Determination of the Falciparum Malaria Resistance to Artemisinin-based Combination Therapies in Pesawaran, Lampung, Indonesia

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Research Article

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INTRODUCTION

Malaria remains one of the parasitic diseases that life-threatening of human in most part of the world mainly in tropical region. In 2015 malaria caused 438,000 death (range: 236,000-635,000) worldwide with the most cases were in African region (90%), followed by the South-East Asia region (7%) and the Eastern Mediterranean region (2%)[1].

Indonesia is one among the Southeast Asian countries with high incidence of malaria. Based on the data released by Ministry of Health of the Republic of Indonesia[2], during the period of 2014 there were 252,027 confirmed malaria cases in the country. Among the provinces in Indonesia, Lampung is one that categorized as a malaria endemic area with the Annual Parasite Incidence (API) of 0.55 per 1000 people. Hay *et al.*[3] stated that with the API of less than 1 per 1000 people, Lampung province can be categorized as low endemicity of malaria.

Although, in a provincial scale, Lampung categorized as low malaria endemicity but in the district scale some of the districts in this province can be categorized as a moderate or high endemicity. The district of Pesawaran for instance, with an API of 7.26 should be categorized as moderate endemicity. According to the Lampung Provincial Health Office report[4], some of the sub districts in Pesawaran, in fact, showed an API upto 51.58 namely the sub-district of Padang Cermin.

There are factors that have long been identified contributing to the high incidence of malaria in Indonesia, including physical and socioeconomic environmental factors. The physical factors included rainfall, mosquito breeding and resting sites. Social and economic factors included income, education, use of bednets and pattern of outdoor activities, especially at night and the use of repellents, mosquito coils and sleeping arrangements[5]. Later, it was realized that one of the biggest challenges in the malaria eradication program is the emergence and spread of drug resistance in malaria parasites[6].

In relation to drug resistance, Indonesia is the country with a long history of malaria drug resistance, mainly *Plasmodium falciparum* and *Plasmodium vivax* to chloroquine, which in turn becoming the most important barrier to malaria control in the country. In the province of Lampung, during 2000-2004, the chloroquine resistance was high indicated by a low adequate clinical and parasitological response (ACPR)[7] which was only 33%. In the neighboring province of Lampung, the province of South Sumatra, the failure of treatment with the standard dose of chloroquine allegedly related to polymorphism in the *pfcrt* and *pfmdr1* genes in *P. falciparum* isolates[8].

To minimize the burden caused by *P. falciparum* resistance to conventional anti-malarial medications, since the year 2004 Indonesia adopted artemisinin-based combination therapies (ACTs) into the national malaria control program. Three types of ACTs have been used to treat uncomplicated malaria namely dihydroartemisinin-piperaquine, artemether-lumefantrine and artesunate-amodiaquine[9].

In the future, the use of artemisinin seems to face similar challenges with the application of chloroquine, i.e., the resistance. The declining efficacy of the artemisinin family drugs i.e., artesunate/mefloquine combination and the clinical resistance to artesunate, manifested as delayed clearance of parasitaemia after therapy was noted in the Cambodia-Thailand border region of Southeast Asia[10]. In the province of Lampung, exactly in the district of Pesawaran, the indication of resistance to artemisinin was evidenced by failure of efficacy of ACT treatment, which reach 11.59%, in patients with falciparum malaria[11].

This study was conducted to ascertain whether there has been a resistance of *P. falciparum*, known as the most deadly malaria parasite[12], to the ACTs treatment, especially dihydroartemisinin-piperaquine (DHP) in the area of highest malaria endemicity in the province of Lampung, Indonesia. A total of 52 falciparum malaria patients of both sexes and all age groups have examined for the parasitaemia count and the clinical symptom before and after the treatment.

MATERIALS AND METHODS

Area and time of the study: The study was conducted in the district of Pesawaran (5E5'42"S, 105E10'47"E) one of the district known as a highest malaria-endemic areas in Lampung Province, Indonesia. This study included 13 villages in the sub-district of Padang Cermnin and lasted 6 months from February to July, 2016.

