EFFICACY OF ARTESUNATE MONOTHERAPY AND ARTEMISININE-BASED COMBINATION THERAPIES (ACTs) IN THE TREATMENT FOR UNCOMPLICATED FALCIPARUM MALARIA IN SOME CENTRAL & WEST-HIGHLAND PROVINCES, VIETNAM

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Abstracts

Background: Malaria still a public health problem in the tropics and subtropics areas, particularly in South East Asia (SEA) and Subsaharan, Africa. Achievements of National Malaria Control Program in Vietnam cannot be made without significant contribution of highly effective antimalarial drugs. The introduction of artemisinine helped to contain resistance in the context of *P. falciparum* showing low response and high resistance to quinine, chloroquine, and mefloquine. During 2000 – 2011, some *in vitro* and *in vivo* results found artemisinine and its derivatives had declined efficacy against *P. falciparum* (WHO., 2011), in line with some health facilities in Central and Westhighland provinces reported that reduced efficacy of ACTs on several clinical cases (H.H.Quang *et al.*, 2011). The emergence and spread of drug-resistant malaria parasites, especially in dangerous *Plasmodium falciparum* species, is the major threat to effective malaria control. The artemisinin derivatives have had an important clinical impact both on the treatment of multi drug resistant falciparum malaria. However, experience has shown that resistance eventually curtails the life span of drugs. If measures are not applied to contain resistance, the investment put into the development of new drugs will be squandered. Therefore, in parallel with the monitoring of the efficacy of currently-used drugs, the evaluation of novel ACTs for replacing and overcoming the backwards of the old ones is really essential to suggest possible changes in the national drug policy. **Objectives:** The study is designed to evaluate the efficacy of artesunate monotherapy 7 days regimen and some artemisinine based combination therapies (ACTs) on uncomplicated *P. falciparum* malaria patients, and assess some possible side effects from these regimens M**ethods:** with study design of clinical trials of *in vivo* in the community based-fields, and treatment outcomes classification by the latest update WHO guidance in 2009, including ACPR (Adequate clinical and parasitological response), ETF (Early treatment failure), LTF (Late treatment failure, including LPF_Late parasitological failure and LCF_Late clinical treatment). **Results:** the study was conducted according to the WHO guideline protocols 2009, the results showed that: (1) The ACPR efficacy of artesunate monotherapy were 82.2% and 87.2% in Quang Tri (with cross-border of Laos) and Ninh Thuan (without cross-border) sentinel site, respectively. Benny notes in proportions of LCF of 15.1% and LPF of 2.7% (in Quang Tri), and LCF of 8.5% and LPF of 4.3% (in Ninh Thuan). Both of them were decreasing efficacy with artesunate alone regimes; (2) The ACPR efficacy of ACTs (dihydroartemisinine plus piperaquine) were in ranged 94.8% to 100% during 2007-2011 in

Quang Tri, Ninh Thuan, Gia Lai. In which, especially notes in Gia Lai sentinel, there was ETF of 3.4% and LCF of 1.7%, and accompanied with nearly 17% positive parasite on or after D_3 post-treatment of ACTs as indirect clinical marker for resistance high risk (WHO definition 2011). Three cases of LCF were corrected by PCR technique and reinfection (new infection) or recrudescence were indetified entirely; (3) Both of artesunate monotherapy and ACTs regimes were high safety, no significant serious side-effects related to these drugs. **Conclusions:** The efficacy of artesunate alone with treatment failure rate more than 10%, need to be alter for the better, and *vice versa*, the efficacy of ACTs (dihydroartemisinine plus piperaquine) are extremely high (ACPR = 94.8-100%), ideal for falciparum malaria treatment, but positive parasite on day 3 (prolonged parasite clearance time) > 10% as clinical marker of resistance in the near future. Side-effects of the regimes were reported but did not health care interventions.
Keywords: Malaixia, Artesunate

