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Review Article

Grasp of dihydroartemisinin resistance in Indonesia: Focused on genetic polymorphisms and new antimalarial

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Abstract

The eastern region of Indonesia is endemic to malaria, a tropical parasitic infection that causes significant mortality. The Sustainable Development Goals (SDGs) encompass the global commitment to prevent and eliminate malaria by the end of 2030. Nevertheless, the biggest issue lies in the antimalarial drug resistance in Indonesia. Genetic polymorphism has been a considerable factor in the mechanism of antimalarial drug resistance of which could lead to inadequate activity of antimalarial drugs to undertake *Plasmodium* infection by several molecular mechanisms. Hence, first-line therapy for malaria in Indonesia such as dihydroartemisinin, piperaquine, and primaquine, becomes ineffective. However, the resistance is unavoidable. This review aims to summarize the genetic polymorphism possible mechanisms contributing to antimalarial resistance in the Indonesian population and to discuss the potential new antimalarial drug candidates.

Keywords: Antimalarial resistance, dihydroartemisinin, eastern Indonesia, imidazole, polymorphism

Introduction

Malaria is an infectious disease that causes hematological disorders leading to death but the disease is often neglected in tropical countries including Indonesia where the disease is endemic [1,2]. Malaria is caused by the *Plasmodium* parasite that is transmitted through the bite of infected female *Anopheles* mosquitoes [2]. Four *Plasmodium* species are commonly associated with human infections: *P. falciparum, P. vivax, P. ovale,* and *P. malariae*. In Southeast Asia, a fifth species, *P. knowlesi* can rarely infect humans and result in severe illness. However, it primarily affects non-human primates. *P. falciparum* is the most hazardous, exhibiting the highest incidence of complications and fatalities [2]. *P. falciparum* can have dangerous consequences, such as brain fever and death [1]. The vast majority of global malaria-related health issues are attributed to *P. falciparum* and to a lesser extent, *P. vivax* [3,4]. The malaria mortality rate due to *P. falciparum* was reported higher than *P. vivax* [5], because of its ability to develop severe anemia infecting all development stages of red blood cells [6]. The fatality rate is substantial (ranging from 15% to 25%) and those who survive may experience lasting neurological impairments [7].

Accurate and rapid diagnosis is crucial for effective malaria treatment, control, and elimination. The World Health Organization (WHO) recommends prompt malaria diagnosis in all patients through rapid diagnostic tests (RDTs). The vast majority of RDTs used worldwide are based on detecting *P. falciparum* histidine-rich protein 2 (*PfHRP2*). However, there is an issue when some isolates of *P. falciparum* lack the *Pfhrp2* gene, which can result in false negative results in RDTs [8]. In fact, Indonesia experienced a 30% decrease in diagnosing malaria cases in

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2020 [9]. The treatment of malaria also plays a crucial role in efforts to reduce the severity and spread of malaria. Unfortunately, incorrect usage has contributed to the widespread development of resistance to these medications [10].

Instead of becoming a public health concern, malaria could be a concern in the military. The world recorded malaria noticeably infected US soldiers during World War II, the Korean War, and Vietnam's conflict [11]. Moreover, relapses and new malaria cases significantly affect military personnel's productivity [12,13]. In the Lao People's Democratic Republic, military personnel in the forest are vulnerable to malaria infection and it is challenging to get the proper diagnosis and self-medication using antibiotics [11]. The Indonesian National Army is also vulnerable to malaria due to frequently operating in endemic areas [12]. Therefore, the Sustainable Development Goals (SDGs) encompass the global commitment to prevent and eliminate malaria by the end of 2030 [13]. Indonesia can potentially serve as a primary research hub for the disease due to its ability to significantly reduce malaria prevalence from 2003 to 2010 [14].

Although the malaria morbidity rate has decreased globally from 78.85 to 24.5 per 100,000 population in 2016–2018, the emerging resistance is concerning [5,13]. Antimalarial drug resistance was found in over 25% of Indonesia during the last decade, including artemisinin, the first line for malaria treatment [15]. Antimalarial drug resistance is defined by the ability of *Plasmodium* parasites to survive under proper antimalarial drug treatment in the customarily recommended dose without any absorption disorder [16]. Genetic polymorphism is one reason behind the rise of antimalarial drug resistance [17]. A previous study has identified genetic polymorphisms of the *P. falciparum* multidrug resistance gene 1 (*Pfmdr1*) gene that attenuate accumulation concentration levels in parasites for dihydroartemisinin (DHA), chloroquine, mefloquine, lumefantrine, quinine, and mono-desethyl-amodiaquine. The *Pfmdr1* gene encodes p-glycoprotein homolog 1 (*Pgh-1*), an ATP-dependent efflux pump for antimalarial drugs [18]. Several genetic polymorphisms protect host cells against malaria such as human red blood cell polymorphisms (*GYPCΔex3, CR1, CD35, G6PD*), *ADAMTS13* genetic polymorphisms, immune system genetic polymorphisms (T regulatory cells, toll-like receptors, T helper, interleukin-13), and the Duffy antigen receptor for chemokines (*DARC*) polymorphisms [19]. The *GYPCΔex3* genetic polymorphism invasively protects a subset of the parasite by associating merozoite erythrocyte-binding antigen 140 (*EBA-140*) [19]. Antibodies targeting *EBA-140* suppressed the invasion of *Plasmodium* [20]. Another mechanism involves inhibiting merozoite entry into red blood cells, disrupting intracellular parasite growth, and preventing erythrocyte lysis that occurs with parasite maturation, leading to the release of merozoites into the bloodstream [19]. Furthermore, the diversity of ethnicities and regions contributes to genetic variability and polymorphisms involving antimalarial drug regimens [21].