Study participants: It is a cross sectional study targeting malaria patients in which study samples was set using both inclusive and exclusive criteria. The inclusive criteria are new falciparum malaria patient with the fever symptoms (>38EC) for more than 2 days with or without chills, aged more than 1 year and the patient or parent/guardian willing to provide informed consent. The malaria patients excluded if he/she has previously received malaria treatment for at least 1-2 weeks and the patient's home is difficult to be accessed. With reference to those criteria, 52 malaria patients were eligible to be the study samples.

Prior to the clinical examination and blood sampling, the patients or their guardian notified in advance whether they are willing to volunteer in this study or not. Informed consent that must be understood and signed by patients or their guardians contains a dialogue regarding the study's purpose, duration, experimental procedures, alternatives, risks and benefits. In addition, to the patients also be informed that the participation is voluntary and any kind of refusal will not result in any consequences and all the data regarding the patient private information will kept secret. The informed concent used in the study was approved by Ethics Committee for Medical and Health Research the Faculty of Medicine, University of Lampung. The ethics committee are appointed by the dean Decree No. 155/UN26/8/KP/2014.

Blood samples: Blood sample was collected from the patient's third finger of the left hand, using finger prick technique with disposable needle/lancet after the fingers peak was wiped with 70% alcohol. The blood sample was then dropped on 2 separate object glasses, on which the samples identity codes had been written, to make thick and thin film. A thick film, used for quantifying parasite density, was made by drying the blood smears for 30 min without using any fixative. While, the thin film, used for parasite identification, was prepared by drying blood smear for 10 min. After drying, the thin blood smear was fixed by dipping it into methanol for 5 sec. To make blood films ready for microscopic examination, both thin and thick smears were stained with 5% Giemsa's stain for 30 min.

Study parameters: To identify the parasites and assess *Plasmodium* density per 1 μL of blood, microscopic examination was carried out using light microscope with 1000x magnification. Parasitaemia count was assessed by counting the number of asexual parasites against 200-300 the number of white blood cells (WBCs). The parasite density was calculated by dividing the number of asexual parasites by the number of WBCs counted and then multiplying by 8000 (the assumed WBC snormal density) and expressed as the density per micro liter. Along with the parasitaemia examination, clinical symptoms namely fever, chills, headache, nausea and vomiting, myalgia, diarrhea, impaired consciousness and seizures were also observed. All parameters above noted before (day-0) and after (day-1, 2, 3, 7, 14 and 28) DHP treatment.

Statistical analysis: Both descriptive and analytical statistics were used. Demographic data of the falciparum malaria patients and the clinical symptoms as well as the parasite counts in their blood samples are presented descriptively. While, ANOVA and linear regression were used for describing the relationship strenght between parameters.

RESULTS

Patient demographics: There were 52 patients who met the inclusion criteria in this study. They consisted of 24 women and 28 men in the age range 3-65 years distributed in 13 villages. Details of the distribution of residence, gender and age range of study participants presented in Table 1.

Clinical symptoms and parasite counts: Clinical symptoms of falciparum malaria patients and the asexual parasitaemia and gametocyte counts before artemisinin treatment are presented in Table 2. Fever is the only symptom experienced by all (100%) patients, followed by headaches (98.08%), chills (94.23%), myalgia (84.62%), nausea and vomiting (78.85%), diarrhea (25%), impaired consiousness (7.69%) and seizures (3.85%). Asexual parasitaemia counts ranged 196-210880 μLG^{1} impaired consiousness experienced by patients with a parasitaemia count of more than 40,000 μLG^{1}, while seizures experienced only by the patients with parasitaemia count of more than 100,000 μLG^{1}.

