

Plasmodium Resistance to Artemisinin Derivates due to Kelch-3 Gene Mutation

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Abstract. Artemisinin class of antimalarial drugs play an important role in controlling falciparum malaria after the emergence of resistance of *Plasmodium falciparum* to other antimalarial drugs such as chloroquine, sulfadoxine-pyrimethamine and mefloquine. Therefore, the presence of *Plasmodium falciparum* resistance to this class of drugs is threat to global efforts to eliminate this disease. Resistance of *Plasmodium falciparum* to artemisinin recently known to be associated with mutations in the propeller domain of the kelch-13 (K13) *Plasmodium falciparum* gene. The incidence of *Plasmodium falciparum* resistance due to mutations in the K13 gene, among others, can be found in Cambodia, Laos, Vietnam, China, Myanmar, Thailand and Africa. The presence of mutations in this gene will change the response of *Plasmodium falciparum* against oxidative stress induced by artemisinin by involving the proteasome-ubiquitin pathway. In addition, mutation K13 will also change the levels of PI3K and PI3P in the body of *Plasmodium falciparum*. PI3K and PI3P are lipids that essential for the development of *Plasmodium falciparum* from ring stage to schizont. Resistance to artemisinin will also provide phenotypic changes in the life cycle of *Plasmodium falciparum* in the form of elongation at the stage ring and transient shortening in trophozoite development. This resistance incident can be overcome, among others by prolonging the duration of treatment (from a 3-day regimen to a 4-day regimen) and combining artemisinin with proteasome inhibitors.

Keyword: Antimalaria, resistance, artemisinin, plasmodium, mutation

Abstrak. Artemisinin sebagai obat antimalaria memegang peranan penting dalam mengontrol malaria falciparum setelah timbulnya resistensi *P.falciparum* terhadap obat-obat antimalaria golongan lain seperti klorokuin, sulfadoksinpirimetamin dan meflokuin. Oleh karena itu, adanya resistensi *P.falciparum* terhadap obat golongan ini menjadi ancaman terhadap upaya global dalam mengeliminasi penyakit ini. Resistensi *P. falciparum* terhadap artemisinin belum lama ini ini diketahui berkaitan dengan mutasi pada domain propeller dari gen kelch 13 (K13) *P.falciparum*. Kejadian resistensi *P.falciparum* akibat mutasi gen K13 antara lain dapat ditemui di Kamboja, Laos, Vietnam, Cina, Myanmar, Thailand dan Afrika. Adanya mutasi pada gen ini akan mengubah respon *P. falciparum* terhadap stres oksidatif yang ditimbulkan oleh artemisinin dengan melibatkan jalur proteasome-ubiquitin. Selain itu, mutasi K13 juga akan mengubah kadar PI3K dan PI3P di dalam tubuh *P. falciparum*. PI3K dan PI3P adalah lipid yang esensial bagi perkembangan *P. falciparum* dari stadium cincin menjadi skizon. Resistensi terhadap artemisinin juga akan memberikan perubahan fenotip pada siklus hidup *P. falciparum* berupa pemanjangan pada stadium cincin dan pemendekan sementara pada perkembangan trofozoit. Kejadian resistensi ini dapat ditanggulangi antara lain dengan memperpanjang

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durasi pengobatan (dari regimen 3 hari menjadi 4 hari) dan mengkombinasikan pemberian artemisinin dengan obat golongan proteasome inhibitor.

Kata Kunci: *Antimalaria, resistensi, plasmodium, artemisinin, mutasi*

Received 5 May 2021 | Revised 30 August 2021 | Accepted 28 September 2021

1. Introduction

Malaria is an infectious disease caused by protozoan parasites of the genus *Plasmodium* which are inoculated to humans by the *Anopheles sp.* female. Since 2000, significant progress has been made in the fight against malaria. According to data, between 2000 and 2015, the incidence of malaria decreased by 41% and the malaria mortality rate fell by 62%. However, malaria is still an important cause of morbidity and mortality in children and adults in countries endemic to this disease. As of early 2016, malaria was still considered an endemic disease in 91 countries and territories. [1]

The World Health Organization (WHO) reports that 212 million cases of malaria occurred globally in 2015 and caused 429,000 deaths, the majority of which occurred in children under 5 years old in Africa, while in Indonesia, the incidence of malaria in 2013 decreased compared to 2007 from 2.9% to 1.9%, except in West Papua which experienced a sharp increase. The dominant parasite species causing malaria in Indonesia is *Plasmodium falciparum* (86.4%) while the rest are *Plasmodium vivax* and a mixture of *Plasmodium falciparum* and *Plasmodium vivax*. [2]

