation at point Asn86Tyr in gene *Pfmdr1*.

• Resists atovaquone and similar drugs:

The resistance to atovaquone caused by a single mutation in gene cytochrome B (*cytB*) that includes 3points: Tyr268Asn, Tyr268Ser or Tyr268Cys.

• Resists quinine:

Quinine resistance mechanism of *P. falciparum* is very complicated. By using electron microscopy, some studies show that a gene called *Pfnhe-1* can affect H^+/Na^+ exchange process; a mutation at point ms4760 in this gene changes the effects of quinine malaria parasite.

• Resists artemisinine and its derivative:

The reduction of the sensitivity of ART and its derivative has been mentioned in some recently researches. By using radioisotope method, researcher indicates that a genetic mutation occured in gene *Pfmdr1*can be the result of multiple drug resistance.

1.1.3.2. P. vivax ‘s resistance

• Resist chloroquine:

The first study on chloroquine resistance of *P. vivax* was made in Indonesia and Papua New Guinea in 1980. Chloroquine resistance mechanism of *P. vivax* related directly to a mutation at point *Tyr976Phe* in gene *Pvmdr*-1.

1.1.3.3. *Malariae* and *P. ovale* ‘s resistance

Research by Maguire and partners in 2002 reported on the state of chloroquine resistance of *P. malariae* and *P. ovale* in Indonesia.

1.1.3.4. Some factors that affect anti-drug process

Some popular factors are: taking insuffcient drug doses or poor quality drugs, using a drug that resists malaria drugs and each individual’s reaction.

1.2- STATE OF DRUG-RESISTANCE MALARIA IN GREATER MEKONG SUB-REGION AND PREVENTIVE STRATEGY

1.2.1. State of drug-resistance malaria in Greater Mekong Sub-region (GMS)

1.2.1.1. Resists monotherapy regimen

- **Resists Chloroquine:**

The first time *P. falciparum* resists chloroquine has been reported in Nha Trang in 1961. The resistant up to 100 percent in 1982. From 1996 until now, chloroquine tends to be sensitive back in some region.

- **Resists sulfadoxine – pyrimethamine:** resistant up to 73,6%.

- **Resists quinine:** resistant up to 100% in Cambodia and 27,7% in Vietnam.

- **Resists mefloquine:** Rate of MEF resistance up to 40% in Vietnam and Myanmar.

- **Resists artemisinine and its derivative:** Border Thailand-Cambodia is the first place that showed evidences of ART resistance of *P. Falciparum*.

1.2.1.2. Reduction of sensitivity with ACT drugs

- **Artesunate-amodiaquine:**In GMS, it appears in Vietnam and Myanmar

- **Artesunate-mefloquine:** Cambodia and Thailand has high rate of "treatment failure" that up to ≥ 10%.

- **Artemether - lumefantrine:** Cambodia and Myanmar has high rate of "treatment failure" that up to 26,1% (2001) and 28,9% (2002).

- **Dihydroartemisinine-piperaquine(Arterakine):** In Vietnam, the efficacy of Arterakine is still high but its sensitivity with *P. falciparum* began to decrease.

1.3/ ARTERAKINE AND ITS EFFECTS IN TREATMENT OF UNCOMPLICATED P. *falciparum* MALARIA

1.3.1. Information about Piperaquine (a component of Arterakine)

PQ tends to be sensitivity back as a component of ACT drug-combination, piperaquine has the molar mass M=535,5g/mol and its molecular structure is 1,3-bis-{4-(7-chloroquinolyl-4)-piperazinyl-1}-propane. In base form, its chemical formual is $C_{29}H_{32}Cl_2N_6$, in the form of tetraphosphate (PQP) its chemical formula is $C_{29}H_{32}Cl_2N_{6\,.}$, $4H_3PO_4$.