1. BACKGROUNDS

Malaria, the "King of diseases", continues to haunt and taunt mankind. More than a century after identification of the causative parasites, and more than half a century after finding effective drugs and insecticides, the disease as old as humanity itself, affects more than 500 million and kills more than 3 million people every year. The successful events of National Malaria Control Program (NMCP) in Vietnam cannot be made without significant contribution of highly effective antimalarial drugs, especially in artemisinine and its derivaties. The emergence and spread of *Plasmodium falciparum* resistance to commonly used antimalarials such as chloroquine (CQ), sulphadoxine/ pyrimethamine (SP) and mefloquine (MEF) has posed major challenges to malaria control in sub-Saharan Africa and South East Asia, including Vietnam. In the face of escalated resistance to these widely used and long utilized antimalarial the World Health Organization (WHO) and NMCP (2009) currently recommends the use of artemisinin combination therapies (ACTs) as the first line treatment of malaria in Vietnam. Despite these recommendations, country specific evidence-based data to support antimalarial first line treatment policy change to ACTs is still limited.

The artemisinin derivatives have had an important clinical impact both on the treatment of multi-drug resistant (MDR) falciparum malaria. However, experience has shown that resistance eventually curtails the life span of drugs. If measures are not applied to contain resistance, the investment put into the development of new drugs will be squandered. Therefore, a number of lessons were learnt after this policy revision. in parallel with the monitoring of the efficacy of currently-used drugs, the evaluation of novel ACTs for replacing and overcoming the backwards of the old ones is really essential to suggest possible changes in the national drug policy. In the aftermaths of interim policy inception (e.g. artesunate monotherapy → artemisinine based combinations), several major steps were taken including conducting *in vivo* studies on efficacy of ACTs and other newly registered antimalarials geared to increase choices and preparedness should the need for policy revision arise. In this framework, hence, an *in vivo* study was carried out on the efficacy, tolerability and safety of some artesunate alone, ACT drugs with a view of supporting the National Malaria Control Programme

(NMCP) in reviewing the antimalarial drug treatment policy in Vietnam.

2. GENERAL OVERVIEWS

2.1. Artesunate monotherapy and nouvel ACTs

Artesunate (AS) is recommended by the World Health Organization (WHO) in replace to some traditional antimalarial drugs for the treatment of uncomplicated and/ or severe falciparum malaria and has been used worldwide for many years. Artesunate is in the class of medications known as artemisinins, which are derivatives from the "quinghaosu" or sweet wormwood plant (*Artemisia annua*). Most of clinical studies in multicenters in the world showed that AS has rapid action, quick clearance of parasite and resistance retardation, but due to AS's short half-life, this will be recrudescence post-treatment, and therefor, easy to develop resistance trends.

Although artemisinins are potent and rapidly acting antimalarial drugs, their widespread use for treating patients with *Plasmodium falciparum* malaria raises the question of emerging drug resistance. Artemisinin monotherapy should not be used in areas where malaria is endemic; it requires an extended administration period and may lead to treatment failure, most frequently because of problems with compliance. Recent reports of high failure rates associated with artemisinin-based combination therapy along the Thai–Cambodian border, Myanmar, Thailand (WHO., 2011), and in Binh Phuoc, Gia Lai, Vietnam (T.T.Tinh *et al.,* 2011; H.H. Quang *et al., 2011,* T.T. Hien *et al., 2012),* as well as *in vitro* drug susceptibility data, suggest the possibility of clinical artemisinin resistance.

ACTs are the first-line treatments for uncomplicated *P. falciparum* malaria in most malaria endemic countries. Recently, partial artemisinin-resistant *P. falciparum* malaria has emerged in SEAs, especially in borderlines. Exposure of the parasite population to artemisinin monotherapies in subtherapeutic doses for over 30 years, and the availability of substandard artemisinins, have probably been the main driving force in the selection of the resistant phenotype in the region. A multifaceted containment programme has recently been launched, including early diagnosis and appropriate treatment, decreasing drug pressure, optimising vector control, targeting the mobile population, strengthening management and surveillance systems, and operational research.

2.2. The potential of nouvel ACTs

So far, ACTs have amply shown how they can benefit both in clinical trials and clinical hospital facilities. It cures more people than other widely available treatments, thus reducing the risk that the patient will get severe malaria or die. And at a community level, ACTs have helped to stem transmission of the disease by lowering the number of gametocytes, hence the malaria parasites in infected people taken in by *Anopheles* mosquitoes when they feed, and then transmitted to other humans.