The genetic polymorphisms of *Pfmdr1*, which were associated with antimalarial resistance respectively in *P. vivax* parasites, were reported in most patients from northeastern Myanmar [21]. On the other hand, studies in the absence of *Kelch13*-resistant genotypes in *P. falciparum* isolates from malaria patients in Cameroon were associated with total parasite clearance, high reduction of parasite loads, and resolved symptoms [22]. Other studies concerning polymorphism have reported that genetic variations in the *Pfmdr1* and *Pfcrt* genes are associated with reduced susceptibility to aminoquinoline antimalarial drugs, especially when compared to parasites that have not been exposed [23]. In addition, a study reported the prevalence of polymorphisms in the *Pfkelch13*, *Pfcrt*, and *Pfmdr1* genes among Chinese migrant workers who returned to Guangxi Province from Africa. However, its finding had no any clinical evidence related to artemisinin resistance but suggested a positive response to chloroquine [24]. A study reported that there were differences in the efficacy of DHA-piperaquine on the genetic polymorphism of *FcγRIIA* gene between sample populations in several provinces of Indonesia such as Lampung, NTT, North Maluku, and North Sulawesi [25]. The prevalence of *Kelch13* mutations and allele diversity has been found to vary in a remote part of eastern Myanmar and this variation is associated with artemisinin resistance [26]. In Africa, out of the 240 patients who were administered intravenous artesunate, 14 exhibited signs of artemisinin resistance in vivo, characterized by a prolonged parasite clearance half-life of more than 5 hours [27]. Among these 14 patients, 13 were infected with *P. falciparum* parasites carrying mutations in either the *A675V* or *C469Y* allele within the *Kelch13* gene [27]. The aim of this review is to discuss the genetic

polymorphisms in Indonesia that contributing to antimalarial drug resistance and to present strategies for overcoming this issue including to discuss the potential new antimalarial drug candidates.

Malaria in Indonesia

Malaria poses a notable public health concern in Indonesia, impacting individuals from diverse age groups and socio-economic statuses. A study has revealed that factors like socio-demographic elements, environmental conditions, economic circumstances, cultural aspects, and behavioral patterns play a pivotal role in shaping the occurrence, intensity, and ultimate results of malaria infections [28]. The majority of Indonesia's population lives in rural areas (57%) compared to urban areas (43%) [29]. The population's age composition consisted of 30% in the young age category (0–14 years old), 65% in the productive age range (15–64 years old), and 5% in the old age group (65 years old and above) [30]. Economically, a study estimated that 73 million Indonesians (32%) lived in poverty in 2006 [31]. Furthermore, the situation in Indonesia's health sector is also worrying of which its number of doctors per population is lower (2 to 15 times fewer) than other countries [30]. These demographics can contribute to the risk of malaria outbreaks in several ways. Infants, children below the age of 5, and pregnant women have an elevated vulnerability to severe malaria symptoms and a greater likelihood of mortality because their immune systems are not fully developed or are weakened by pregnancy [32-34]. The risk of malaria transmission and the severity of the disease can be heightened by poverty and restricted healthcare access. Insufficient housing and a lack of proper sanitation in impoverished living conditions can additionally facilitate the proliferation of malaria [34]. Elevated population density, particularly in urban regions, can heighten the risk of malaria transmission because it creates a conducive environment for the proliferation of malaria-carrying mosquitoes [35,36].

Over 65 million people in Indonesia still live in malaria-endemic areas [37]. With Asmat's prevalence of 12.4%, this endemic area became the target of the Indonesian malaria elimination program [38]. The most common malaria parasites found were *P. falciparum* and *P. vivax*, with the median prevalence of 5% and 3%, respectively, in the eastern region of Indonesia [39]. Malaria outbreaks occurred almost every year in Indonesia with an increasing number of cases. Malaria cases reached 19,483 in 1998–1999, killing 66 individuals [40]. Between 2000 and 2005, the total number of cases increased to 58,152 cases and 536 deaths [41]. Malaria cases decreased during the years 2006 and 2007, with 3,705 and 1,664 cases, respectively [41-44]. At the national level, in 2021, 347 districts or cities, constituting 67.51% of the overall total were declared malaria-free. During the same year, Indonesia documented a total of 304,607 malaria instances, with the most significant concentration recorded in Papua Province, where there were 275,243 cases. This was followed by East Nusa Tenggara Province which reported 9,419 cases, and West Papua Province with 7,628 cases [45].