For more than 50 years, *Plasmodium falciparum* has developed resistance to antimalarial drugs used to eradicate this parasite such as chloroquine, sulfadoxine-pyrimethamine, quinine, piperazine and mefloquine. Therefore, WHO recommends the use of artemisinin combination therapy (ACT) as a drug of choice for malaria, including malaria with parasites that are resistant to several antimalarial drugs. ACT is generally very effective and well tolerated. However, recently, in Southeast Asia, many cases of *Plasmodium falciparum* resistance have been reported to this drug. The emergence of *Plasmodium falciparum* resistance to various types of antimalarial drugs is related to the genetic condition of *Plasmodium falciparum*. The genetic factors that play a role in forming the resistance of *Plasmodium falciparum* to artemisinin are mutations in the Kelch-13 gene. [3]

2. Artemisinin and its Derivates

Artemisinin is an antimalarial drug derived from *Artemisia annua*, an herb that has long been used in traditional Chinese medicine to treat intermittent fever. There are several theories regarding the mechanism of action of this class of drugs, but in general the theory is related to the formation of artemisinin free radicals. Artemisinin enters the body in an inactive form and is

activated by reductive cleavage of the endoperoxide group. The activation of artemisinin then causes the formation of free radicals. [4]

The free radicals formed will mediate the eradication of *Plasmodium* by altering the biochemical pathways in the parasite, which include alkylation of heme molecules and interference with heme detoxification pathways, inactivation of sarcoplasmic, endoplasmic reticulum PfATPase calcium pump (SERCA), alkylation of cytosolic proteins, such as PfTCTP, a tumor potential protein possibly associated with parasite replication, and disruption of mitochondrial function. Currently, WHO has determined artemisinin as the drug of choice for malaria therapy. However, in 2008, in Cambodia, it was reported that there were 2 isolates of *Plasmodium falciparum* affected by artemisinin which were observed to have a prolonged parasite clearance time. This finding indicates the resistance of *Plasmodium falciparum* to this class of drugs and this finding is the first finding related to the resistance of *Plasmodium falciparum* to the artemisinin group. Starting from Cambodia, artemisinin resistance has now spread to other countries, be it neighboring countries such as Vietnam, Laos, Myanmar and Thailand, as well as distant countries such as China, Bangladesh and India. [5]

Artemisinin resistance was defined as slowed parasite clearance. This represents partial/relative resistance which has so far only affected *Plasmodium falciparum* at the ring stage. Artemisinin is usually combined with other drugs (partner drugs) to eradicate *Plasmodium falciparum* rapidly. Therefore, despite slowing of parasite clearance, patients with artemisinin resistance can still eradicate infection as long as the combination drug is still working effectively. Artemisinin resistance rarely causes therapeutic failure, but the presence of artemisinin resistance also has certain implications, namely the risk of developing partial resistance to total resistance, failure to eradicate the parasite quickly, which can jeopardize the use of artemisinin in the treatment of severe malaria, slow parasite clearance in patients receiving currently on artemisinin treatment, which may result in more parasites exposed to the combination drug only after the artemisinin component is rapidly eliminated after 3 days of treatment. This can facilitate the occurrence of resistance of *Plasmodium falciparum* to the combination drugs used. [6]

3. Kelch-13 Mutation

Artemisinin resistance and its derivatives in *Plasmodium falciparum* are known to be related to multiple single nucleotide polymorphisms (SNPs) in a gene on chromosome 13 of *Plasmodium falciparum*, namely kelch13 (K13). The K13 protein consists of a *Plasmodium*-specific domain, a BTB-POZ domain, and a 6-blade propeller domain, in which the majority of mutations associated with artemisinin resistance occur in the propeller domain of the protein. In the eastern sub-region of the Greater Mekong, including Cambodia, Laos and Vietnam, the most common mutations found were in the alleles C580Y, R539T, Y493H and I543T, while in the

western sub-region of the Greater Mekong, including China, Myanmar and Thailand, the most common mutations were found in alleles F446L, N458Y, P574L and R561H. While in Africa, the most common K13 mutation found was in the A578S allele. [7]

The K13 mutation causes *Plasmodium falciparum* resistance to artemisinin in several ways. First, the inactive form of artemisinin will be activated by a source of Fe (II) (such as heme from hemoglobin degradation) to produce active antiretroviral therapy (ART*). ART* is reactive, and will react with parasite proteins, causing protein alkylation. In normal *Plasmodium falciparum*, protein alkylation will cause cell death and parasite death. However, in *Plasmodium falciparum* with K13 mutation, there is an increase in stress response due to involvement of the proteasome-ubiquitin pathway, so that the cells survive. [8]