But this is not all. As with combination therapies against tuberculosis and HIV/AIDS, ACTs also delay antimalarial resistance by eradicating organisms that may be resistant to one of the drugs in the combination, through the action of the other. ACTs thus, have real and proven potential to combat the deadly form of malaria. But to optimise cure rates, 4 particularly challenging requirements must be fulfilled: (i) the patient must complete the entire course of treatment; (ii) the drugs

need to be good-quality; (iii) they also need to be appropriately selected; (iv) and the vast majority of malaria infections need to be treated with ACTs.

3. METHODS

3.1. Study sentinel sites, times and designs

- The study was conducted within the *invivo* efficacy testing framework of the Vietnam NMCP/WHO Network for Monitoring Antimalarial. Total 5 health facilities took part in the study:

+ Ma Noi commune (Ninh Son district, Ninh Thuan province);

+ Phuoc Chien commune (Thuan Bac district, Ninh Thuan province);

+ Xy and Thanh commune (Huong Hoa district, Quang Tri Province);

+ An Trung communee, Konch'ro district, Gialai province;

+ Ia Ke commune (Phu Thien district, Gia Lai province).

- This study was conducted in 2007 - 2011, belonging to malaria transmission peaks, after the long seasonal rainfall;

- Following to the WHO standardized protocol for the assessment of therapeutic efficacy of antimalarial drugs (WHO 2007, 2009), or *in vivo* clinical trials in the fields;

3.2. Inclusion and exclusion criteria

- Age, 6-59 months, i.e. under 5 years in areas of high transmission, and all patients over 6 months of age in areas of low to moderate transmission;

- Mono-infection with P. falciparum deteced by microscopy;

- Asexual parasite count of 2.000 - 200.000/ μL in areas of high trans and 1.000-100.000/ μL in areas of low to moderate transimission;

- Axillary temperature ≥ 37.5 °C or history of fever during the 24 h before recruitment;

- Ability to swallow oral medication;

- Ability and willingness to comply with the protocol for the duration of the study and to comply with the study visit schedule;

- Informed consent from the patient or from a parent or guardian in the case of children;

- Absence of general danger signs in children under 5 years or signs of severe falciparum malaria according to the definitions of WHO (2000);

- Absence of severe malnutrition according to WHO child growth standards;

- Absence of febrile condition due to diseases other than malaria (e.g. measles, acute lower respiratory tract infection, severe diarrhoea with dehydration) or other known underlying chronic or severe diseases (e.g. cardiac, renal or hepatic diseases, HIV/AIDS);

- Absence of regular medication, which might interfere with antimalarial pharmacokinetics;

- Absence of history of hypersensitivity reactions or contraindication to any medicine being tested or used as alternative treatment;

- and a negative pregnancy test or not breastfeeding.

3.3. Antimalarial drugs interventions

The patients were randomized to receive either AS monotherapy (7 days) or the ACTs (DHA+PPQ_dihydroartemisinine plus piperaquine) in 3 days regimes according to the NMCP dosage schedule of sentinel testing. At each sentinel site AS alone and DHA+PPQ were used. AS monotherapy was given on day 0 of 4mg/kg, on 1, 2, 3, 4, 5, 6 with dose of 2mg/kg body weight;

All treatments were supervised by study nurse, assisstant doctor or princible investigators and patients were observed for 30 minutes in the aftermath of drug intake. All patients who vomited within 30 minutes

intervals were re-administered another full dose of the same medicine. All treated patients were followed for 28, or 42 days to assess clinical and parasitological responses;

Treatment outcomes were classified as early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF) and adequate clinical and parasitological responders (ACPR) using the (WHO 2003) guidelines. Clinical therapeutic outcomes were adjusted by genotyping the *P. falciparum* merozoite surface protein 2 (*msp2*) and glutamate rich protein (*glurp*) on admission (Day 0) and any day of infection recurrences (Day 7, 14, 21, 28, 35 or day 42). Recrudescence was differentiated from new-infections as described by PCR.

