EVALUATION OF THERAPEUTIC EFFICACY AND SAFETY OF DIHYDROARTEMISININ-PIPERAQUINE IN UNCOMPLICATED *PLASMODIUM FALCIPARUM* MONOINFECTION IN TIMOR TENGAH SELATAN DISTRICT, NUSA TENGGARA TIMUR, INDONESIA

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Abstract. Malaria is still a health problem in Nusa Tenggara Timur (NTT), especially in Timor Tengah Selatan (TTS) District, Indonesia. Since 2004, the Ministry of Health of Indonesia has recommended ACTs including dyhidroartemisinin-piperaquine (DHP) as an antimalarial in Indonesia and used in NTT since 2011. According to WHO (2010), the policy to replace antimalarial agents in the region is when the failure rate of therapy is more than 10%; however, information on the therapeutic evaluation of DHP is still very sparse. This quasi experimental study aimed to assess the efficacy of DHP as a therapy for uncomplicated falciparum malaria patients at Boking Community Health Center and Noeumuke Community Health Center, TTS District, NTT Province. A total 63 patients were treated with DHP, and clinical and parasitological observations were conducted for 42 days. Up to day 42, 61 patients were in the ACPR classification (adequate clinical and parasitological response), while the remaining two patients experienced failure early in the treatment and were included in the ETF classification (early treatment failure). There was no clinical or parasitological failure of treatment from day 4 until the last day of observation with a successful cumulative incidence of 0.968 with 95% CI of 0.892-0.99. Based on this study, DHP is still recommended as an antimalarial therapy for uncomplicated falciparum malaria in the area of Timor Tengah Selatan District, Nusa Tenggara Timur, Indonesia.

Keywords: clinical and parasitological response, efficacy, dihydroartemisinin, piperaquine, Indonesia

INTRODUCTION

Malaria is still a world health problem including in Indonesia (WHO, 2015). According to WHO Malaria Report 2010, Indonesia is one of the 10 countries in Southeast Asia classified as a high endemic area (WHO, 2010a). In 2013, Nusa Tenggara Timur (NTT) was included in the five provinces with the highest incidence and prevalence following Papua, West Papua, Central Sulawesi and Maluku (Research and Development Department, Ministry of Health Indonesia, 2013). The district of

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Timor Tengah Selatan (TTS) is included in the 6 districts in NTT Province that has the highest number of malaria-positive cases compared to the 16 other districts in NTT Province. In 2014 the number of malaria positive cases in TTS district was 3,313 cases with the dominant species being *Plasmodium falciparum* (Health Ministry of NTT Province, 2014).

The purpose of this study was to evaluate the therapeutic efficacy and safety of dihydroartemisinin +piperaquine therapy in an uncomplicated falciparum malaria in TTS district of NTT Province.

MATERIALS AND METHODS

This study used an experimental quasi design with the number of patients enrolled in accordance to the protocol of WHO antimalarial efficacy test, which requires at least 50 subjects with an expected failure rate of 5%, 95% confidence level and 5% precision level (WHO, 2009). This research was conducted from 1 February 2016 until 31 December 2016 at Boking Community Health Center, Boking Village, Boking Sub-district, and Noemuke Community Health Center in Noemuke Village, Noebeba District, TTS District, NTT Province.