1.3.2. The similarity between chloroquine and piperaquine

- **The similarity between their structures**

They both contain a functional group 7-chloro-4 aminoquinolein

- **The similarity between their mechanisms of action**
- **Cross-resistance of piperaquine and chloroquine.**

1.3.3- The effects of Arterakin drug combination in treatment of uncomplicated *P. falciparum* malaria

Arterakine tablets containing DHA (40 mg) + PQ (320 mg) have been tested and evaluated its safety and efficacy since 2001 . Arterakine has a high efficacy with cure rate ranges from 90,4% to 98,3%. However, after a short time of using Arterakine, its efficacy with *P. falciparum* began to reduce.

1.4. MOLECULAR BIOLOGICAL TECHNIQUE IN RESEARCHING DRUG-RESISTANCE

1.4.1. Principle of PCR technique

Polymerase Chain Reaction (PCR) technique had been proposed by Karry Mullis – an U.S biochemist in 1985. The specific DNA sequence that has to be researched will be multiplied by 2^n.

1.4.2. Applications of PCR technique in researching malaria

• PCR technique in distinguishing between relapse and reinfection

By defining the genotype in 3 locus: MSP1, MSP2, GLURP of malarial parasite, we can determine whether it is relapse or reinfection.

• PCR technique in detecting anti-drug malarial parasites:

By using PCR technique we can find out that the most important mutation that determines chloroquine - resistance is in gene *Pfcrt* in chromosome number 7 of *P. falciparum*.

CHAPTER II

OBJECTS AND METHOD OF STUDY

2.1. OBJECTS AND PATIENT SELECTION CRITERIA

2.1.1. Objects of study

Objects for both clinical test and chloroquine - resistance evaluation are all the patients that were diagnosed with uncomplicated P. *falciparum* malaria.

2.1.2. Patient selection criteria

- Age from 6 months to 60 years.
- Have fever with average body temperature > $37^0$5
- Density of *P. falciparum* in blood $\geq$ 1000-200.000/mm^3

2.1.3. Elimination standards

- Children under 6 months of age and adults over 60 years old
- Have signs of severe and complication malaria.
- Coordinate infected or single infected by other types of *Plasmodium*

2.1.4 Location and time of study

- Location of study

The study was conducted in 2 areas that have malaria: Quangtri and Daknong.

- Execution time of study: From 2008 to 2010

2.2. METHOD OF STUDY

2.2.1- Design of the study The study is a one-arm and open clinical test. That design has been used mostly in monitoring the eficacy of malarial drugs (WHO 2005).

Monitoring the efficacy of Arterakine in malarial patients caused by uncomplicated *P. falciparum* is a 28 days process that used PCR technique to determine relapse-reinfection.

To evaluate the chloroquine-resistance condition of *P. falciparum* from sub-divided parts in monitoring the efficacy of malarial drugs section, we used PCR technique.

2.2.2- Sampling method

Recently researches showed that the "treatment failure"'s rate with Arterakin is <5%. With the reliability up to 95%, we chose p = 0,1, the accuracy d = 10%. The sample size of this study that can be calculated based on the standard sample size table of WHO (1987) is 35 but 30% of the patients can be lost in monitoring process that is 11 so we got n = (1+0.30) x 35 = 46

Rounded number of selected patient in each research point is 50. The sample size of 2 points is 100 patients.

Population ratio (*P*), reliability = 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

2.2.3.Drugs and regimen of the study

Arterakine:

Arterakine contains 40 mg of dihydroartemisinine (DHA) and 320 mg of piperaquine (PIP) produced by Central Pharmaceutical Enterprise No.1.

Regimen:

Age groups	Amount of Arterakine tablets used per day			
	0h	8h	24h	48h
2 - 3 years old	0.5	0.5	0.5	0.5
3 - < 8 years old	1.0	1.0	1.0	1.0
8 - < 15 years old	1.5	1.5	1.5	1.5
≥ 15 years old	2.0	2.0	2.0	2.0

2.2.4. Research and monitoring process

2.2.4.1. Evaluating clinical effects of Arterakine process

The study was conducted according to a standard process of World Health Organization (WHO/MAL/96.1077).

Qualified patients will be put on study list. Density of parasite and body temperature will be checked once a day until the patient totally recover from fever and there is no parasite detected inside his or her body 2 days in a row. Afterwards, density of parasite and body temperature will be checked once a week in $D_{7,}D_{14}D_{,21}D_{,28}$.