3.4. Clinical and laboratory assessment procedures in follow-up duration

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 AS) and and 42 (with ACTs). The day the patient is enrolled and receives the first dose of medicine is traditionally day 0. A follow-up of 28 days is recommended as the minimum duration for medicines with elimination half-lives of less than 7 days (AS). For medicines with longer elimination half - life (piperaquine phosphate), longer follow-up periods are necessary;

Although a follow-up period of 42 days is optimal for most medicines, long periods of follow-up for routine monitoring might not always be feasible for national malaria control programmes. A longer study follow-up increases the risk that more patients will be lost to follow-up, reducing the study's validity and subsequently its sensitivity to reveal the true level of failure. Thus, as a compromise, a 28-day follow-up is recommended as the minimum standard to allow NMCP to capture most failures with most medicines, except piperaquine, for which the minimum follow-up should be 42 days (Stepniewska *et al*., 2004).

3.5. Classification of responses to treatment outcomes, included ACPR, ETF, LPF, LCF

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 early treatment failure; 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 early treatment failure.

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 early treatment failure or late clinical failure.

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 early treatment failure, late clinical failure or late parasitological failure.

3.6. Data analysis and study end-points

- Data generated in patient's case record forms were entered on WHO_GMP_ Ringwald Pascal database software version 7.1. Descriptive analysis was done and differences in proportions of treatment outcome were compared using above software for proportions.

- The Kaplan-Meier curves method is that preferred for statistical analysis of data on drug efficacy. The advantage of survival analysis is that it takes into account data on patients who were lost to follow-up or withdrawn from the study, in particular patients with reinfection;

- A study end-point is the classification assigned to a patient. Valid study endpoints include treatment failure (early treatment failure, late clinical failure, late parasitological failure), completion of follow-up without treatment failure (adequate clinical and parasitological response), loss to follow-up, withdrawal from the study and protocol violation;

- Results should be expressed as the cumulative success rate (or the cumulative failure rate) and the proportion of adequate clinical and parasitological response (or proportion of early treatment failure, late clinical failure or late parasitological failure) before and after adjustment by PCR.

4. RESULTS

4.1. General study profile and patient's records

A total of 764 patients who have uncomplicated falciparum malaria were enrolled at 6 sentinel sites in Gialai (westhighland zone), Ninh Thuan (Central southern part), and Quang Tri (Central northern part).

Table 1. Kinds and regimes of antimalarial drugs intervention

	Antimalarial drugs	Subtotal	LOF /WITH
1	- Artesunate monotherapy	212	11
2	- Artemisinine monotherapy	31	0
3	**Dihydroartemisinin plus piperaquine (DHA+PPQ)** - Artekin - Arterakine - CV-Artecan	 51 196 19	 0 9 4
4	**Artequick** - Artequick tablets - Artequick granule - Artequick-primaquine	 52 107 96	 3 3 4

Of that 243 patients who received artesunate alone and artemisinine monotherapy, 266 patients received ACTs in kind of dihydroartemisinine plus piperaquine (DHA+PPQ) with different trade marks of Artekin, Arterakine, CV-Artecan, 159 patients received Artequick regimes (both in tablets and granules), and 96 patients received Artequick-primaquine combinations. All of patient's mean age, body weights, clinical and parasitological parameters at enrollments. There were 42 patients who were loss to follow up (LOF); withdraw (WITH) of 2 cases. Overall 720/764 (94.24%) patients were available for the assessment of therapeutic endpoints.

4.2. Artesunate alone and artemisinine monotherapy treatment outcomes

Table 2. Efficacy of Artesunate and Artemisinine monotherapy regimes

Efficacy index	Artesunate monotherapy (7 days regimes)			Artemisinine monotherapy
n	43	75	94	31
ETF	0	0	0	0
LCF	0	15.1%	8.5%	0
LPF	0	2.7%	4.3%	0
ACPR *(in vivo analysis)*	100%	**82.2%**	**87.2%**	100%
ACPR *(PCR adjustment)*	√	**90.2%**	**90.4%**	√
LOF (cases)	3	2	6	0
WITH (cases)	0	0	0	0
Years	2007	2009	2010	2007
Sentinel site	Ninh Thuan	Quang Tri	Ninh Thuan	Ninh Thuan

Both drugs were tolerated; there was no report of significant adverse drug reaction (ADR). Table 2 shows the crude ACPRs in 2007 both of artesunate and artemisinine monotherapy were 100%, but two year later in the same sentinel sites, efficacy of artesunate down to 82.2% (in 2009) and 87.2% (in 2010) with the 7 days regime. After PCR adjustment however, the corresponding figures rose to 90.2% in 2009 and 90.4% in 2010, respectively. Most of the recurrent infections at were due to LPF and LCF. Interestingly, after genotyping all these were found to be due to new infection. These showed that artesunate potential declined from 100% to around 90%, or treatment failure rate nearly was 10%.

