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ASEXUAL BLOOD STAGE PROFILES OF *PLASMODIUM FALCIPARUM* MALARIA AFTER TRIPLE ARTEMISININ-BASED COMBINATION THERAPY

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ABSTRACT

Artemisinin-based combination therapy (ACT) is the first-line drug of choice recommended by WHO for *Plasmodium falciparum* treatment. The artemisinin resistance was reported, particularly in Greater Mekong Subregions, including Thailand. We investigated the parasite density and stage distribution of *Plasmodium falciparum* in blood films of 40 uncomplicated falciparum malaria patients who were admitted at Phusing Hospital and Khun Han Hospital from Srisaket as a part of the Tracking resistance to artemisinin collaboration II (TRAC II) clinical study in 2015-2018. Parasitemia and parasite stages (ring, trophozoite, and schizont) were evaluated before and after being treated with standard and triple-ACT by using the microscopic examination. Nineteen and 21 cases were treated with dihydroartemisinin/piperaquine (DHA/PPQ) and dihydroartemisinin/piperaquine plus mefloquine (DHA/PPQ plus MQ), respectively. Most of the patients 90.4% who received DHA/PPQ plus MQ were successfully cured. Parasitemia was cleared by almost 94.6% and 53.8% after DHA/PPQ plus MQ and DHA/PPQ treatment, respectively (P = 0.01). The ring-stage parasites were cleared completely 100% at 120 hours after DHA/PPQ plus MQ treatment whereas 61.5% after being treated with DHA/PPQ (P = 0.02). Trophozoites were disappeared after 4 hours after treatment in both groups. Schizonts were not observed in all blood films. Fifteen out of 19 cases (78.9%) who received DHA/PPQ were recrudescence. These results demonstrated that the ring-stage parasites were less susceptible to artemisinin derivatives. The DHA/PPQ plus MQ is an alternative treatment for artemisinin-resistant falciparum malaria.

Keywords: *P. falciparum*, artemisinin resistance, artemisinin combination therapy

INTRODUCTION

Malaria is a significant mosquito-borne tropical disease that causes morbidity and mortality all over tropical and sub-tropical areas in 91 countries worldwide (Ashley, Pyae Phyo, & Woodrow, 2018). According to the World Health Organization (WHO), an estimated 228 million cases of malaria occurred worldwide in 2018, compared with 251 million cases in 2010 and 231 million cases in 2017 (WHO, 2019). It is transmitted by the bite of infected female anopheline from human to human. A Causative agent is a blood parasitic protozoan in the genus Plasmodium (Maier, Matuschewski, Zhang, & Rug, 2019). Nowadays, there are 6 species that cause health impacts in humans which are *P. falciparum*, *P. vivax, P. ovale curtisi, P. ovale wallikeri, P. malariae,* and *P. knowlesi* (Calderaro *et al,* 2013). The most severe infection is *P. falciparum* which most important cause of

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death so called "malignant malaria" (Liu, Miao, & Cui, 2011). Reducing this disease burden has serious concern from the past to the present. Hence, the availability and proper use of effective antimalarial drugs were provided for the treatment and elimination of the disease. Artemisinin combination therapy (ACT) is introduced and recommended as the first-line antimalarial drugs treatment for uncomplicated *P. falciparum* malaria worldwide. ACTs are co-formulated with fast-acting, highly-potent artemisinin (artesunate, artemether, and dihydroartemisinin) and a slow-acting, less-potent partner drug (mefloquine, piperaquine, lumefantrine, and sulfadoxine/pyrimethamine). The action of artemisinin-derivatives kills the majority of parasites and the partner drug eliminates the remaining parasites by a different mechanism. They are believed to protect each other from the spontaneous development of resistance (Fairhurst & Dondorp, 2016). Artesunate/mefloquine (AS/MQ) and dihydroartemisinin/ piperaquine (DHA/PPQ) have been the ACTs of choice in Cambodia, Thailand, and Vietnam for lengthy periods during the past decade (Hamilton *et al,* 2019). However, loss of artemisinin efficacy in ACTs has emerged to mefloquine resistance on the Thailand-Myanmar border and contributed to the emergence and spread of piperaquine resistance in *P. falciparum* in Cambodia and southern Vietnam (van der Pluijm *et al,* 2019). Artemisinin resistance manifest as a slower rate of parasite clearance associated with enhanced survival of ring-stage parasites after exposure to the drug in vitro, a parasite clearance half-life ≥ 5 hours in patients treated with an artemisinin-derivatives or an ACT (Nsanzabana, 2019; Tilley, Straimer, Gnädig, Ralph, & Fidock, 2016) whereas that for sensitive parasites is around 2 hours (Siddiqui *et al,* 2020). As the result, artemisinin-derivatives are less effective to kill whole parasites within 3 days (WHO). Triple artemisinin-based combination therapies (TACTs) are a combination of existing ACT with a second slowly eliminated partner drug, added additional antimalarial activity, and provide mutual protection for the partner drugs (van der Pluijm *et al,* 2020). Aim of this study was to investigate the parasite density and asexual erythrocytic stages distribution of *P. falciparum* in uncomplicated malaria patients treated with ACT and TACT.

