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Evaluation of artemisinin-based combination therapy (ACT) to uncomplicated falciparum malaria patients in Purworejo District, Central Java, Indonesia

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ABSTRACT

Artemisinin-based Combination Therapy (ACT) to treat uncomplicated *Plasmodium falciparum* malaria has been applied in Purworejo District, Central Java, Indonesia, since 2004. However evaluation of the two ACT regimens used ie: Artesunate Amodiaquine (AAQ) and Dihydroartemisinin-Piperaquine (DHP) co-administered with Primaquine (PQ) has not been performed. This study aims to evaluate the efficacy and adverse events of AAQ + PQ and DHP + PQ treatment in uncomplicated falciparum malaria in Purworejo. In this descriptive and observational study, 46 *Pf* infected patients who fullfill the inclusion and exclusion criterias were recruited from December 2010 to August 2011. Standard ACT treatment were given to the patients followed by WHO drug efficacy evaluation for 28 days. The clinical symptoms and adverse events was also evaluated over the course of the treatment. From all recruited subjects, 37 patients received DHP + PQ and 9 patients received AAQ + PQ. On the DHP + PQ treated patient, all subjects were free of asexual and sexual parasites by Day-3 while on AAQ + PQ treated patient, this parasite clearance was achieved faster as early as on D-2 at the latest. On the otherhand, the disappearance of fever was also last longer in DHP + PQ treated patient which in one patient last on D-14, while in AAQ + PQ treated patient, the symptom of fever dissappeared by D-2 at the latest. No Early or Late Treatment Failures were found on either DHP + PQ or AAQ + PQ treatment as well as clinical and parasitological failures. However, the presence of adverse events cause by both drugs should not be ignored to ensure drug compliance.

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ABSTRAK

Terapi malaria dengan *Artemisinin-based Combination Therapy* (ACT) untuk malaria tanpa komplikasi diterapkan di Kabupaten Purworejo, Jawa, Indonesia, sejak tahun 2004. Namun demikian evaluasi terhadap efektivitas dua regimen ACT yang digunakan yaitu Artesunate Amodiakuin (AAQ) dan Dihydroartemisinin-Piperaquine (DHP) disertai Primakuin (PQ) belum pernah dilakukan. Penelitian ini bertujuan untuk mengevaluasi efikasi dan *adverse events* AAQ + PQ dan DHP + PQ pada penderita malaria tanpa komplikasi di Purworejo. Penelitian ini merupakan penelitian deskriptif dan observasional. Subjek penelitian adalah pasien yang terinfeksi *P. falciparum* yang memenuhi kriteria inklusi dan eksklusi, direkrut dari Desember 2010 hingga Agustus 2011. Status resistensi obat, dan *adverse events* dievaluasi menggunakan cara evaluasi baku WHO selama 28 hari. Dari semua subjek yang direkrut (46), 37 penderita diobati dengan DHP + PQ dan 9 penderita dengan AAQ + PQ. Pada kelompok yang mendapat pengobatan DHP + PQ, semua subyek bebas dari parasit stadium aseksual dan seksual pada hari ke 3, sedangkan kelompok yang mendapatkan pengobatan AAQ + PQ, parasit sudah menghilang dari sirkulasi darah pada hari-2. Semua penderita yang diobati DHP + PQ gejala demam menghilang lebih lama yaitu sampai hari ke 14, sedangkan pada penderita yang diobati dengan AAQ + PQ gejala demam sudah menghilang sejak hari ke 2. Tidak ada kegagalan pengobatan dini maupun kegagalan pengobatan lanjut baik secara klinis maupun parasitologi pada penggunaan kedua obat tersebut. Walaupun demikian, *adverse events* sebaiknya tidak diabaikan untuk memastikan kepatuhan obat.