- Blood samples are tested to find parasite, counting the number of parasite from 2 individuals then encoding this information by location and time of getting those samples from D_0 to D_{28}

- Using PCR technique for blood samples in D_0 to make sure that the patient was not infected by *P. vivax*.

- If the parasite still survives inside the body in D_3, take more blood samples in D_4, D_5..(stop whenever the result is negative 2 days in a row)

- If the patient get fever again in any day of $D_{7,}$- D_{28}**,** his or her blood will be taken to test for malarial parasite and to use PCR technique to differentiate relapse and reinfection in accordance with the process.

2.2.4.2. Process of discriminate relapse and reinfection by using PCR

- Compare genotype of malarial parasite (D_0) with genotype of it in the day that fever returns (D_{14}-D_{28}).

- Like blood sample, blotting-paper was encoded by location and time of the study. Blood samples were preserved in special nilon bags at -20^{0}C.

- Analyze genotypes of *P. falciprum* in 3 locus by using PCR technique. If genotypes of *P. falciprum* in 3 locus are the same, it is relapse. If one genotype is different from the other two, it is reinfection. Primer pair is:

• ***For locus MSP1, using primer pair that has order below:***

M1-OF: 5'- CTA GAA GCT TTA GAA GAT GCA GTA TTG -3'

M1-OR: 5'- CTT AAA TAG TAT TCT AAT TCA AGT GGA TCA

• ***For locus MSP2, using primer pair below:***

M2-OF: 5'- ATG AGG GTA ATT AAA ACA TTA TCT ATT ATA -3'

M2-OR: 5'- CTT TGT TAC CAT AGG TAC ATT CTT -3'

• ***For locus GLURP, using primer pair below:***

G-OF: 5'- TGA ATT TGA AGA TGT TCA CAC TGA AC -3'

G-OR: 5'- GTG GAA TTG CTT TTT CTT CAA CAC TAA -3'

2.2.4.3. Determination of anti-drug mutation at point 76 of *P. falciparum* by using PCR

- Taking blood samples from all patients and put them on the absorbent paper Whatman 3MM

- Keeping blood samples in good condition to use PCR technique in accordance with the process.

- Determining mutation at point 76Lys→76Thr by using nested PCR technique with outside primer pair to find chloroquine resistance of *P. Falciparum*.

Reaction Nest1

TCRP1: 5'-CCG TTA ATA ATA AAT ACA CGC AG-3'

TCRP2: 5'-CGG ATG TTA CAA AAC TAT AGT ACC C-3'

Reaction Nest2:

• Determine lysine at point 76 by using primer pair

76Com: 5'-CGA GCG TTA TAC AGA ATT AG -3'

76W: 5'-TTA AAG TTC TTT TAC CAA AAA TTT-3'

• Determine threonine at point 76 by using primer pair

76Com: 5'-CGA GCG TTA TAC AGA ATT AG-3'

76M: 5'-TTA AAG TTC TTT TAC CAA AAA TGT-3'

Based on standard ladders and LabWorks 4.0 program with *Digital Imaging Systems SDS – 8000,* measuring the size of PCR products after using electrophoresis.

2.3. TECHNIQUES USED IN THE STUDY

2.3.1 Technique to find malarial parasite

Taking blood sample:

Take blood from fingertips, stain blood using Giemsa stain and find malarial parasite by using microscopy.

Density of parasite (per mm^3) is calculated by using equation below:

$$\text{Density of parasite} = \frac{\text{Number of parasite counted x 8000}}{\text{Number of leukocyte counted}}$$

2.3.2. Technique to discriminate relapse, reinfection and to determine anti-drug mutation at point 76 of *P. falciparum* by using PCR

Extraction of DNA:

Plowe and partners 's technique (1995):

- Cut blood sample on Whatman paper into smaller pieces and put them into 1.5 ml test tubes.
- Add 1ml mixture of Saponin 0,5% and PBS (pH = 7,4).
- Keep it at 37°C in 30 mins then use centrifuge to remove upper liquid layer.
- Wash the test tube with 1ml of PBS (pH = 7,4) 3 times.
- Add 150µl Chelex - 100 (10%)
- Boil the test tube in 8 mins then use centrifuge to remove upper liquid layer. Preserve it at -20°C.