MATERIALS AND METHODS

A retrospective study as part of multicenter clinical trials from Tracking resistance to artemisinin collaboration II (TRAC II) in 2015-2018. All 1,062 peripheral blood films were collected from 40 Thai patients who enrolled at Phusing Hospital and Khunhan Hospital, Srisaket province, Thailand. They were received either DHA/PPQ or DHA/PPQ plus MQ. Thin and thick blood films were examined for asexual erythrocytic stages (ring, trophozoite, and schizont) on admission, 4, 8, 12 hours after treatment, then 6 hours-interval until 2 consecutive consequences of asexual erythrocytic stages were showed negative result and 42 days of treatment. The number of asexual erythrocytic parasites had calculated to estimate the level of parasitemia per blood microliter (/μL) in thin and thick blood film examination as follows theses formulas by WHO guidelines, 2016. The asexual erythrocytic parasites were recorded staging per 100 infected red blood cells (iRBCs). The *P. falciparum* positivity was determined by nested PCR assay (Singh *et al,* 2004)

RESULTS

Nineteen and 21 cases (47.5 and 52.5%) were received DHA/PPQ and DHA/PPQ plus MQ, respectively. On admission, the parasite counts in DHA/PPQ and DHA/PPQ plus MQ treatment groups were 3,000-128,000 and 4,000-160,000/μL, respectively. After 42 days of treatment, *P. falciparum* positivity was determined and corrected by nested PCR assay (Singh *et al,* 2004). In DHA/PPQ plus MQ treatment group; 19 cases (90.4%) were successfully cured. In DHA/PPQ treatment group; 15 cases (78.9%) were recrudescence. The parasite clearance and stages were analyzed by Kaplan-Meier's survival analysis with observing

period since first dose administration until showed first negative result. We found that parasite density was slowly decreased in the first 6 hours and took time over 3 days that presented negatively in both treatment groups. DHA/PPQ plus MQ could clear parasites completely as 94.6% meanwhile DHA/PPQ could clear the parasites 53.8% within 120 hours (P = 0.01). Ring-stage parasites were slowly decreased and presented negatively on hours 60 and 72 after being treated with DHA/PPQ and DHA/PPQ plus MQ, respectively. Ring-stage parasites were negative within 120 hours after DHA/PPQ plus MQ treatment (100%), not in DHA/PPQ treatment group (61.5%) (P = 0.02). Trophozoites were not detectable in the blood films after 4 hours in both treatment groups. No schizonts were observed in all blood films.

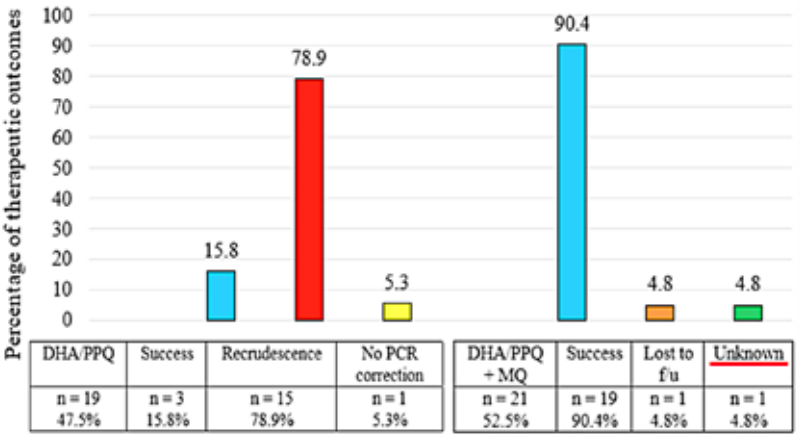

Fig 1- Therapeutic outcomes in 40 uncomplicated *P. falciparum* malaria patients after DHA/PPQ and DHA/PPQ plus MQ treatment. Unknown outcome was due to non-compliance with study procedures/discontinued study drugs.