To find out if there is a relationship between the patient's age and severity of malaria they suffered, as well as between the parameters measured, linear regression analysis and analysis of variance was performed which results are presented in Table 3. The results showed there is no significant correlation between patient age and clinical symptom as well as parasite counts (all p#0.05). Also, there is no correlation between the total of symptomatic types and gametocyte counts. However, there is a strong correlation between asexual parasitaemia counts and the total of symptomatic types with an coefficient of correlation R = 0.5347 and the p<0.001.

Table 1: Falciparum malaria patients participated in the study

Name of villages	N	Male	Female	Youngest	Oldest
Lempasing	16	9	7	3	65
PPI Lempasing	9	4	5	5	41
Arnas	7	5	2	18	36
Hanura	5	1	4	35	60
Gebang	4	2	2	13	60
Mutun LPS	3	1	2	13	30
Kampung sawah	2	2	0	16	40
Sukabumi LPS	2	1	1	11	32
Ringgung	1	0	1	13	
Sidodadi	1	1	0		47
Sukamulya LPS	1	1	0	7	
Tirtayasa	1	1	0		24
Total	52	28	24		

Table 2: Description of clinical symptoms and parasite counts of falciparum malaria patients examined before DHP treatment

Patients			Clinical symptoms**									Parasite counts	
No	Sex*	Age	Fever	Chills	Headache	NI	Myalgia	Diarrhea	IC	Seizures	E	Parasitemia	Gametocyte
1	2	24	1	1	1	1	1	1	0	0	6	192	0
2	1	36	1	1	1	0	1	0	0	0	4	367	0
3	2	45	1	1	1	1	1	0	0	0	5	400	0
4	2	13	1	1	1	1	0	0	0	0	4	618	0
5	1	47	1	1	1	0	1	1	0	0	5	692	0
6	1	17	1	1	1	1	1	0	0	0	5	1000	0
7	1	60	1	1	1	0	1	0	0	0	4	1080	400
8	1	60	1	1	1	0	0	0	0	0	3	1120	0
9	2	26	1	1	1	1	1	1	0	0	6	1120	720
10	1	40	1	1	1	1	0	1	0	0	5	1160	0
11	1	19	1	1	1	0	1	0	0	0	4	1282	0
12	2	30	1	1	1	1	1	0	0	0	5	1400	0
13	1	35	1	1	1	1	1	0	0	0	5	1739	39
14	2	31	1	1	1	1	1	1	0	0	6	1961	0
15	1	37	1	1	1	0	1	1	0	0	5	1990	0
16	1	3	1	0	0	0	0	0	0	0	1	2167	0
17	2	11	1	1	1	1	1	0	0	0	5	2275	0
18	1	17	1	0	1	0	1	0	0	0	3	3280	0
19	2	15	1	1	1	1	1	0	0	0	5	3960	0
20	2	65	1	1	1	1	1	0	0	0	5	4134	0
21	2	13	1	1	1	1	0	0	0	0	4	5360	0
22	1	7	1	1	1	1	0	0	0	0	4	5560	2960
23	1	32	1	1	1	1	1	0	0	0	5	5800	0
24	1	32	1	1	1	1	1	1	0	0	6	7801	0
25	2	13	1	1	1	1	1	0	0	0	5	8314	0
26	2	8	1	1	1	1	0	0	0	0	4	9490	118
27	2	18	1	1	1	1	1	0	0	0	5	11760	0
28	2	13	1	1	1	1	1	0	0	0	5	12600	0
29	2	30	1	1	1	1	1	0	0	0	5	14595	0
30	2	22	1	1	1	1	1	0	0	0	5	14800	0
31	1	16	1	1	1	1	1	1	0	0	6	15098	0
32	1	16	1	1	1	0	1	1	0	0	5	15490	40
33	1	15	1	1	1	1	1	0	0	0	5	20079	0
34	1	41	1	0	1	0	1	0	0	0	3	20720	0
35	1	35	1	1	1	1	1	1	0	0	6	21647	0
36	1	20	1	1	1	1	1	0	0	0	5	21804	0
37	2	41	1	1	1	1	1	0	0	0	5	23323	0
38	1	11	1	1	1	1	1	0	0	0	5	27360	280
39	2	15	1	1	1	1	1	0	0	0	5	29280	0
40	1	43	1	1	1	1	1	0	0	0	5	30600	0
41	1	32	1	1	1	1	1	0	0	0	5	40800	0
42	1	24	1	1	1	1	1	0	0	0	5	42440	0
43	1	20	1	1	1	1	1	0	0	0	5	44296	0
44	1	35	1	1	1	0	1	0	0	0	4	44480	0
45	1	15	1	1	1	1	1	1	1	0	7	49280	0
46	2	38	1	1	1	1	1	0	0	0	5	50240	0
47	2	33	1	1	1	1	0	1	0	0	5	54080	0
48	2	22	1	1	1	1	1	0	0	0	5	60000	0
49	1	40	1	1	1	1	1	0	0	0	5	61680	0
50	2	13	1	1	1	1	1	0	1	0	6	85600	0
51	2	35	1	1	1	1	1	0	1	1	7	114800	0
52	2	5	1	1	1	1	1	1	1	1	8	210880	0