Antimalarial regimen in Indonesia and its emerging resistance problem

The malaria treatments used in Indonesia were chloroquine and sulfadoxine-pyrimethamine based on national guidelines [39]. A study revealed *P. falciparum* resistance to chloroquine in eastern Indonesia was significantly higher than in western Indonesia, with in vivo and in vitro data was 56% vs 43% and 64% vs 54%, respectively [29]. Sulfadoxine-pyrimethamine resistance was also reported in Indonesia but was not very significant, with in vivo and in vitro data of 18% and 64%, respectively [29]. *P. vivax* resistance to chloroquine was also commonly found in eastern Indonesia and was significantly higher than in western Indonesia, with in vivo data of 57% vs 23% [29].

Artemisinin-combination therapy (ACT) and primaquine have been replaced with chloroquine, sulfadoxine-pyrimethamine, and quinine due to the growing number of cases of antimalarial drug resistance in the past ten years [15,46]. However, ACT and primaquine regimen was given based on the high efficacy of ACT and primaquine in some studies in Indonesia [47-50]. A study from West Papua, Indonesia, reported that using ACT at a dose of 0.25 mg/kg body weight could cure malaria patients due to *P. vivax* in an abbreviated time if the patient complied

[51]. Furthermore, injection and oral ACT reduced the treatment time of uncomplicated *P. falciparum* malaria patients by 20.8% and 18.0% [52]. In addition, a study reported 95% effectiveness of ACT regimens for pediatric malaria patients in West Papua in 2016 [53]. In 2019, the Indonesian Ministry of Health made a policy of using ACT for uncomplicated malaria and intravenous artesunate for severe malaria in all types of plasmodia [54]. As a result, a study showed that the prevalence of hospitalized patients had decreased significantly, especially malaria patients due to *P. falciparum* [55]. This policy contributes significantly to the morbidity and mortality of malaria patients in Papua [55].

ACT, with DHA-piperaquine and primaquine, is the standard regimen for treating uncomplicated cases of *P. falciparum* and *P. vivax* malaria. However, it is not allowed for infants under 6 months, pregnant women, breastfeeding mothers, and G6PD-deficient individuals [46]. The mechanism of DHA induces protein damage in the *Plasmodium* and inhibits protein folding by initiating transcription factors bound to Kelch 13 proteins sensitive to DHA [56]. Resulting in disturbing protein folding progress and then triggering a stress response in the endoplasmic reticulum of the *Plasmodium* [56]. Consequently, the *Plasmodium* life cycle is discontinued. DHA also inhibits proteasomes in *Plasmodium* by generating protein breakdown in amino acid homeostasis and inhibits important cellular factors to diminish *Plasmodium* sp. infection [57].

Primaquine is another drug of choice for antimalarial, particularly in Indonesia. The liver metabolizes primaquine through two steps: (1) primaquine is oxidized into hydroxy metabolite by the enzyme CYP2D6, and (2) the hydrogenase-like carbon monoxide dehydrogenase and pyruvate ferredoxin oxidoreductase enzyme induced the second oxidation for hydroxy metabolite into quinonimines and hydrogen peroxide (H_2O_2). The human cytochrome P450 reductase (huCPR) enzyme provides quinine electrons through flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) cofactors, converting it back to hydroxy metabolite [58]. This cycle continuously brings in H_2O_2 to the liver and bone marrow, thus the accumulation of H_2O_2 leads to the death of *Plasmodium* parasites in the liver [58].

Antimalarial medications are vital tools in the battle against malaria, but their effectiveness is being undermined by the emergence of drug resistance [59]. This persistent drug resistance poses a significant hurdle to malaria control and puts at risk the renewed hopes for eradicating the disease [60]. The rise of resistance to antimalarial drugs has become a primary threat to the ongoing efforts to control malaria [61]. The development of drug resistance stands as one of the most formidable obstacles to malaria control, leading to an increase in both the incidence and death rates associated with malaria [62]. The impact of resistance on disease incidence and mortality is often underestimated [62]. Anticipating the emergence and spread of resistance to current antimalarials and newly introduced compounds is imperative for planning malaria control strategies and implementing measures that may delay the onset of drug resistance [63].