Keywords : falciparum malaria – artesunate + amodiaquine – dihydroartemisinin + piperaquine - treatment failure - adverse events

INTRODUCTION

Malaria remains a public health problem in more than 100 countries, including Indonesia. It is estimated that 300-500 million clinical cases occurs worldwide annually.[1] Among four species of Plasmodium that infect humans, *P. falciparum* is the most virulent species and cause high morbidity and mortality (1 million per year). Malaria incidence in Indonesia had been reported to decline in the last decade. Compared to 2008, the Annual Parasite Incidence (API) in Indonesia in 2009 has decreased from 3.82 to 1.85 per million population. Java and Bali were classified as low endemic areas. However, despite of a continuous control measures malaria outbreaks have occurred sporadically in Indonesia in 2009, including Java Island.[2]

Malaria control efforts in Indonesia have faced many constraints such as shortage of budgets, human resourse, insecticide-resistent vectors, ineffective intervention strategies and treatment failure due to parasite resistance to many antimalarial drugs.[3] Parasite resistance is defined as the Plasmodium parasites ability to survive or proliferate within a theuraphetic or higher of the recommended dosage but still within patient tolerance.[4] Based on the WHO and Indonesian Malaria Diagnosis Expert Committee (KOMLI), Artemisinin-based combination therapy (ACTs) should be implemented in Indonesia in 2004. In Indonesia, there are currently two ACT regimens used in the malaria program i.e. artesunate-amodiaquine (AAQ) and dihydroartemisinin-piperaquine (DHP). Malaria parasite are known very adaptive and the reduction of

sensitivity to anti malaria drug is predicted to appear along with the widespread use of ACT.[5] The indication of the reduction of sensitivity of *P. falciparum* to AAQ/ DHP was reported by Hasugian *et al.*[6] in Southern Papua, AAQ with treatment failure of 45% and treatment failure for DHP was 13%.. Similar studies on AAQ resistance were also reported in Gabon, Ghana, Madagaskar, Rwanda, Sierra Leone, Zanzibar and Myanmar with treatment failure ranged from 8.7% to 27%; and in Kenya the treatment failure of DHP was reported 8.3%. [4]

Purworejo is a district in Central Java that was classified as low-endemic areas in Indonesia.[7] Incidence of malaria in Purworejo is influenced by several factors. These include. high mobility of people to and from disease endemic areas, geography, behavior of the population and also the presence of efficient malaria vector mosquitoes.[8] Malaria control efforts were emphasized in 2002 by *Gebrak Malaria* programme that includes the strengthened on surveillance, vector control, and treatment. The success of this program was demonstrated by the decrease in malaria cases from 2002 to 2004. However, malaria cases have been increasing afterwards and outbreaks have been common in some villages[9] starting in 2006.

Purworejo was confirmed as a region with multidrug resistance parasite[10,11] in 2003, and therefore the used of ACT (Artesunate-Lumefantrine, Coartem) was recomended to overcome treatment failure.[12] When this study started in 2010, ACT regimen used in Purworejo were AAQ and DHP regimens.[9] Evaluation of AAQ efficacy in Purworejo has been performed by Kusumaningsih.[13] However, the AAQ used was without primaquine (PQ) as recommended.[14] The results found that the AAQ treatment failure was 12.9%, indicating reduced sensitivity to the AAQ that may cause malaria cases continue to appear.

Therefore, the objectives of this study were: 1. To evaluate the efficacy of AAQ+PQ and DHP+PQ on the treatment of uncomplicated falciparum malaria in Purworejo. 2. To evaluate possible adverse events on the used of AAQ+PQ and DHP+PQ on the treatment of uncomplicated falciparum malaria in Purworejo. The results will provide valuable information regarding the status of ACT regimens and the used of this drug as effective and safe regimen for the population of Purworejo.

MATERIALS AND METHODS

This is an observational analytic study and was conducted in Purworejo District from December 2010 to August 2011. Subjects were patients with uncomplicated *P. falciparum* malaria who met the inclusion and exclusion criteria[15]. The study was conducted in coordination with District Health Office of Purworejo and local health workers of related Primary Health Centers in Purworejo. Subjects were malaria patients identified during passive or active case finding. Subjects were treated with DHP/Artekin® **(**Dihydroartemisinin 2-4 mg/kg body weight (BW) and Piperaquine 16-32 mg/kg BW); or with AAQ/Arsuamon® (Amodiaquine 10 mg/kg BW and artesunate 4 mg/kg BW); co-administered with Primaquine (0.75mg/kg BW) on the first day as suggested by the Indonesian National Policy.[14] The administration of AAQ or DHP depended on the availability of either AAQ or DHP in each Primary Health Center. Collection and examination of peripheral blood smear (both thin and thick) were conducted from Day 0 (D0) to D28 for patients being treated with AAQ and D0 to D42 for patients being treated with DHP. Stained blood films were examined by laboratory personnel of a related Primary Health Centers and cross checked by the Department of Parasitology, Faculty of Medicine, Universitas Gadjah Mada,