Conducting PCR reaction:

Blood samples was used to analyze genotypes of *P. Falciparum* in 3 locus MSP1, MSP2, GLURP. Based on this analysis, we can determine the mutation at point 76 of *P. falciparum* by using Eppendorf with GenAmp PCR System 9700.

2.3.3. Electrophoresis technique for PCR products

Products from PCR process will be analyzed by using electropherosis with agarose gel - containing 25µg ethidium bromide. The products will be showed in ladders as bright lines that correspond with their specific weight.

Evaluate electrophoresis result.

- For Locus MSP1, electrophoresis technique was used for PCR products with agarose gel 3% - containing 25µg ethidium bromide:

Genotype K1 weighs	142-295 bp
Genotype MAD20 weighs	100-250 bp
Genotype RO33 weighs	160 bp

- For Locus MSP2, using electrophoresis technique with agarose 2,5%

Genotype FC weighs	220-550 bp
Genotype IC weighs	400-750 bp

- For Locus GLURP, electrophoresis technique was used with agarose gel 1,5% and the genotype weighs 470-1200bp

2.4. Materials and mediums used in this study

2.4.1. Materials and mediums used to evaluate efficacy of Aterakine

- Original Giemsa stain
- Potassium dihydrogen phosphate (KH_2PO_4)
- Disodium hydrogen phosphate (Na_2HPO_4)
- Microscopy with oil lens and magnification of 10 x 100
- Micro pregnancy test and HIV test strips

2.4.2- Materials and mediums used in PCR technique

Sample: 30-50 µl blood of malarial patient put on Whatman 3MM paper.

**** Primer system:***

- Primer system to determine mutation of *P. falciparum*. TCRP1, TCRP2, TCRP3, TCRP 4W, TCRP 4M

- Primer system to determine genotype of *P. falciparum*. M1-OF, M1-OR, M1-2MF, M1-2MR, M1-2KF, M1-2KR, M1-2RF, M1-2RR, M2-OF, M2-OR, M2-ICF, M2-ICR, M2-FCF, M2-FCR, G-OF, G-OR, G-FN.

**** Chemicals used in extraction, purification of DNA and PCR reaction***

2.5. IMPORTANT INDEXES

- Pre-treatment information
- Time to get out of fever and malarial parasite
- Rate of anti-drug parasite followed by geography
- Connection between drug resistance and age, gender.
- Connection between number of parasite and body temperature (D_0)
- Bodytemperature and parasite development from D_0 to D_3.
- Compare parasite development between 2 different groups: resist chloroquine and do not resist chloroquine.
- Treating requirement: according to WHO criteria

2.6. ANALYZES DATA

Use Statar program to analyze the data taken by 2 indiviuals.

2.7. ETHICAL ISSUES IN THE STUDY

2.7.1. Voluntariness

- All the patients participating in this study will be explained thoroughly about the program and they will be free to take this study or not (Appendix 10)

2.7.2. Healthcare services

- All the patients was provided malarial drugs, intravenous and vitamins for free, If unwanted reactions happen, the patient will be brought to the hospital as fast as possible.

-. Patients with other health problems will be consulted to treated.

2.7.3. Dealing with "treatment failure" cases

Using other drugs as needed like quinnine sulphate combine with doxycycline clindamycine or Artesunate according to Department of Health's instruction.

CHAPTER III

RESULT

3.1. EVALUATING THE EFFICACY OF ARTERAKINE

3.1.1. Information about objects of the study

Table 3.1 Patients's Information

Location	Quangtri	Daknong	Total
Number of patients	65	59	124
Male (%)	29 45%	49 82%	78
Female (%)	36 55%	10 18%	46
Average Age (Min-Max)	16,9 0,8-55	25,1 2 - 60	20,8 0,8-70
< 5 years old n	6	3	9
5 - 15 years old n	29	6	35
> 15 years old n	30	50	80

All qualified cases that were researched in Quangtri and Daknong are 124 which has 65 cases from Quangtri and 59 cases from Daknong.