Mechanism of dihydroartemisinin (DHA) resistance in Indonesia

DHA, a derivative of artemisinin, is part of a class of drugs used in the treatment of malaria caused by *P. falciparum* [64]. It rapidly converts into DHA within the bloodstream, demonstrating 5 to 10 times greater effectiveness against malaria than artemisinin and its other variants [64]. DHA tends to accumulate predominantly within red blood cells, with infected red blood cells accumulating approximately 300 times more DHA than their uninfected counterparts [65]. The action of DHA seems to involve the production of free radicals that damage the parasite's cell membrane. This leads to the rapid destruction of all stages of malaria parasites [65].

One of the mechanisms of DHA resistance involves the efflux pump, a protein responsible for expelling drugs from the parasite's cells [66]. This efflux pump can diminish the concentration of DHA inside the parasite, thereby reducing its effectiveness [67]. Tolerance to DHA refers to the parasite's ability to survive exposure to the drug at concentrations that would typically be lethal [68-70]. The relationship between tolerance and the efficacy of DHA is intricate and multifaceted, encompassing various metabolic and cellular factors [68-70]. Factors that may contribute to tolerance include the upregulation of genes associated with apicoplast metabolism and the downregulation of genes involved in the drug's uptake and activation [68-70]. The development

of tolerance can result in reduced DHA efficacy and contribute to the emergence of resistance [71].

Genetic polymorphism and antimalarial resistance in Indonesia

The issue of genetic variation and resistance to antimalarial treatments in Indonesia is highly significant due to the considerable threat it presents to the effectiveness of the nation's malaria control efforts [72]. Genetic variations in specific genes linked to drug resistance have been recognized as a primary factor contributing to antimalarial resistance in the Greater Mekong Sub-region, encompassing Indonesia [73]. Kelch13 proteins play a critical role in DHA's drug mechanism of action. Its mutations induce the onset of drug resistance, the two pathway mechanisms are presented in **Figure 1**. The first pathway is the *C580Y* mutation which is correlated to the increased levels of phosphatidylinositol-3-kinase (PfPI3K) and lipid phosphatidylinositol-3-phosphate (PI3P) in *P. falciparum*. Hence, the failure to carry out PfPI3K proteolysis results in DHA resistance [74]. The second pathway is by the mutation in the Kelch 13 protein compartment, preventing endocytosis of the host cell, and DHA resistance occurs [75].