Yogyakarta. Patient complaints and clinical symptoms were documented and observed during follow up as well as during blood sample acquisition. Therapeutic responses assessed were Early Treatment Failure and Late Treatment Failure (Late Clinical Failure/ Late Parasitological Failure) as defind in WHO guidelines.[15] Side effects or adverse event were defined as any clinical sign or symptom that did not exist during first visit (D0) but appeared after, or already existed and were aggravated during the follow up period.

This study was reviewed and approved by Institutional Review Boards for the ethical conduct of research on human subjects at the Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta. Informed consent was collected from subjects before their participation in this study.

RESULTS

Of the 77 malaria patients recruited, only 46 were followed until day 28 for AAQ and day 42 for DHP. The reasons for that is mostly related with geographical situation, a number of patient refused to be visited or migrated to other place of residency. Patients came from 14 villages spread over 7 sub-Districts in Purworejo i.e. Kaligesing, Bener, Bagelen, Pituruh, Pecan, Loano, and Gebang sub-District.

The characteristics of the subjects were shown in TABLE 1. The major complaints of subjects on the commencement of this study are shown in TABLE 2. Fever is the most common clinical manifestation among 46 uncomplicated falciparum malaria patient in Purworejo (91.3%) follwed by dizziness, headache and muscle pain, however, 4 patient

TABLE 1. The characteristics of uncomplicated falciparum malaria patient in the Purworejo District included in the ACT efficacy study

Characteristics	Patient treatment regimen		
	AAQ (n=9)	DHP(n=37)	Total (n=46)
Age			
• 5-15	2	6	8
• 16-45	4	18	22
• ≥46	3	13	16
Sex			
• Male	7	18	25
• Female	2	19	21
***Plasmodium* stage**			
• Trophozoit +gametes	3	9	12
• Trophozoit	6	28	34
Parasite density			
• 1000-10,000	7	22	29
• >10.000-50,000	2	12	14
• >50,000-100,000	0	3	3
Axilla temperature (^{0}C)			
• ≥ 37.5	8	34	42
• < 37.5	1	3	4

n=number of samples; parasite density is the number of asexual parasite per µl blood

(87%) did not experience any clinical sign (asymptomatic). Parasite density did not seem to correlate much with fever, 25 out of 29 (86%) patients with parasite density between 1000-10.000 parasite/ul experiencing fever, as well as all patients with higher parasite density (14 and 3 patients with parasite density > 10,000-50,000 and 750,000-100,000 respectively). (TABLE 3).

TABLE 2. Clinical symptoms of uncomplicated falciparum malaria patient in Purworejo District involved in the ACT efficacy study conducted from December 2010 to August 2011

Patients' clinical symptoms	(N= 46) %
Fever	91.30
Dizziness	91.30
Headache	89.13
Shivering	71.74
Anorexia	63.04
Muscle pain	78.26
Coughing	4.35
Vomiting	15.22

TABLE 3. The present or absent of fever in uncomplicated falciparum malaria patient in Purworejo District involved in the ACT efficacy study conducted from December 2010 to August 2011 according to parasite density

Parasite density (no. of asexual/ul)	Symptom of fever		
	Fever	No fever	Total
1000-10,000	25 (86%)	4 (14%)	29
>10,000-50,000	14 (100%)	0	14
>50,000-100,000	3 (100%)	0	3

The percentage of patients that were free of fever following DHP+PQ treatment (n= 37) was 41%, 76%, 89%, 97% on day 1, 2, 3, 7 respectively. All patients were fever-free on day 14. The percentage of patients who were free of asexual parasites was 76%, 95.6% on day 1 and 2 and all patients (100%) were free of parasites on day 3 of follow-up (FIGURE 1). Different results were shown by subjects treated with AAQ+PQ (n = 9), i.e. the percentage of patients with free fever is 67% on day 1 and reached 100% on day 2, while the percentage of patients with free asexual parasites is 90% on the first day and reached 100% on day 2 (FIGURE 2).