3.1.2. Information about malaria

Table 3.2 Information about malarial patient

Location	**Quangtri**	**Daknong**	**Total**
Number of patients	65	59	124
Average temp (D_0)	39,0± 0,9	38,8±0,9	38,9 ± 0,9
t, p	t = 1,79 p> 0.05		
Temp < 39^{0}C (%)	23 (35,4%)	29 (49,1%)	52 (42%)
Temp ≥ 39^{0}C (%)	42 (64,6%)	30 (50,9%)	72 (58%)
$\chi 2$, p	$\chi 2$ = 2,4076; p > 0,05		
Density of parasite D_0 (Geometric) 95 % CI	12.230 (9.066 - 16.513)	17.074 (13.022 -23.268)	14.470 (11.745 - 17.827)
t, p	t = - 0,65; p > 0.05		

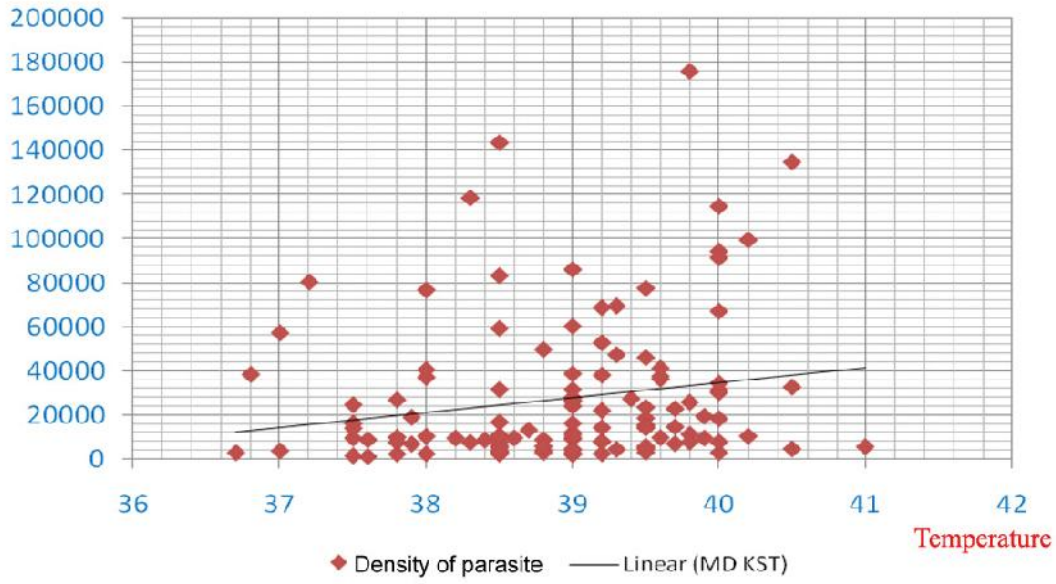

Figure 3.6 Relationship between density of parasite and temperature(D_0)

3.13. Efficacy of Arterakine in uncomplicated *P. falciparum* malarial patient

3.1.3.1. Time to stop fever

Bảng 3.8 Time to stop fever of Arterakine

Location	Quangtri		Daknong		Total	
D_0	65/65 (100%)		59/59 (100%)		124/124 (100%)	
D_1	22	34%	16	27%	38	31%
D_2	0		0		0	-
D_3	0		0		0	-
Average time to stop fever (days)	1,33 ± 0,47		1,27±0,45		1,31 ± 0,46	
P	> 0.05					

Average time to stop the fever is 1,31 ± 0,46 days with p > 0,05.