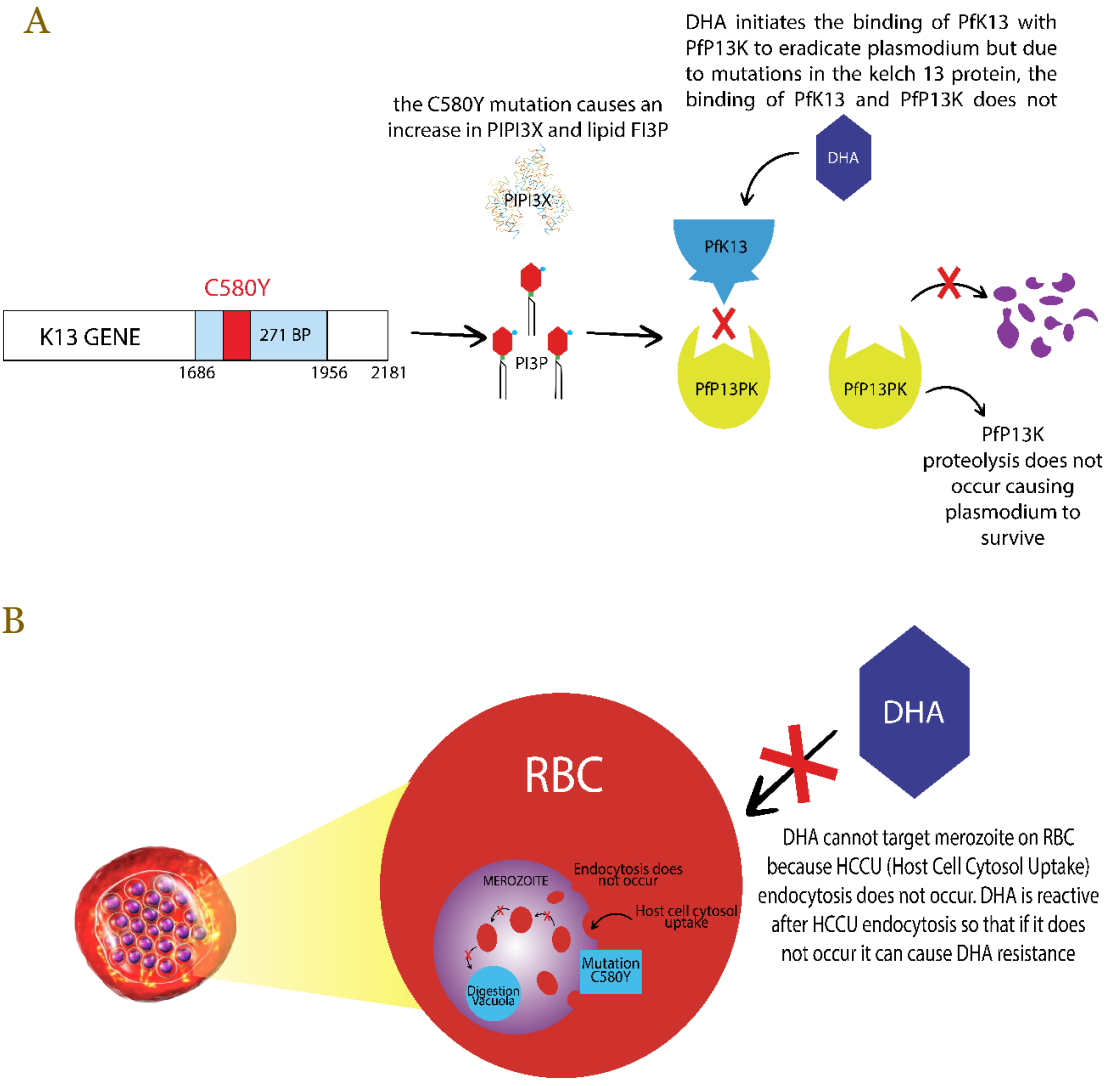

Figure 1. *Kelch13* mutation pathways inducing drug resistance. (A) *C580Y* mutation. The elevated levels of phosphatidylinositol-3-kinase (PfPI3K) and lipid phosphatidylinositol-3-phosphate (PI3P) in *Plasmodium falciparum* lead to dihydroartemisinin (DHA) resistance due to the inability to perform PfPI3K proteolysis. (B) Kelch 13 protein compartment mutation. The second pathway involves *Kelch13* gene mutations, which block host cell endocytosis and result in DHA resistance.

Kelch-like ECH-associated protein 1 (KEAP 1) is also similar to Kelch 13 protein, its mutations can also lead to artemisinin resistance. Mutations in KEAP 1 proteins induce the protein damage encoded by *KEAP1* and nuclear factor erythroid 2-related factor 2 (*Nrf2*) genes, which reduces the oxidative stress response of the *Plasmodium* and avoids death (**Figure 2**) [76].

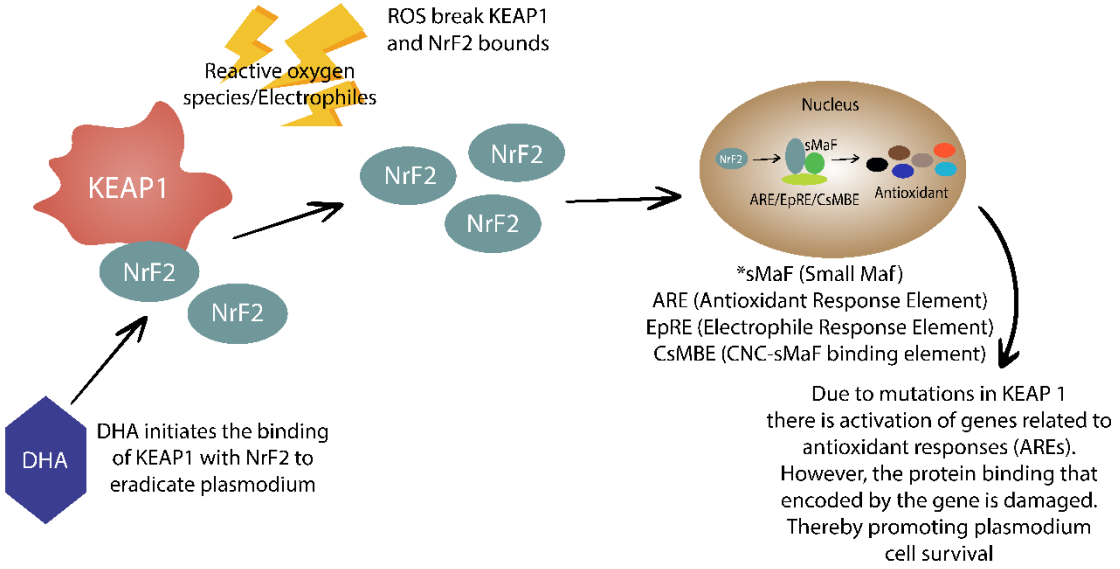

Figure 2. Dihydroartemisinin (DHA) resistance mechanisms linked to mutations in Kelch-like ECH-associated protein 1 (KEAP 1). Mutations in KEAP1 protein can lead to artemisinin resistance, similar to Kelch 13 protein, by causing protein damage encoded by *KEAP1* and nuclear factor erythroid 2-related factor 2 (*Nrf2*) genes, thereby diminishing *Plasmodium*'s oxidative stress response and preventing its death.