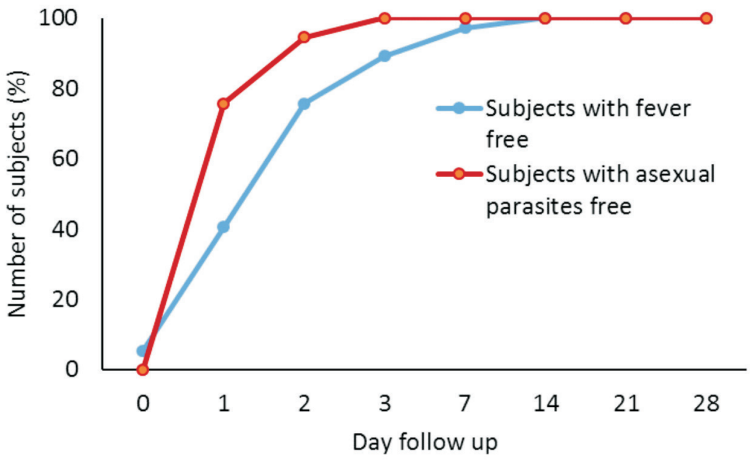

FIGURE 1. The disappearance of fever and asexual parasite in uncomplicated malaria patients following DHP+PQ treatment.

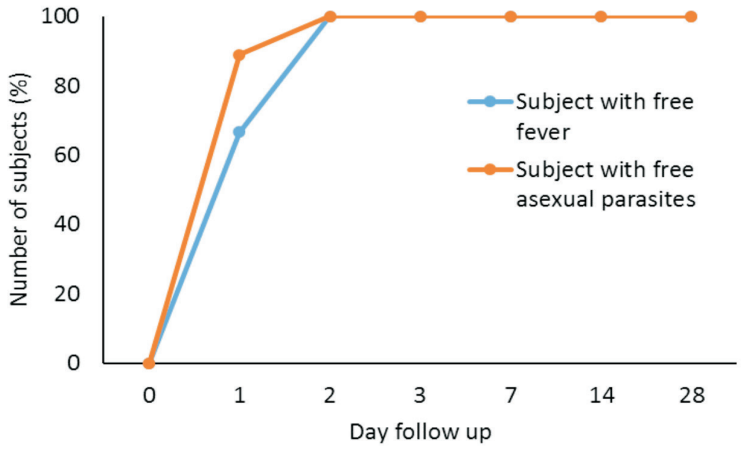

FIGURE 2. The disappearance of fever and asexual parasites in uncomplicated falciparum malaria patients following treatment with AAQ+PQ.

On the first day patients were recruited in the study, 25% and 35% subjects carried gametocytes before the administration of DHP and AAQ respectively. On treatment with DHP+PQ, number of gametocytes-carrier patients reduced from 24.32% on day 0 to 21.62% and 10.81% on day 1 and 2 follow-up, and reached 0% (cleared-up) by the third day follow-up. On treatment with AAQ+PQ, number of gametocytes carrier patients reduced from 33% on day 0 to 22% both on day 1 and 2 follow-ups respectively, and 0% (cleared-up) on the third day post treatment (FIGURE 3).

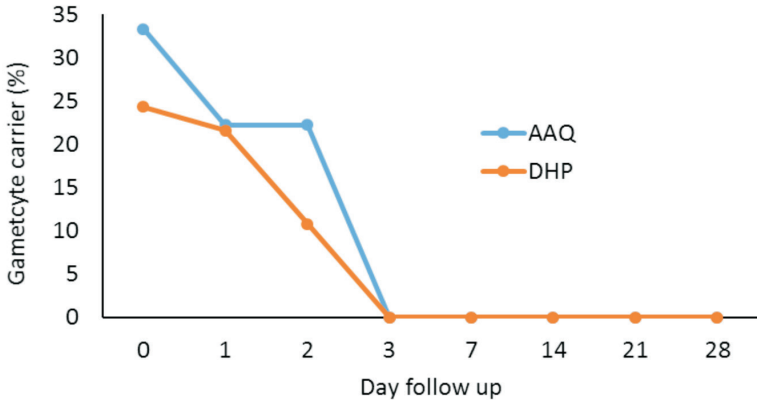

FIGURE 3. The disappearance of gametocyte in P. falciparum-infected subjects treated with AAQ+PQ or DHP+PQ during follow up.