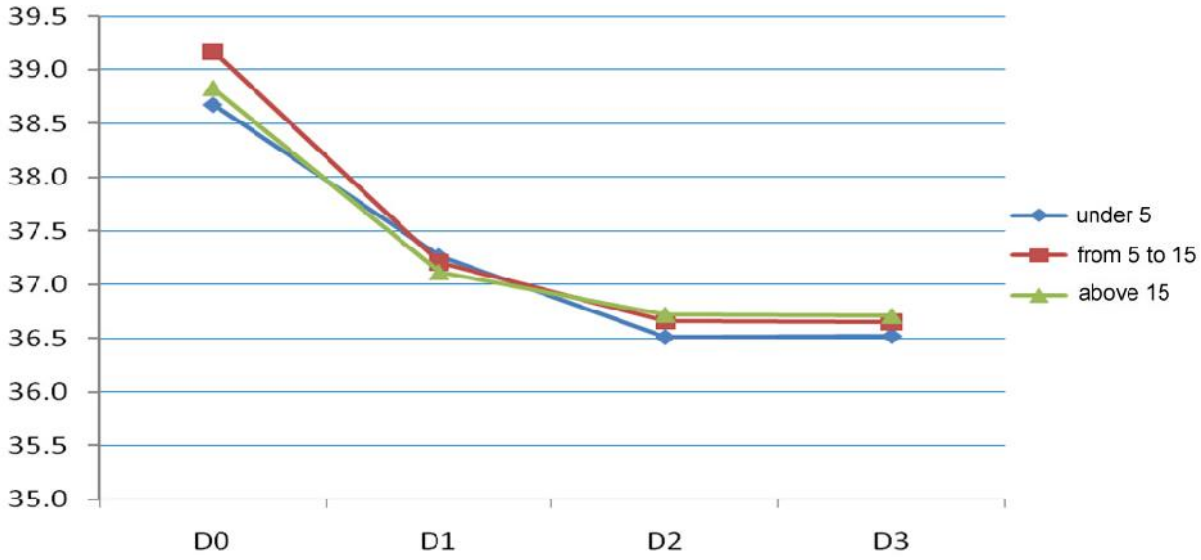

Figure 3.13 Distribution of temperature/day by age groups.

There are no fever cases from D_3

3.1.3.2 Time of parasite-clearance

Table 3.10 Time of parasite-clearance of Arterakin

Location	Quảng Trị		Đăk Nông		Tổng số	
n	65		59		124	
D1	42	64,6%	27	45,8%	69	55,7%
D2	64	98,7%	53	89,9%	117	94,4%
D3	65	100%	56	94,2%	121	97,6%
Average time of parasite-clearance (days)	1,37 ± 0,52		1,69 ± 0,79		1,52 ± 0,68	
p	< 0.05					

3.1.3.3. Clinical efficacy of Arterakine

Table 3.12 Efficacy of Arterakin after using PCR

Classification / Location	ACPR (%)	ETF (%)	LCF/LPF (%)
Quangtri	65/65 (100 %)	0	0
Daknong	59 /59 (100 %)	0	0
Total	124/ 124 (100%)	0	0

Rate of ACPR for 2 locations is 100%

3.2. THE RESULT OF EVALUATING CHLOROQUINE RESISTANCE BY USING PCR TECHNIQUE

3.2.1. Rate of gene S/R

Table 3.14 Percentage of gene R, R + S and S

Genotype / Location	Gen R		Gen R và S		Gen S	
	n	%	n	%	n	%
Both 2 locations	78	62,9	19	15,3	27	21,8
Total	78 + 19 =97 97/143(68%)		19		27+19 = 46 46/143 (32%)	

3.2.2. Rate of gene S/R according to total number of blood samples

Table 3.16 Distribution of gene S/R followed by geography

Point / Cases	Quangtri		Daknong		Total	
	n	%	n	%	n	%
R	49/65	75	48/59	81	97/124	**78**
S	16/65	25	11/59	19	27/124	22
Total	65	100	59	100	124	100
	χ^2= 0,65 (p > 0,05)					

There is no difference of chloroquine resistance ratio between 2 locations (p > 0,05).