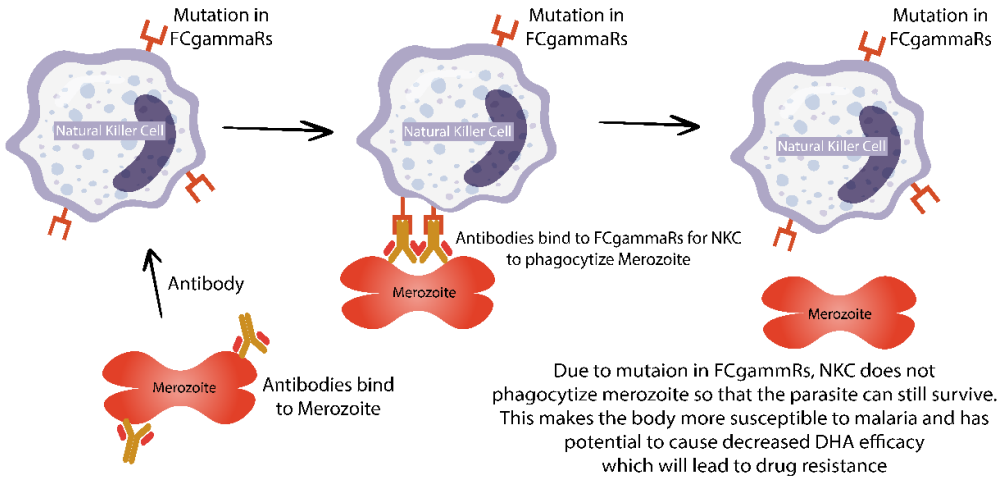

Figure 3. Dihydroartemisinin (DHA) resistance pathway associated with *FcγRIIA* gene mutation. Susceptibility to *Plasmodium* infection is a result of *FcγRIIA* polymorphisms leading to the ineffectiveness of antibodies.

A study reported *FcγRIIA* polymorphisms in the Indonesian population from different cities, including Lampung, Southwest Sumba, South Halmahera, Sikka, and Southeast Minahasa [25]. Genotypes R/R, R/H, and H/H reported in the study exhibited the presence of the R and H alleles, polymorphisms in these alleles are associated with density parasitemia; red blood cells infected with *Plasmodium*. Parasite density is classified into high and low density. High-density parasitemia occurs when the density of parasites in red blood cells counts more than 5,000 parasites/µL. The number of high-density parasitemia (HDP) prevalence obtained in the reported study was 33, where the H allele is higher than the allele R. The H allele dominates in Eastern

Indonesia (Southwest Sumba, South Halmahera, Sikka, and Southeast Minahasa). In contrast, the R allele dominates among the people in western Indonesia (Lampung). This presents that Eastern Indonesians are more susceptible to malaria compared to the western region of Indonesia [25]. Based on the study findings, the H allele could affect the efficacy of DHA by *FcγRIIA* polymorphisms causing susceptibility to *Plasmodium* infection due to ineffective antibodies (**Figure 3**). **Table 1** summarizes the mechanisms of DHA resistance and the responsible polymorphisms. The mutations not only affect DHA drug metabolism but also have implications for inhibiting parasite proteolysis by PfPI3K, disrupting host cell endocytosis, reducing the *Plasmodium* oxidative stress response, and impeding merozoite degradation.

Table 1. Summary of dihydroartemisinin (DHA) resistance

Polymorphisms	Mechanism effected	References
C580Y mutation in *Kelch13*	The elevation of PfPI3K and PI3P levels has implications for the inability to bind PfPI3K that has been polyubiquitinated with Kelch 13 protein, subsequently hindering the inhibition of PfPI3K proteolysis.	[74]
	The endocytosis of the host cell is unfeasible. In the absence of host cell endocytosis, DHA becomes resistant.	[75]
Mutation in *KEAP1*	Disrupt the interaction between the protein encoded by KEAP1 and *Nrf2*, thereby diminishing the *Plasmodium* oxidative stress response. This occurs because *Plasmodium* resides within cells.	[76]
G1937A mutation in CYP2D6*4 *C34T* mutation in CYP2D6*10	Poor and intermediate metabolizers	[77,78]
FcγRIIa-R131 and *FcγRIIa-H131* mutations in *FcγRIIa*	The eradication of merozoites within red blood cells is disrupted, resulting in the persistence of *Plasmodium* infection within the red blood cells.	[25]

Natural products as new antimalarial drug candidates

The need for new antimalarial drug candidates has risen as a result of increasing resistance to existing drugs [79]. Natural products are considered promising reservoirs of potential candidates due to their wide array of chemical structures and biological effects [80]. The ethyl acetate and methanol extract of the *Callistemon citrinus* plant showed antiplasmodial activity with a half maximal inhibitory concentration (IC50) value of around 8.4–13.0 µg/mL [81]. *Helianthus annuus* roots also revealed antimalarial activity, proven through in vivo and in vitro tests by evaluating β-hematin levels, results reported to inhibit heme detoxification [82]. A study conducted on herbal-based traditional medicine in Papua Island, Indonesia, reported that *Alstonia scholaris* L., *Carica papaya* L., *Andrographis paniculata*, and *Physalis minima* L. were often used as antimalarials [83].