Table 3. Dihydroartemisinine plus piperaquine (different trade marks) efficacy in treatment for uncomplicated falciparum malaria

Efficacy index	Artekine® (DHA+PPQ)	Arterakine® (DHA+PPQ)	Arterakine® (DHA+PPQ)	CV-Artecan® (DHA+PPQ)	Arterakine® (DHA+PPQ)
n	51	55	65	19	76
ETF	0	0	3.4%	0	0
LCF	0	0	1.7%	0	1.3%
LPF	0	0	0	0	1.3%
ACPR *(in vivo)*	100%	100%	**91.67%**	100%	97.40
ACPR *(PCR corrected)*	√	√	**94.8%**	√	N/A
LOF	0	0	5	4	2
WITH	0	0	2	0	0
Periods	2007-2008	2009-2010	2010-2011	2011	2011
Sentinel sites	Phuoc Chien Ninh Thuan	An Trung Gia Lai	IaKe Gialai	Iato, Grai Gia Lai	Thanh, Quang Trị

Table 3. showed that efficacy of DHA+PPQ in Phuoc Chien, Ninh Thuan (2007-2008), in An Trung, Gia Lai, and Iato, Gia Lai were stable with ACPR of 100%, but during 2010-2011

in Iake sentinel site, seem to be decline in DHA+PPQ efficacy (91.67%). One case of ETF involved in a patient who had total spleenectomy 12 years ago.

Table 4. Comparision DHA+PPQ (2010-2011) efficacy before and after PCR analysis

Pt's code	Parasite density		Classification after *in vivo* analysis	Classification after PCR corrected
	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})	Recrudescence

Yet, after PCR correction the true efficacy rose to 94.8%. In which of 2 cases re-infection at day 42 (GLAK29 and GLAK48), one case of recrudescence at day 26 (GLAK62) in above table 4.

Table 5. Parasite clearance time analysis in DHA+PPQ (2010-2011) efficacy

Patient's code		Malaria parasite clearance process				
	Code	D_0	D_1	D_2	D_3	D_4
Arterakine regime in Iake (2010-2011)						
1	GLAK05	95.049	35.584	2.445	141	8
2	GLAK14	48.875	2.130	165	48	
3	GLAK 15	75.559	16.395	360	6	
4	GLAK23	46.941	29.480	102	537	
5	GLAK27	56.303	6.225	66	12	
6	GLAK44	43.385	17.139	1.875	66	
7	GLAK46	29.919	17.213	1.695	162	
8	GLAK60	16.493	1.155	54	24	
9	GLAK61	31.014	11.333	756	267	
10	GLAK62	40.320	6.120	201	150	
11	GLAK65	49.673	12.300	483	36	
Asexual parasite/μL		***44.121***	***9.633***	***358***	***66***	***8***

Analysis of parasite clearance time (PCT) of 65 cases indicated DHA+PPQ during 2010-2011 at Iake sentinel site, Gia Lai, there was 11/65 (≈ 17%) cases with positive malaria parasite on day 3 (D_3), as high risk for resistance followed by drug pressure. Moreover, positive parasite on or after D_3 as indirect clinical marker for resistance (WHO definition, 2010).