The K13 protein from artemisinin-sensitive parasites binds to a transcription factor and regulates its degradation, whereas in artemisinin-resistant parasites, K13 cannot bind to the transcription factor. This then leads to upregulation of genes involved in antioxidant-associated cell responses. Under these conditions, the parasite is able to cope with oxidative stress from artemisinin better, one example is by repairing and renewing proteins damaged by oxidants. Another resistance mechanism is that the K13 protein in artemisinin-sensitive parasites will bind to phosphatidylinositol-3-kinase (PI3K) and regulate its degradation. The bond between K13 and PI3K will also reduce the number of functional PI3K. With low amounts of functional PI3K, the parasite cannot produce sufficient PI3-phosphate (PI3P) for growth. PI3P is involved in membrane biogenesis and fusion and plays a role in increasing the number of parasites that develop from ring stage to schizont. In artemisinin-resistant parasites, K13 cannot bind to PI3K, causing increased activity and accumulation of PI3K. The accumulation of PI3K will further increase the basal level of PI3P. Under these conditions, high basal levels of PI3P were able to promote the survival and growth of parasites exposed to artemisinin. [9]

K13 mutations will also provide changes in the phenotype of the *Plasmodium falciparum* life cycle. The most prominent phenotypic changes seen from *Plasmodium falciparum* with resistance against artemisinin is a change in the pattern of development in the intraerythrocytic phase in the form of an elongation of the ring stage, either with or without artemisinin. In the absence of drug stress, in *Plasmodium falciparum* with artemisinin resistance, a prolongation of ring stage development was observed up to 30 hours of its life cycle. This duration was 14 hours longer than the duration in the control group (the normal artemisinin sensitive group). The elongated ring phase is followed by a temporally shortened trophozoite stage although previously preceded by normal development in the schizont. Overcoming resistance (slowed parasite clearance) can be done in several ways. The methods include extending the duration of treatment and combining artemisinin with other drugs (not drugs combination). [10]

In malaria with K13 mutation, administration of dihydro-artemisinin (DHA) for 3 days resulted in 50 times lower parasite load reduction compared to parasite reduction in normal conditions. However, data from a clinical trial conducted in an area with a high prevalence of the K13 mutation showed that 98% treatment efficacy was achieved with extended artemisinin therapy for 6 days. Therefore, based on available data, lengthening the duration of artemisinin administration to 4-6 days in parasites with K13 mutations is predicted to give results equivalent to 3 days of artemisinin administration in normal parasites. In addition to prolonging the duration of artemisinin administration, overcoming artemisinin resistance can also be done by combining artemisinin with drugs that can reverse the resistance. The drugs commonly used are drugs from the proteasome inhibitor class such as Carfilzomib and Bortezomib. The combination of artemisinin and proteasome inhibitors has been shown to provide therapeutic efficacy against parasites with artemisinin resistance. [11]

4. Conclusion

WHO defines artemisinin resistance as slowed parasite clearance. Resistance of *Plasmodium falciparum* to artemisinin was first reported in 2009 in Cambodia and now has spread to several other countries such as Vietnam, Thailand, India, Africa, etc. One of the known causes of artemisinin resistance in *Plasmodium falciparum* is a mutation in the propeller protein of the *Plasmodium falciparum* K13 gene. Mutations were found in several alleles, including C580Y, R539T, Y493H, I543T F446L, N458Y, P574L, R561H, A578S, where each malaria endemic area with artemisinin resistance had a different pattern of mutation positions. Mutations in this gene will cause an increase in cell response to oxidative stress so that cells are not degraded even after artemisinin administration. In addition to modifying the stress response of cells, K13 mutations can also increase PI3K levels and activity thereby indirectly affecting basal levels of PI3P which are essential for parasites to continue progression from ring stage to schizont. Phenotypic changes in artemisinin resistance that appear include elongation of the ring stage and temporary shortening of trophozoite development. Resistance to artemisinin can be overcome, among others, by extending the duration of treatment (from a regimen of 3 days to 4 days) and combining artemisinin with proteasome inhibitor drugs. Based on the explanation above, it can be concluded that mutations in the *Plasmodium falciparum* K13 gene contribute to the mechanism of *Plasmodium falciparum* resistance to artemisinin drugs by increasing *Plasmodium falciparum* resistance to oxidative stress.

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