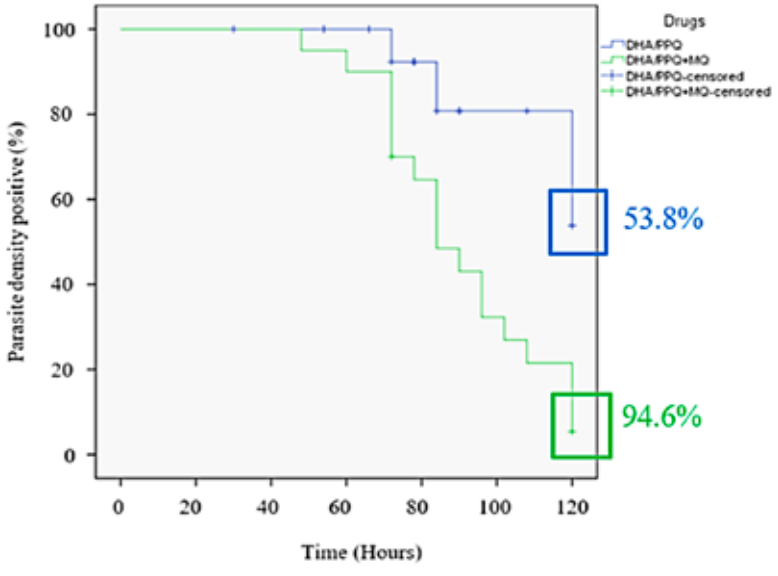

Fig 2- Kaplan-Meier's survival analysis of parasite density clearance in DHA/PPQ (blue line) and DHA/PPQ plus MQ (green line) treatment groups.

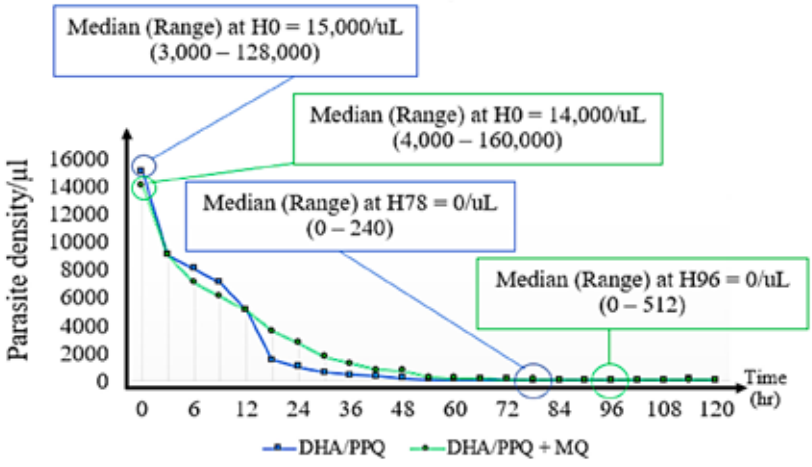

Fig 3- Parasite density reduction within 120 hours after DHA/PPQ and DHA/PPQ plus MQ treatment.

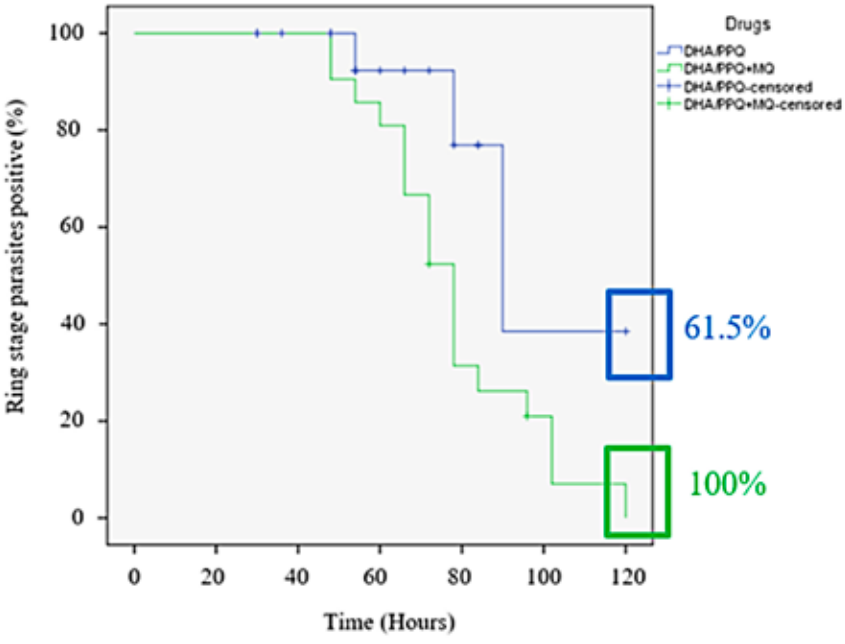

Fig 4- Kaplan-Meier's survival analysis ring-stage parasites clearance in DHA/PPQ (blue line) and DHA/PPQ plus MQ (green line) treatment groups.