*1: Male, 2: Female, **0: Absent, 1: Observed, NV: Nausea and vomiting, IC: Impaired consiousness

Therapeutic responses: Parasitaemia and gametocyte counts of falciparum malaria patients before and after artemisinin treatment are presented in Table 4. Among a total of 52 study participants, only one patient (1.92%) showed signs of ACTs therapy failure. It was evident from the increased number of asexual parasites on day 21 and 28. While, more than 98% of them still show the effectiveness of DHP treatment, characterized by loss of parasites since the 2nd day.

Table3: Results of regression analysis and ANOVA between research parameters

	Statistical parameters		
Correlationship	R	F	p-value
Patient age vs Esymptom	0.0508	0.1293	0.7206
Patient age vs parasitaemia	0.1658	1.4129	0.2402
Patient age vs gametocyte	0.1659	1.4158	0.2397
ESymptoms vs parasitaemia	0.5347	20.0232	0.0000
ESymptoms vs gametocyte	0.1031	0.5370	0.4671
Parasitaemia vs gametocyte	0.1019	0.5245	0.4723

R: Coefficient of correlation, F: F-value of ANOVA

DISCUSSION

Based on Table 1, it is known that the distribution of malaria patients were not evenly distributed among the villages. Lempasing is the village with the highest number of malaria patients (16 patients), followed by its neighboring village, PPI Lempasing (9 patients). It is difficult to ascertain the causes of these differences, but the most likely is the socio-economic factors of the society. According to the

Table 4: Parasitaemia and gametocyte of falciparum malaria patients before (D0 = day-0) and after (D1-28 = day 1-28) DHP treatment

	Asexual parasitaemia counts								Gametocyte counts							
Patients	D0	D1	D2	D3	D7	D14	D21	D28	D0	D1	D2	D3	D7	D14	D21	D28
1	192	32	0	0	0	0	0	0	0	16	0	0	0	0	0	0
2	367	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	400	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	618	80	0	0	0	0	1585	5675	0	0	0	0	0	0	0	79
5	692	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	1000	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	1080	0	0	0	0	0	0	0	400	0	0	0	0	0	0	0
8	1120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	1120	0	0	0	0	0	0	0	720	0	0	0	0	0	0	0
10	1160	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	1282	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	1400	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	1739	0	0	0	0	0	0	0	39	160	0	0	0	0	0	0
14	1961	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	1990	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	2167	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	2275	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	3280	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	3960	119	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	4134	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	5360	160	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	5560	0	0	0	0	0	0	0	2960	3264	0	0	0	0	0	0
23	5800	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	7801	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	8314	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	9490	440	0	0	0	0	0	0	118	120	0	0	0	0	0	0
27	11760	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	12600	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	14595	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	14800	640	0	0	0	0	0	0	0	40	0	0	0	0	0	0
31	15098	199	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	15490	80	0	0	0	0	0	0	40	0	0	0	0	0	0	0
33	20079	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	20720	112	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	21647	4880	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	21804	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	23323	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	27360	0	0	0	0	0	0	0	280	16	0	0	0	0	0	0
39	29280	520	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	30600	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	40800	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	42440	64	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	44296	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	44480	0	0	0	0	0	0	0	0	400	0	0	0	0	0	0
45	49280	48	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	50240	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4: Continue