As shown on FIGURE 4, the clinical symptom of malaria dissapeared following ACT treatment. In group treated with DHP+PQ, the percentage of subjects have no clinical symptoms was 5%, 25%, 70%, 80%, and 90% on day 2, 3, 7, 14 and 21 follow-up. In group treated with AAQ+PQ, all subject still have clinical symptoms until day 3 follow-up, and afterwards, the percentage of subjects with clinical symptoms began to decrease on day 7 and 14, to 40%, and 70% respectively (FIGURE 5). All subjects were free of clinical symptoms on day 28 for DHP and on day 21 for AAQ treatment. The dominant clinical signs were headache, dizziness and muscle pain for subjects treated with DHP+PQ and headache, dizziness and loss of appetite for subjects treated with AAQ. Both regiments showed neither early or late treatment failure (TABLE 4).

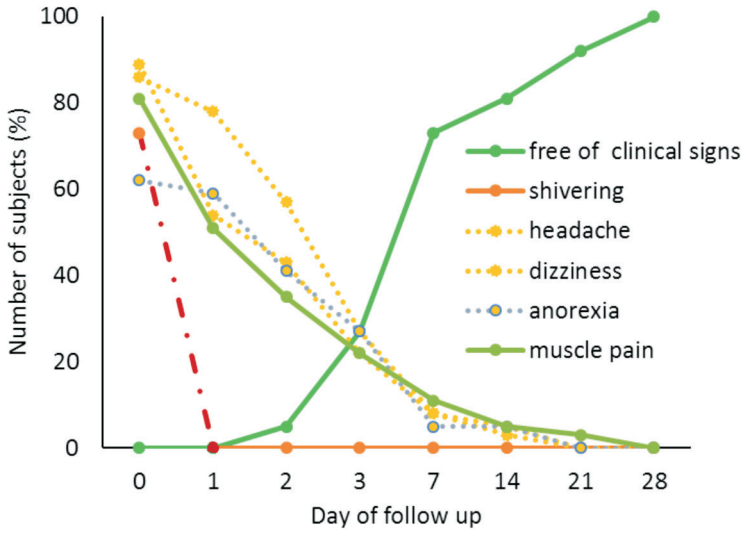

FIGURE 4. The disappearance of clinical symptoms in uncomplicated falciparum malaria patients treated with DHP + PQ during follow up.

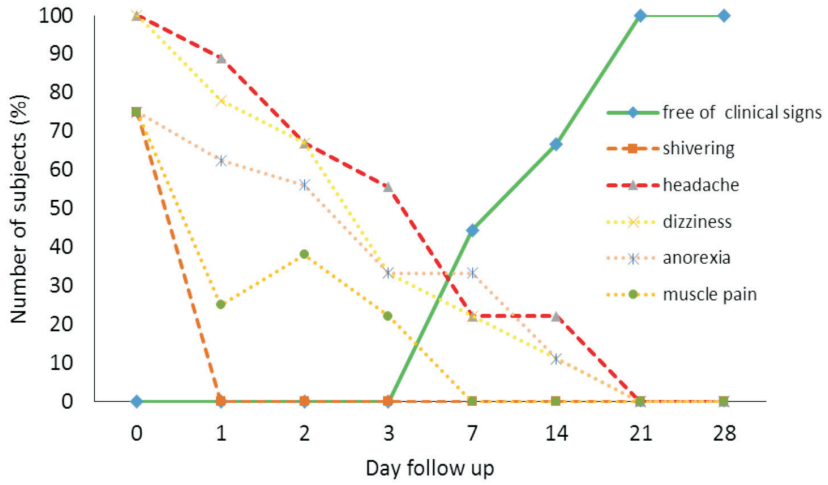

FIGURE 5. The disappearance of clinical symptoms in uncomplicated falciparum malaria patients treated with AAQ+PQ during follow up.

TABLE 4. The result of the evaluation of ACT treatment to uncomplicated falciparum malaria patient in the Purworejo District from December 2010 to August 2011.