Bảng 3.18 **Tỷ lệ % kháng CQ theo nhóm tuổi**

Location / **Age**	**Quangtri** n 49/65	**Dacknong** n 48/59	**Total** n 97/124
< 5 year	4/6	2/3	6/9
%	66,6	66,6	66,6
5 – 15 year	24/29	6/6	30/35
%	83	100	85,7
> 15 year	21/30	40/50	61/80
%	70	80	76,3
Total %	75%	81%	78%

Rate of chloroquine resistance for age groups from 5 year to 15 years is very high which is 85,7% in Quangtri and 100% in Dacknong.

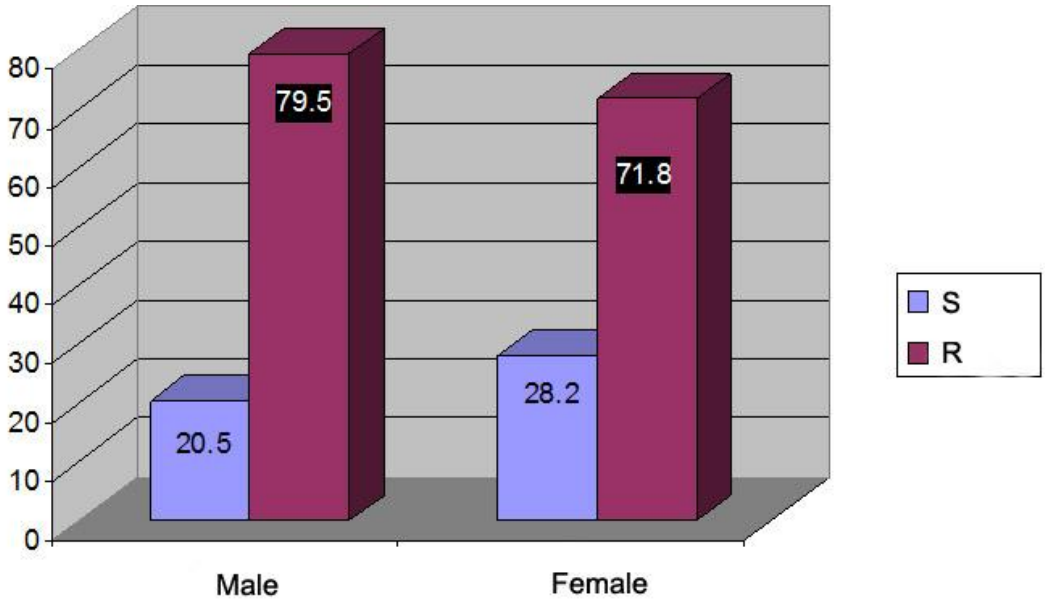

Figure 3.18 Percentage of sensitivity/resistance in male/female

Rates of drug-resistance in both male and female are high >70%

There is no difference between two gender p > 0,05

3.2.3. Compare efficacy of Arterakine in chloroquine-sensitive *P. falciparum* with chloroquine- resisted *P. Falciparum*.