	Asexual parasitaemia counts								Gametocyte counts							
Patients	D0	D1	D2	D3	D7	D14	D21	D28	D0	D1	D2	D3	D7	D14	D21	D28
47	54080	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	60000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	61680	160	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	85600	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0
51	114800	637	0	0	0	0	0	0	0	0	0	0	0	0	0	0
52	210880	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0

village headman concerned, most of the villagers (>72%) livelihood of farmers, ranchers and fishermen who require them spending more time outside the home. As suggested by Monroe *et al.*[13], outdoor sleeping and other night-time social, cultural and economic activities contributed significantly to malaria prevalence due to increase exposure to infective mosquito bites.

Table 2 shows that there were 8 clinical symptoms of malaria observed in this study but only one patient that experienced all the symptoms, i.e., the patient with the highest parasitemia counts. There was also a positive correlation between parasitemia counts and the type number of clinical symptoms (Table 3), that the higher the parasitemia density, the higher the type number of symptoms experienced by the patients. This result seems to be reasonable according to the previous findings, that fever, rigors, headache, myalgia, impaired consciousness, jaundice or malarial hepatitis, thrombocytopenia, acute renal failure and mortality were all strongly associated with the density of *Plasmodium falciparum*, while the parasite density was not related to age and gender[14,15].

Regarding the gametocyte counts shown in Table 4, that except for the patient number 4, all samples with gametocytes on day 0 has totally lost their parasites on day 3 indicated that DHP was effective to inhibit the development of gametocytes. This finding seem to be contradictory to the some previous reports which indicate that most of antimalarial drugs tend to increase the viability of late-stage gametocytes[16]. But then, it is known that some chemical compounds are now widely used in the treatment of malaria, such as hydroxychloroquine, artesunate, pyronaridine can inhibit the development of falciparum gametocyte[17]. Later, Sutanto *et al.*[18] reported that DHP treatment effectively reduce development of gametocytes in falciparum malaria patients without gametocytes on day 3.

Table 4 shows that the artemisinin treatment failed on one of the 52 patients (1.92%), so this study failed to confirm the findings of Suwandi *et al.*[11] that in Pesawaran the failure of ACTs treatment was 11.59%. Yet still the question arises whether this is the evidence of resistance to artemisinin? Referring to the guideline of decision-making process regarding the efficacy of artemisinin treatment, issued by WHO[19], the results of Therapeutic Efficacy Studies (TES) should be interpreted as follows. If the patients parasitemic on day 3 less than 10% and treatment failures less than 10% on day 28 and 48, it means no evidence of resistance to artemisinin and no change in treatment policy required.

Still based on data shown in Table 4, in more than 98% of patients artemisinin is proven to reduce the number of asexual parasitaemia to less than 20% a day after the treatment and completely eliminate the parasite after 2 days of treatment. These results indicate that the falciparum malaria parasite gives a good response to artemisinin. Based on the WHO standard field test, an antimalarial drug can be classified as good response when the drug able to decline the parasite density to less than 25% of the pre-treatment level by day 2 and no parasites are seen by Rieckmann[20] on day 7.

CONCLUSION

Severity of falciparum malaria determined by the density of asexual parasitemia, not on the gametocyte counts. Given the treatment failure in this study is very low it can be concluded that falciparum malaria resistance to artemisinin treatment has not been proven in the disrict of Pesawaran and ACT, particularly dihydroartemisinin-piperaquin, remain effective as an anti falciparum malaria drug.

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