Drugs	Status of Resistance (%)					Total
	Sensitive	Early RI	Late RI	R II	R III	
DHP+PQ	100	0	0	0	0	100
AAQ+PQ	100	0	0	0	0	100
Total	100	0	0	0	0	100

On treatment with DHP+PQ, side effects disappeared by D-1 in 5% subjects. This percentage increased to 25% on D-3, and to 75% on D-7. All subjects were free of side effects on D-28 (FIGURE 6). The dominant side effects with DHP+PQ treatment were nausea, stomach pain and dark urine. A small proportion of subjects (3%) complained of discomfort while breathing after the administration of DHP+PQ. All subjects complained of having adverse events with AAQ+PQ from D1 to D3. On D-7, +50% subjects were free of adverse events and this percentage increased on D-14 (80%) and all subjects (100%) were free of adverse events on D-21 (FIGURE 6). The dominant adverse events in order of occurrence were nausea, dark urine, stomach pain, vomit, and discomfort while breathing.

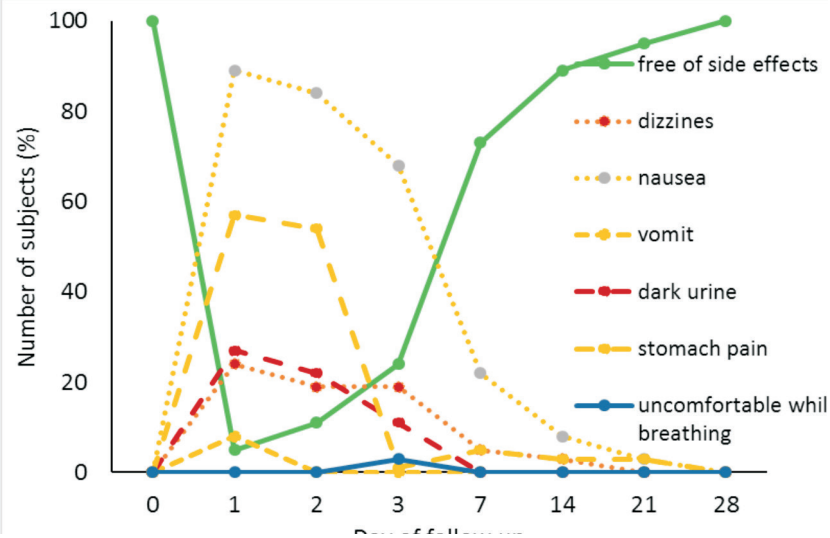

FIGURE 6. Adverse events seens in patients treated with DHP+PQ during follow up

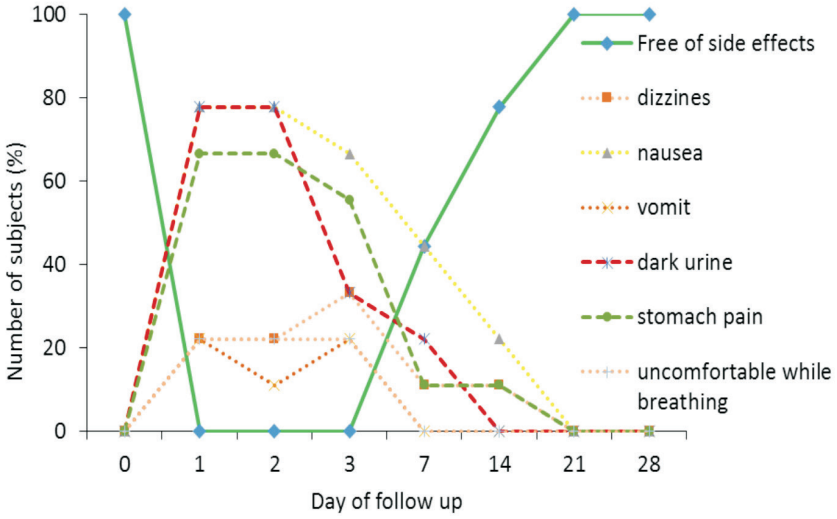

FIGURE 7. Side effects seens in patients treated with AAQ + PQ during follow up

DISCUSSION

From 46 subjects observed in this study, 48% were in productive age (16-45 years old). These probably related with the chance of getting the infection since people in this group of age are more active compared to childhood and elderly. The majority of malaria patient in Purworejo were symptomatic with predominat symptom of fever, diziness and muscle pain and only less than ten percent were asymptomatic. These clinical picture are representing characteristic of malaria patient from low endemic areas where the transmission are low and may be seasonal which do not stimulate effective immune

responses and therefore symptomatic malaria patients must be common in this such low endemic areas. These condition are also in accordance with the low seroprevalence and antibody levels of population in Purworejo.[16] Asymptomatic patient were expectedly be found in area of higher endemicity such as in Lampung[17], where higher level of malaria transmission will stimulate higher antibody responses, resulting in higher seroprevalence and higher antibody level in the serum[16] that might suppressed the clinical manifestation of the diseases.