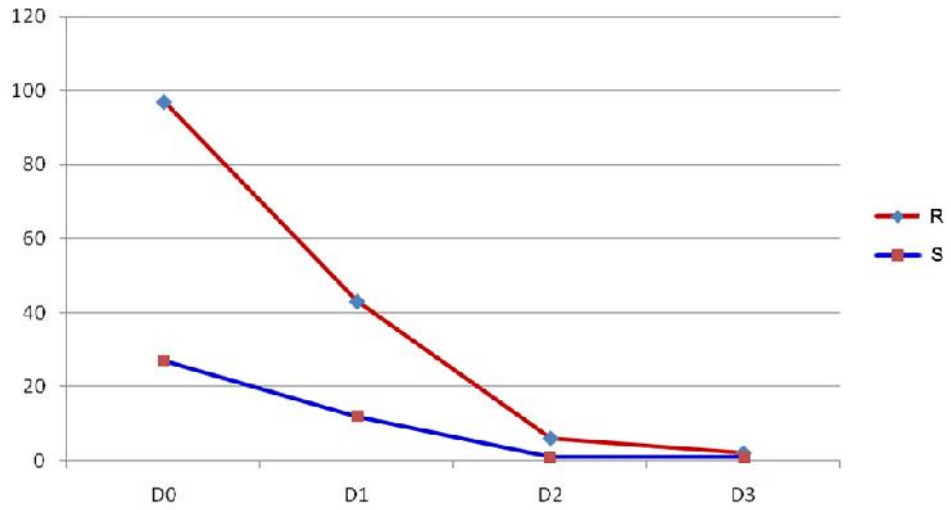

Figure 3.20 Relationship between S/R Chloroquine and parasite clearance day

Chloroquine-resisted group takes a longer time to clear malarial parasite than chloroquine-sensitive group takes.

Table 3.20 Relationship between efficacy of Arterakine, parasite (+) in D_3 and chloroquine-resistance

PCR RESULT	CASES THAT HAS PARASITE (+) IN D_3	REINFECTION	ACPR
CHLOROQUINE SENSITIVE	0	0	100%
CHLOROQUINE RESISTED	3	2	100%

All the cases that still has parasite in D_3 and be reinfected from D_{21} to D_{28} are of chloroquine-resisted group

CHAPTER IV

DISSCUSSION

4.1. Efficacy of Arterakine on uncomplicated *P. falciparum* patient in Quangtri and Daknong.

4.1.1. Time to stop fever

Average time to stop fever is 1,3. This result is similar with the result of Hoang Ha in a study of malaria in Quangtri (Vietnam) and Savannakhet (Laos).

4.1.2. Parasite-clearance time

Parasite-clearance time in 2 locations is 1,5±0,7 days. There is difference between parasite-clearance times in 2 location ($p < 0,05$) because the immunity to malaria of non-resident is lower.

4.1.3. Efficacy of Arterakine (ACPR) in 28 days process:

ACPR percentage is 98,4% and percentage of LTF/LPF is 1,6%, similar with the results of Looareesuwan S and Wilairatana P (1999) in Thailand. Moreover, is also similar with the result of Nong Thi Tien at Quangtri.

4.2. CHLOROQUINE-RESISTANCE CONDITION IN TWO LOCATIONS

There is difference between chloroquine-resistance rate in our study (78%) and in other studies. Ngo Viet Thanh did a research in Binhphuoc that shows chloroquine-resistance rate is 37,93%. Trieu Nguyen Trung did a research in Mid-Taynguyen indicating that chloroquine-resistance rate is only 12,5%.

This difference may be caused by different location of study.

CONCLUSION

Efficacy of Arterakine on uncomplicated *P. falciparum* patient in Quangtri and Daknong.

1/ Arterakine tablet produced by Central Pharmaceutical Enterprise No.1 is still effective in treating uncomplicated *P. falciparum* malaria in Quangtri and Daknong with ACPR percentage is 100%.

2/ Average time to stop fever is 1,3 days. Average parasite-clearance time is 1,5 ± 0,7 days.

3/ Percentage of parasite(+) in D_3 is 2,4%.

4/ Arterakine do not have unwanted side-effects.

5 Efficacy of Arterakine has not been affected by chloroquine-resistace of *P. falciparum.*

Results of evaluating chloroquine-resistance by using PCR

1/ The rate of a mutation to be occurred at point 76 in gene *Pfcrt* that resists chloroquine is 63%, sensitive with/resists to chloroquine is 15% and sensitive with chloroquine is 22%.

2/ Percentage of blood samples that contains chloroquine-resistance mutation in two locations is 78% - Daknong (81%) and Quangtri (75%).

3/ Group of ages 5 -15 has highest chloroquine-resistance rate that is 86%, group of ages above 15 has the rate that is 76% and group of ages below 5 has the lowest chloroquine-resistance rate that is 67%.

PETITION

1/ Annually evaluate the effcacy of ACT drugs and Arterakine to detect the reduction of sensitivityof the drugs to make early treatment.

2/ Continue to monitor the development of anti-drug condition of malarial drugs like chloroquine … to detect the sensitivity of the drugs and use them as components of ACT drug-combination.