DISCUSSION

It has been reported that the youngest as tiny rings stage was the lowest percentage of reduction in parasitemia level at 6 hours when compared with older stages (small ring to middle trophozoite stages) after ACT treatment, $P < 0.001$ (Intharabut *et al*, 2019). They also suggested that PfK13-wild type and mutants associated with artemisinin resistance appear to have arisen independently along Thailand-Cambodia and Thailand-Myanmar border regions. A Recent study by Theerayot

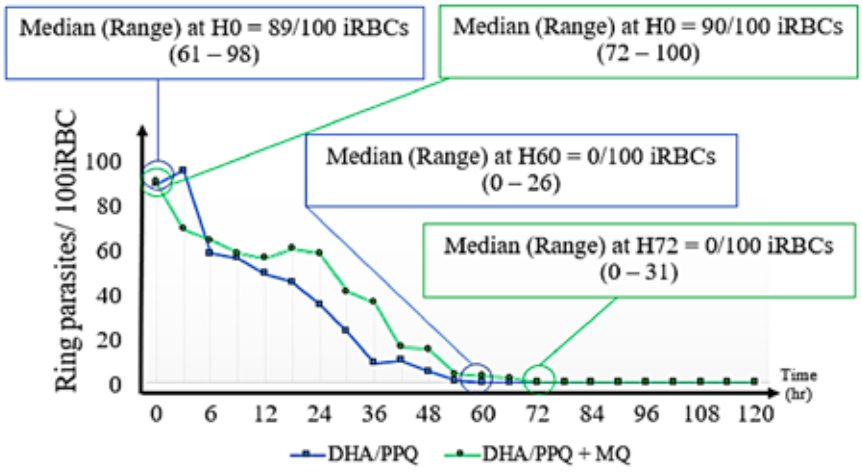

Fig 5- Ring-stage parasites reduction after DHA/PPQ and DHA/PPQ plus MQ treatment.

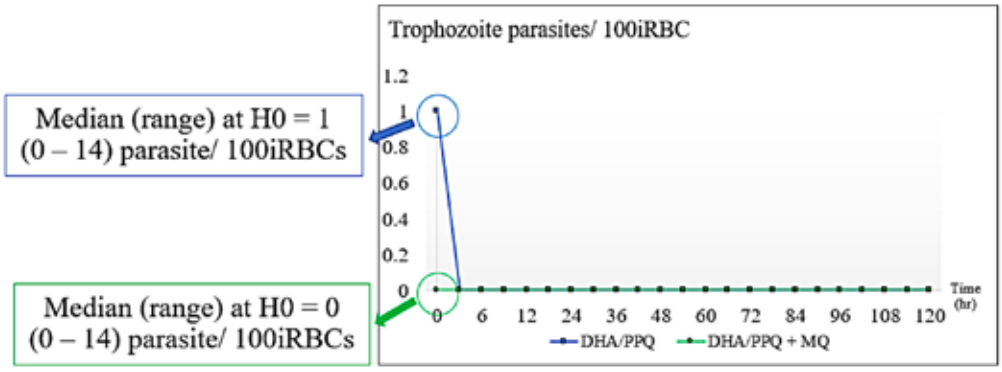

Fig 6- Trophozoite-stage parasites reduction after DHA/PPQ and DHA/PPQ plus MQ treatment.

Kobasa *et al*. investigated the prevalence of the PfK13 mutations in many provinces in Thailand. They found that PfK13-wild type and C580Y mutant were associated with artemisinin resistance in Srisaket province, Thailand (Kobasa *et al,* 2018). Even though DHA/PPQ was introduced and widely replaced artesunate/mefloquine (AS/MQ) in many parts of Thailand for the last decade ago because of mefloquine resistance. However, the resistance of DHA/PPQ has been reported the increased rate of treatment failure caused by a single lineage of a multidrug-resistant that has spread across Cambodia, northeast Thailand, southern Laos, and southern Vietnam. Plasmepsin II-III gene amplification, a molecular marker of piperaquine resistance were also present in high frequencies in Cambodia, Thailand, and Vietnam whereas pfmdr1 gene amplification, a molecular marker of mefloquine resistance was not observed anywhere (van der Pluijm *et al,* 2020). The results in this study are consistent with the previous report that the ring-stage parasites were less susceptible to artemisinin derivatives. The mechanisms of resistance need to be further investigated. It suggested that DHA/PPQ plus MQ might be an alternative treatment for artemisinin-resistant falciparum malaria in the multi-drug resistance area.

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