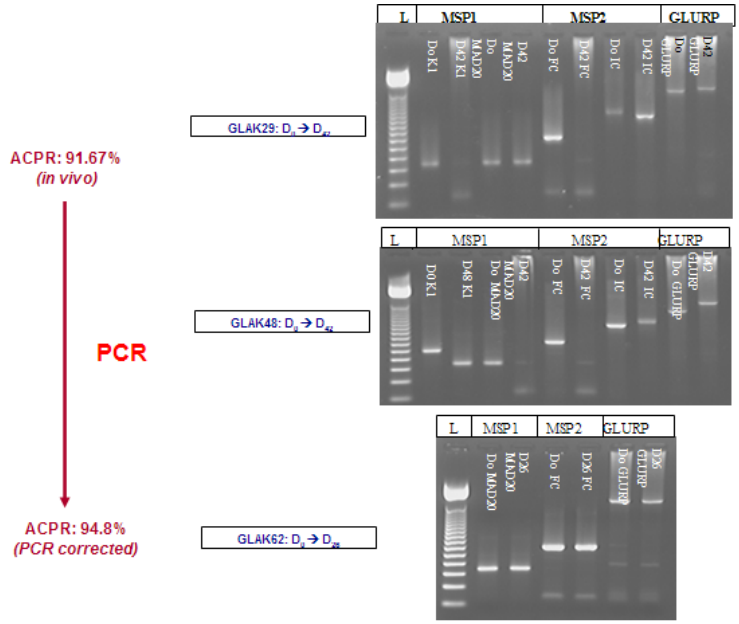

Figure 1. Differentiation of the reinfection from recrudescence cases by PCR technique

Table 6. Efficacy of artemisinine based combination (Artequick & Artequick-primaquine)

Efficacy index	Artequick (tablets)	Artequick (Granules)	Artequick-Primaquine	Artequick-Primaquine
n	52	107	36	60
ETF	0	0	0	0
LCF	0	0	0	0
LPF	0	1.9%	0	0
ACPR	100%	98.1%	100%	100%
LOF	3	3	2	2
WITH	0	0	0	0
Periods	2007-2008	2007-2008	2010-2011	2011
Sentinel sites	Phuoc Chien Ninh Thuan	Phuoc Chien Ninh Thuan	Ia' Ke Gia Lai	Ia' Ke Gia Lai

The table 6. showed that efficacy in both artequick and modified artequick (artequick-primaquine) in both sentinel sites in Ninh Thuan and Gia Lai were very high, nearly absoluted. Only one case (1.9%) in Phuoc Chien sentinel site of LPF, but after corrected by PCR in China with new infection.

5. DISCUSSION

The main goal of this *in vivo* ACT efficacy study was to support the establishment of evidence-based results that can be used to change malaria treatment policy in Vietnam. This study has demonstrated high therapeutic efficacy and tolerability for artesunate, artemisinine monotherapy in 7 days, and some artemisinine based combinations (ACTs) regimes in some Central -Westhighland sentinels of Vietnam. Both crude and PCR-corrected ACPRs for artesunate alone efficacy

of artesunate down to 82.2% → 90.2% (in 2009) and 87.2% → 90.4% (in 2010). Most of the recurrent infections at were due to LPF and LCF. Importantly, these results showed that artesunate potential declined from 100% to around 90%, or treatment failure rate nearly was 10%. Hence, artesunate or artemisinine monotherapy need to be stop using in treatment for uncomplicated falciparum malaria, this was both suitable for WHO recommendations and delay on resistance development.

Table 3. showed that efficacy of DHA+PPQ at Phuoc Chien, Ninh Thuan (2007-2008), An Trung, Gia Lai, and Iato, Gia Lai (2010-2011) were stable efficacy with ACPRs of 100%, but during 2010-2011 at Iake sentinel site, once problematic phenomenon occurred - seem to be decline in DHA+PPQ efficacy (91.67%), yet after PCR correction the true efficacy rose to 94.8%, accompanied by there was 11/65 (≈ 17%) cases with positive malaria parasite on day 3 (D_3) or prolonged parasite clearance time as high risk for resistance development followed by drug pressure in this zone. According to WHO/Global Malaria Programme and World wide antimalaria drug resistance Network (WWARN) definition with positive parasite on D_3 as indirect clinical marker for resistance (WHO., 2010). Moreover, recently WHO is using working definition as below: (i) an increase in parasite clearance time, as evidenced by greater than 10% of cases with parasite detectable on day 3 following treatment with an ACTs (suspected resistance); or (ii) a treatment failure as evidenced by presence of parasites at days 3 and either parsistence of parasites on day 7 or recrudescence after day 7 of parasites within 28/42 days, after treatment with an oral artemisinin-based monotherapy, with adequate blood concentration (confirmed resistance). In this analysis with proportion of positive parasite on $D_3 \approx 17\% > 10\%$, due to lack of pharmacokinetics and the blood drug /or metabolic concentration data, we can concluded that suspected resistance!