The inclusion criteria in this study include: patients ≥ 6 months of age, *P.falciparum* infection (monoinfection) with asexual parasite density 1,000-100,000 μl, axillary temperature ≥ 37.5ºC or history of fever in the last 24 hours, willing to participate (written informed consent for adults or legal guardians if below legal age of consent) and willing to follow the established procedures during this study. Exclusion criteria include: taking antimalarial drugs in the previous month; pregnant and lactating; sign of severe malaria in children under 5 years of age; presence of severe malnutrition; other *Plasmodium* spp found besides *P. falciparum* in peripheral blood; fever not caused by malaria (*eg*, measles, acute lower respiratory infections, severe diarrhea (with dehydration) or other severe and chronic diseases (*eg*, HIV/AIDS, hepatic, kidney, severe heart dysfunction); severe symptoms and signs of malaria and other symptoms associated with falciparum malaria (coma, respiratory distress syndrome or severe anemia) requiring hospitalization; taking drugs which may interfere with the pharmacokinetics of antimalarial drugs; has a history of allergies to the drug to be given and allergic reactions to medication given during the study; unable or unwilling to use contraception during the study (for women of child-bearing age); and with suspected G6PD deficiency and a history of hemoglobinuria. Patients who met the inclusion criteria were treated with DHP with a dose of 2-4 mg/kg body weight dihydroartemisin and 16-32 mg/kg body weight piperaquine subsequently followed by physical and parasitological examination on days 0, 1, 2, 3, 7, 14, 21, 28, 35 and 42.

Clinical responses were assessed on the basis of the clinical state, the length of the time required for fever clearance, the symptoms that emerged after taking the drug and the presence or absence of severe malaria events during follow-up. The parasitological response was assessed based on the number of Plasmodium parasites, the presence or absence of gametocytes and the length of time required until the absence of Plasmodium asexual forms in the patient's blood [parasite clearance time (PCT)]. Parasite densities were examined on days 0, 1, 2, 3, 7, 14, 21, 28, 35 and 42. All of the peripheral blood preparations were examined by trained personnel, and confirmed at the

TTS District Health Laboratory by certified cross checkers. During the treatment period, patients were monitored for medication adherence, drug side effects, and complications of malaria. After taking the medicine, the patients were observed within 30 minutes. If the patient vomitted during the observation, the drug was given again with the same dose. If during monitoring drug side effects occurred then the patient was given medication to relieve symptoms. If the patient showed signs of severe malaria or malaria complications, the patient was excluded from the study and treated at the TTS District Hospital. Patients who did not follow the schedule/follow-up was categorized as loss to follow-up and were not included in the analysis.

Clinical and parasitological responses were classified as Early Treatment Failure, Late Clinical Failure, Late Parasitological Failure and Adequate Clinical and Parasitological Response, and subsequently analyzed by the Kaplan-Meier method to evaluate cure and failure rates of therapy.

This study has received ethical approval from the ethics committee of the Faculty of Medicine, University of Nusa Cendana, (Register number UN16020001).

RESULTS

From February to December 2016, there were 71 patients who were positive for falciparum malara, with 63 (89%) fulfilling all inclusion criteria. All patients were successfully followed-up with no drop-outs.

Before treatment day (D) 0, all patients had body temperature ≥ 37.5ºC (Table 1) which decline from the first day

Table 1
Patients' baseline characteristics at the start of study.

Characteristics	No. (%)
Community Health Center	
Boking	54 (86)
Noemuke	9 (14)
Gender	
Male	28 (44)
Female	35 (56)
Age group in years	
< 5	1 (2)
5-15	12 (19)
>15	50 (79)
Weight	
Mean ± SD, kg	36.21 ± 10.8
Hemoglobin range	
Mean SD, g/dl	10.92 ± 1.1
Body temperature at day 0	
Median (range), °C	38.5 (38.0-40.0)
Parasite density at day 0	
Median (range), /µl	4,640 (1,040-160,000)

Table 2
Clinical responses of 63 patients on DHP treatment.

Clinical response	No. (%)
Fever[a]	
Day 1	53 (84)
Day 2	22 (35)
Day 3	2 (3)
Day 7 - Day 42	0
Fever clearance time[a]	
Median (Range), day	3 (1-7)
Symptoms after medication	
Nausea	26 (41)
Vomitting	8 (13)
Dizziness	12 (19)
Headache	5 (8)
Allergy reaction	0 (0)
No symptoms	43 (68)
Severe malaria event	0

DHP, dihydroartemisinin + piperaquine.
[a] Fever was axillary temperature > 37.5 °C.

of treatment (D1) (Table 2). At D1 after taking DHP, 84% of the subjects still had fever and only 16% were not feverish. On D2, 35% of the subjects still had fever that decreased to 3% by D2, and from D7 to D42 no subjects has fever.