Table 2. Antimalarial natural products

Natural product	Study design	Result	Mechanism	References
Hydroxytryptamine (5-HT) *Andrographis paniculata* leaf extract	*Plasmodium* is cultivated in vitro in human O^+ red blood cells and diluted to a 1% hematocrit in roswell park memorial institute (RPMI) 1640 medium with gentamycin (20 g/mL), 23.78 mM $NaHCO_3$, 7.68 mM Hepes buffer, and 10% human O^+ red blood cell serum. The observed parameter requires evaluating the number of	IC50 value suggests hydroxytryptamine (5-HT) *Andrographis paniculata* leaf extract's effectiveness as an antiplasmodium agent against chloroquine-resistant *P. falciparum* strain G-2300.	Interfering with the cell membrane of the parasite and preventing it from generating new cells.	[84,85]

Natural product	Study design	Result	Mechanism	References
	infected red blood cells within a 1000-cell sample.			
Dendrophthoe pentandra extract	The study involves 60 Swiss strain white mice as its subjects, which were subsequently infected with *P. berghei*. The experimental solution was given over a span of 4 days and the parameter under observation involves determining parasitemia levels.	The findings indicate that the extract derived from *Dendrophthoe pentandra* exhibits antiplasmodium properties, with an effective dose (ED50) of 146.2 mg/kg of body weight.	Inhibition of the folate pathway or influencing DNA synthesis.	[86]
Isolate bark *Lannea coromandelica*	The evaluation of parasite growth in a controlled culture by employing hypoxanthine isotopes. After a 60-hour incubation with healthy, uncontaminated cultures, a combination of 50 ml of roswell park memorial institute (RPMI) and serum containing 0.25 µCi of isotopes was introduced. The culture was then mixed thoroughly and subjected to an additional 12-hour incubation at 37°C, making it a total of 72 hours of incubation. Parasites were subsequently collected using a semi-automated cell harvesting technique and the incorporation of radiolabel was determined using a Liquid Scintillation Analyzer.	The study's findings reveal that the ethyl acetate isolate exhibits a moderate level of antiplasmodium activity, with an IC50 value of 2,727 µg/ml.	Chelating cations to block various nucleic acid synthesis processes, disrupt protein synthesis, and impede the new permeation pathway (NPP) in infected erythrocyte membranes .	[87]
Carica papaya leaf extract	The experiment involved testing the in vitro antimalarial activity against *P. falciparum* strain G2300 using an extract from papaya leaves dissolved in dimethyl sulfoxide (DMSO). The testing was conducted on a flat titration plate with 24 wells, using a 1% parasitemia level and a hematocrit of 5%. Subsequently, the percentage of parasitemia and growth percentage was calculated.	The antimalarial test outcomes for papaya leaves of the Cibinong variety indicate an IC50 value of 2.7821 nanograms/milliliter, while the Solo variety of papaya demonstrates an IC50 of 2.14279.	Chelating cations to block various nucleic acid synthesis processes, disrupt protein synthesis, and impede the new permeation pathway (NPP) in infected erythrocyte membranes .	[88]
Methanol extract of *Alstonia scholaris* bark	Methanol extracts from various plant species were subjected to desiccation and employed for evaluating their biological activity as antiplasmodial agents in an in vivo context. The in vivo appraisal of antiplasmodial efficacy was carried out through a 4-day suppressive test on Swiss mice that had been infected with *P.*	The in vivo assessment of antiplasmodial activity using methanol extract from *Alstonia scholaris* tree bark yielded an effective dose (ED50) of 29.78 mg/kg body weight, classifying it as excellent.	Chelating cations to block various nucleic acid synthesis processes	[89]

Natural product	Study design	Result	Mechanism	References
	berghei. These mice were categorized into control groups (those lacking test substances) and treatment groups, with each group comprising 5 mice.			