Previous studies by Teuscher et al. have shown that following treatment with AS, *P. falciparum* ring-stage parasites become dormant and approximately 0.001–1.313% recover to resume growth depending on the strain. In those studies, parasite recovery from dormancy was found to be dose - dependent. To determine if this same phenomenon occurs *in vivo*, dormant parasite recovery (time to reach > 5.0% parasitemia) was assessed using *P. v. vinckei*. Results showed that dormant forms, similar to those found *in vitro*, are present in treated mice and that the day of parasite recovery is positively related to the number of dormant parasites present in intact mice; however, this was more variable in asplenic mice. Based on our experimental design we cannot definitively calculate recovery rates. However, we can assume that because groups injected with 40 parasites did not recover, that the recovery rates are in the order of 1 in 400 dormant parasites (0.25%) which are comparable to those found in previous studies.

In which of 2 cases re-infection at day 42 (GLAK29 and GLAK48), one case of recrudescence at day 26 (GLAK62), one case of ETF involved in a special patient who had total spleenectomy 12 years ago → delayed parasite clearance progress. In acute malaria, red blood cells that have been parasitized, but no longer contain a malaria parasite, are found in the circulation. These are thought to arise by splenic removal of dead or damaged intraerythrocytic parasites and return of the intact RBCs to the circulation. In a study of

5 patients with acute falciparum malaria who had previously undergone splenectomy, it was found that none of these 5 patients had any circulating RESA-RBCs, in contrast to the uniform finding of RESA-RBCs in all patients with acute malaria and intact spleens. Parasite clearance after artesunate treatment was markedly prolonged, although the parasites appeared to be dead and could not be cultured *ex vivo*. These observations confirm the central role of the spleen in the clearance of parasitized RBCs after antimalarial treatment with an artemisinin derivative. Current criteria for high-grade antimalarial drug resistance that are based on changes in parasitemia are not appropriate for asplenic patients (*Kesinee Chotivanich et al., 2002*). Determination of resistance or not, next studies must be using *in vivo, in vitro*, molecular aspects, and pharmacokinetic methods specifically designed to address the question of potential artemisinin resistance.

The efficacy in both Artequick and nouvel Artequick (artequick-primaquine) in both sentinel sites in Ninh Thuan and Gia Lai were very high, nearly absoluted 100%. Only 1 case (1.9%) in Phuoc Chien of LPF, but after corrected by PCR in China with new infection, therefor ACPRs of them was 100%.

The testing of each drug independently for each site was due to the arrangements in place that required testing each individual drug as part of antimalarial nationwide drug testing allocation. This allows accumulation of evidence of the performance of the ACT in different settings. It can be argued that there are differences from place to place related to the response to treatment that is observed but treatment policies are formulated at regional or sub-regional level hence the need to get regional summary estimates. The sentinel system is a good approach toward addressing that need. Secondly it will be very difficult to sample all possible places where variation is being expected and under the current malaria transmission intensity in Vietnam, it is hard to follow up enough patients for all treatment groups in a single site during the same transmission year.

Both ACTs drugs (DHA+PPQ and Artequick) have high efficacy profiles in the region that in principal would require huge sample size to be able to show a comparative difference in next plans or studies.

6. CONCLUSIONS

According to all data here, both artesunate and artemisinine monotherapy efficacy were high, with treatment failure nearly 10% with other data from multicenters study as caution level for change policy. DHA+PPQ currently used as first line treatment for uncomplicated falciparum malaria and for stand-by treatment due to quick action and reduce severe malaria, year by year, DHA+PPQ efficacy seem to be declined, more detail in prolonged PCT and proportion of positive parasite on day 3 → easy to develop resistance, and its useful therapeutic life might be compromised sooner following widespread use. Efficacy monitoring and surveillance hence, are very important for policy revision guidelines.

Both Artequick and Artequick-primaquine were high in efficacy, short regime (2-3 days) , we can considered an effective combination therapy and deployed in national malaria diagnosis and treatment guidelines in the next time in line with DHA+PPQ in Vietnam. All these experience have paved the way for adoption of DHA+PPQ and Artequick as well, play a role as the first line antimalarial drug in Vietnam now and then.

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