After treatment 68% of the patients experienced no symptoms while 32% had nausea, vomiting, dizziness, and headache. There were no allergic reactions or severe malaria.

The mean parasitic density on D0 was 4,640 parasites/μl of blood (range 1,040-160,000 parasites/μl of blood) which started to decrease on D1 to an average of 600 parasites/μl of blood (range 0-16,000 parasites/μl of blood); however on D1 98% of the subjects still had parasites in their blood smear (Table 3). On D2, parasitic density decreased to an average of 80 parasites/μl of blood, although 71% of the subjects still experienced parasitemia. On D3, parasitic density declined to 0-1,600 parasites/μl of blood, although 13% of subjects still had parasites in their blood. During D7-D42 no parasites were found in the blood smears of all subjects. Median PCT was 3 days (range 1-7 days).

Up to D42, there were 61 (97%) patients included in the cured category either clinically or parasitological, thus classified in the adequate clinical and parasitological response (ACPR) category, while the remaining 2 (3%) patients were in early treatment failure both clinical and parasitological, thus classified in the early treatment failure (ETF) category (Table 4). Using Kaplan-Meier analysis, cumulative success incidence was 0.968 (95% CI: 0.892-0.993).

DISCUSSION

According to WHO (2009) antimalarial efficacy effectiveness protocol, subjects recruited in this efficacy test should be children 6 to 59 months of age, as this age group has low immunity, so it is less likely to affect treatment outcomes. However in areas of low to moderate transmission, where it is difficult to obtain samples or to include children under 5 years of age, children over 5 years old or adults may be included in the drug efficacy trials even though there may be an underestimation of drug resistance status tested as adults tend to have better response to treatment than children (WHO, 2009). According to Harijanto (2011), malaria sufferers are mainly in the adult age group. This is allegedly caused by more activity outside the home by adults than children, so the possibility of being bitten by infected female Anopheline mosquitoes.

The length of follow-up period

Table 3
Parasitological response to DHP treatment.

Parasitological response	
Parasite density, median (range), /μl	
Day 0	4,640 (1,040 – 160,000)
Day 1	600 (0 – 16,000)
Day 2	80 (0 – 6,400)
Day 3	0 (0 – 1,600)
Days 7, 14, 21, 28, 35 and 42	0
Parasitemia, *n*/*N* (%)	
Day 0	63/63 (100)
Day 1	62/63 (98)
Day 2	45/63 (71)
Day 3	8/63 (13)
Days 7, 14, 21, 28, 35 and 42	0/63 (0)
Gametocytemia, *n*/*N* (%)	
Day 0	3/63 (5)
Day 1	3/63 (5)
Day 2	3/63 (5)
Day 3	0
Days 7, 14, 21, 28, 35 and 42	0
Parasite clearance time median (range), day	3 (1-7)

DHP, dihydroartemisinin + piperaquine.

Table 4
Treatment response classification.

Treatment response	No. (%)
1. Cured (ACPR)	61 (97)
2. Failure	
a. ETF	2 (3)
b. LCF	0
c. LPF	0

ACPR, adequate clinical and parasitological response; ETF, early treatment failure; LCF, late clinical failure; LPF, late parasitological failure.

recommended by WHO (2009) for antimalarials with long half-lives such as piperaquine is 42 days. Piperaquine has a half-life of 28 days.

The clinical responses obtained in this study were similiar to those of Lidia *et al*, (2015) in the DHP efficacy test on vivax malaria in Kupang District, NTT, where on the second day after taking the drug 64% of the subjects have fever and on D7 8% of subjects still have fever; the fever clearance time is a median of 3 days (range 1-21 days). On the other hand Awab *et al* (2010) reported that on the first and second day after therapy, 10.9% and 1.1% of the subjects, respectively have fever. Further studies will be necessary to resolve these differences in clinical response to DHP therapy.