The presence of subject with gametocytes (26%) on day 0 probably suggesting that transmission might be still in progress during the study since Purworejo was a receptive areas where Anopheles mosquito vector are always available in most of the time[8], readily transmits any gametocytes within the blood circulation of the nearby population. The factor that might stimulate the differenciation to gametocytes might varies such as the host immune responses, oxidative stress or other condition that make the parasite in uncomfortable condition.[18] The presence of gametocyte in the blood circulation of malaria patient must be take as highly priority of attention since it is critical for transmission to other people. Anti gametocytocidal drug must be given and prevention to mosquito bite are also very essential.

In this study both DHP+PQ and AAQ+PQ treatment resulted in the rapid clearance of both asexual and sexual form of the parasite by day 3 of the follow-up. ACT is known as a potent blood schizonticides which also have an anti gametocidal effect but only against immature gametocytes, and therefore addition of Primaquine on the first day of ACT treatment are still essential to kill the mature gametocytes.[19] Efficacy study of DHP treatment without PQ had been carried-out in Kalimantan and Sulawesi by Siswantoro *et al.*[20] and reported that in those study gametocytes were still detected on D-28 of the follow-up. The similar result was shown when using Artemisinin-mefloquine without PQ in Thailand.[21] The effect of primaquine on the treatment of malaria using ACT was also shown in Lampung by Inge *et al.*[22] and the result indicated that the addition of primaquin may fastened the gametocyte clearance from the blood circulation as compared to ACT whithout prmaquine. The efficacy of DHP+PQ and AAQ+PQ on the treatment of uncomplicated malaria patient in the Purworejo District indicated that these regiment are still sentitive, no early nor late treatment failure. Although the efficacy of DHP+PQ and AAQ+PQ in this study seems still promising, further study that includes more subject at least 50 subjects as recommended by WHO are highly suggested to make statistically significant conclusion.

Despite the effectiveness of DHP+PQ and AAQ+PQ in the clearance of sexual and asexual parasites, this study also showed that both regiments also rapidly reduce most of the clinical symptoms appeared on the patient with more intensive in DHP+PQ as compared to AAQ+PQ, except for fever which was last longer until day 14 in DHP+PQ treated patients. Shiver was also common symptom appeared in malaria patient in Purworejo, however, this symptom rapidly dissappeared on the following day of the first ACT treatment. Although both treatments also causing some adverse events, i.e. symptoms that initially absent but appeared following treatment or initially present and than agravated following treatment. The major adverse events arosed were nausea, stomach pain and dark urine. These adverse events were less and minimal in DHP treated patients. Furthemore, they also dissapear faster in DHP treated patients.

Similar adverse events during the used of DHP was also repoted by Hasugian *et al.*[6] during treatment against multidrugs-resistant malaria in Papua suah as nausea, vomiting, and anorexia. These adverse events diminished on D-7, and were also milder and better tolerated than those treated with AAQ. Vomiting, stomach pain and discomfort during breathing/dyspnea was also been reported with DHP treatment[23, 24] but there was no evidence of the ECG changes.[23] Adverse events seen during and after treatment needs to be considered as an important factor that might contribute to patient compliance for malaria drugs regimens.[25] The fact that some subjects complained dark urine during treatment or follow-up days suggested that some subjects might experienced hemolysis as a results of enzyme disorder condition (G6PD).

CONCLUSION

Treatment of uncomplicated falciparum malaria patients using DHP+PQ and AAQ+PQ is successful in clearing of the asexual parasites in the Purworejo District with no Early nor Late Treatment Failure, and therefore no artemisinin resistance is observed. The co-administration of primaquine onto these regiments is useful in gametocyte clearance adding the benefit of possibly blocking the transmission. It is recommended that DHP+PQ or AAQ+PQ should still be used in the Purworejo District realizing that adverse events may be an issue with compliance. Dark urine that found in some subjects demands further study on the prevalence of G6PD deficient in this region.

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