Plasmodium is a parasite carried by female *Anopheles* mosquitoes. In the form of sporozoites, they enter the bloodstream while mosquitoes release saliva to prevent blood clotting, making it easier for them to feed on human blood. These sporozoites travel to liver hepatocyte cells where they multiply asexually to form merozoites [90]. Antimalarial drug resistance appears after the sporozoite successfully infects the liver. A new mechanism has been identified in inhibiting sporozoites from bonding with any of the receptors in hepatocyte cells, one of them is CD81 with a binding site of helix D. This receptor is related to HCV and malaria. The receptor interacts with hepatitis C virus-E2 (HCV-E2) when infecting the liver and induces hepatitis C disease [91]. It also interacts with sporozoite in hepatocytes when it develops into merozoite [92]. The 21 amino acid sequences and the D137 amino acid are crucial for the binding of CD81 to the circumsporozoite protein [93]. Therefore, if a chemical compound inhibits the binding site, sporozoites cannot infect the liver. Imidazole is a chemical agent that can inhibit the binding of sporozoites to CD81 [91]. First, imidazole will be synthesized into N,N-substituted imidazole-4,5-dicarboxamides (I45DCs), which resemble helix D. Sporozoite will recognize I45DCs as CD81 and binds to it, consequently, refraining the sporozoite from infecting the liver (**Figure 4**). Additionally, there may be sufficient time for antibodies to phagocytose the sporozoite.

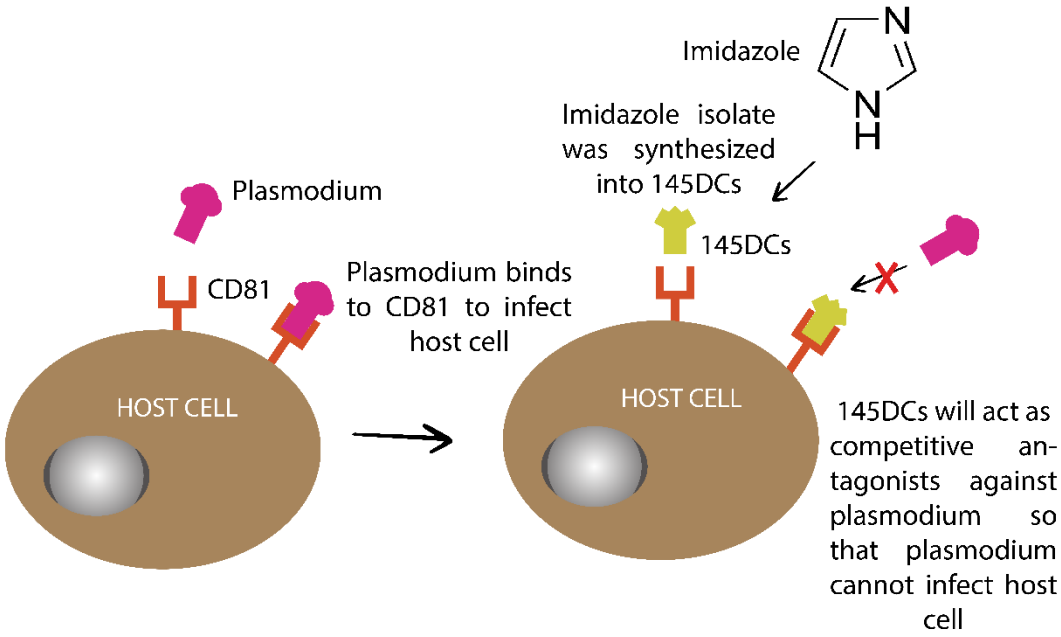

Figure 4. Competitive antagonism mechanism of imidazole against *Plasmodium* at the CD81 receptor. Sporozoites identify I45DCs through CD81 and attach to them, which, in turn, prevents the sporozoite from infecting the liver.

Imidazole has been isolated from the plant roots of *Lepidium meyenii*, *L. culinaris*, and *Sponge dercitus* (Halinastra) japonensis [94-96]. A root extract from *Lepidium meyenii* has yielded two recently discovered imidazole alkaloids. These compounds have been identified as 1,3-dibenzyl-4,5-dimethylimidazolium chloride (1) and 1,3-dibenzyl-2,4,5-trimethylimidazolium chloride (2) [94]. A new imidazole sulfate was isolated from the sponge [95]. 5-Dimethylamino-naphtalene-1-sulphonyl-Imidazole was obtained from the seeds of *Lens culinaris* [96]. Several other studies have conducted extractions or isolations of imidazole from plants commonly found in Indonesia, namely *Zingiber officinale*, *Annona muricata*, *Shorea* sp., and *Melastoma malabathricum* L. [97-100].

Conclusion

Kelch 13 protein polymorphism responsible for DHA resistance in Papua, Indonesia. *FcγRIIA* polymorphism was also a contributing factor in susceptibility to *Plasmodium* infection due to the ineffectiveness of antibodies. In the light of imidazole, this sparks a new strategy in discovering novel compounds for antimalarial, possibly exploring Indonesia's biodiversity for further research. It inhibits the binding of sporozoites to CD81 hence presumably a key step in the malaria infection process. Furthermore, there might be an adequate timeframe for antibodies to phagocytose the sporozoites. Nevertheless, ensuring the safety and efficacy of imidazole as an antimalarial agent requires further research to clarify the specific molecular interactions involved in sporozoite binding to host cells.

Ethics approval

Not required.

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

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