Fever is a classical clinical symptom of malaria. The prevalence of fever in falciparum malaria is milder than in vivax (Anstey *et al*, 2009). Fever associated with malaria infection results from the rupture of blood schizons that release various pyrogens, which stimulate release by the host of chemokines, such as tumor necrosis factor and interleukin-6, which lead to the production of prostaglandins, causing the hypothalamus to change the normal body temperature threshold, thus, fever. DHP administration decreases the number of parasites, thereby reducing the amount of malarial pyrogens released and hence the decline in fever (Anstey *et al*, 2009; Robinson *et al*, 2009).

In addition to fever, clinical responses observed during the follow-up were (in decreasing order of frequency) nausea, dizziness and vomiting. These symptoms could not be ascertained whether they were the symptoms of malaria or side effects of the drug. During follow-up, no subjects had any allergic reactions or severe malarial events. Similar to Awab *et al* (2010) the administration of DHP therapy can be well tolerated by the subjects. An earlier study showed there was a significant difference between the proportion of subjects who experienced symptoms after taking DHP compared to those taking chloroquine ($p = 0.008$), so it is likely that the symptoms observed in our study were due to drug-related effects (Lidia *et al*, 2015). Hasugian *et al* (2012) tested the efficacy and safety of DHP in treating uncomplicated vivax malaria in Pontianak District, West Kalimantan; Katingan District, Central Kalimantan; Southeast Minahasa District, North Sulawesi; and Sigi District, Central Sulawesi and found that fever clearance time of DHP is achieved on the 7th day with 100% of the subjects free of fever. Adverse events that occur after DHP treatment is diarrhea, anorexia and sweating. The efficacy of DHP on D28 is 100% and D42 97.6% (95%CI: 91.5-99.3) indicating the efficacy and safety of DHP according to WHO criteria and can be recommended for widespread use in Kalimantan and Sulawesi (Risniati *et al*, 2011; Hasugian *et al*, 2012). Another study in Timika, Papua found increased incidence of diarrhea and headache complaints on D1 and D2 after DHP administration but concluded that in Timika, DHP is still effective and safe to use as a multidrug-resistant therapy of falciparum and vivax malaria (Ratcliff *et al*, 2007).

Mean PCT in this study was 3 days (range 1-7 days). Although 4.76% of subjects were found with gametocytes in their peripheral blood smears from D0 to D2, on D3 after the treatment, all subjects were gametocyte-free in their peripheral blood smears. Similarly, the study of Lidia *et al* (2015) we noted on D1 and D2 most > 50% subjects in the DHP group were still with parasitemia and were 100% parasite-free on D7. In addition, parasitemia with gametocytes were found on D1 and D3 indicating the failure of DHP as an antigametocytocidal. In contrast to Awab *et al* (2010) our study showed DHP had a faster parasitological effect and no recrudescence by D42. On the other hand, a study conducted by Hasugian *et al* (2012) reported 100% subjects gametocyte-free on D7 and recurrences occur from D21 to D42. While the study by Ratclif *et al* (2007) in Timika, Papua found 73% of subjects in the parasite-free DHP group within 24 hours. Lidia *et al* (2015) reported the DHP group obtained cumulative success incidence of 0.76 (95% CI: 0.542-0.884).

The WHO set a minimum of 10% for treatment failure of antimalarial medication or a cure rate of less than 90% in order to change the treatment policy of malaria

in a region (WHO, 2010b). The efficacy test as performed in this study may predict the presence of drug resistance, another study is required to prove the presence of *P. falciparum* resistant to DHP. In addition to the therapeutic efficacy test, other studies are needed to prove the presence of DHP resistance in a particular area, especially in TTS district, such as molecular marker tests (PCR) for genotyping, *in vitro* testing, and the measurement of drug content or pharmacokinetic analysis (White, 1998; Basco, 2007; Picot *et al*, 2009; WHO, 2010b).

Thus, DHP is still recommended as an antimalarial in uncomplicated falciparum malaria therapy in the area of Timor Tengah Selatan District, Nusa Tenggara Timur, Indonesia.

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