Review Article

A Path Towards Eliminating Malaria: Drug Resistance A Major Roadblock? - 🔓

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ABSTRACT

Malaria has remained a life threatening disease in South-East Asia and Sub-Saharan Africa for decades. Many efforts have been spent on Global and regional surveillance of malaria burden. Over US$ 2.7 billion was invested in malaria control and elimination globally in 2018. Insecticide-treated mosquito nets, rapid diagnostic tests andArtemisinin-based combination therapy are the three major pillars that malaria control and elimination currently relies on. Insecticide resistances as well as the anti-malarial drug resistance have emerged as the major road blocks in winning the war against malaria. Here we discuss in details the artemisinin chemotherapy, the resistance phenotypes associated with its widespread use and the molecular marker associated with artemisinin drug resistance. Specifically we discuss the surveillanceof K13 genotypes as an essential step in tracking the ART drug resistance in malaria laden areas.

Keywords: Malaria; Plasmodium falciparum; Drug resistance; Artemisinin; K13

INTRODUCTION

Malaria has been the widespread and deadly parasitic infection caused by anopheline mosquitoes. It has become a global issue with 214 million recent cases and 438,000 deaths in 2015, mainly in the sub-Saharan African regions [1]. Over the last decade, malaria endemic regions have determined the dropping rate of malaria and shifting the focus from reducing to eliminating this life-threatening disease. It has been shown in the past that the decrease in malarial spread is achievable but very hard to sustain. The worldwide campaign to eradicate malaria has seriously failed due to the development of parasitic resistance to efficient antimalarial drugs and resistance of mosquito to insecticides. Now the global concern remains to introduce efficient drugs as a replacement of old and failed drugs [2,3].

Humans are affected by mainly four types of parasitic Plasmodium, but the falciparum species is notable for the majority of fatalities worldwide. Most of the studies are focused on the falciparum malaria, but more efforts should be directed towards the interpretation of other malaria species that would help us grasp the severity of malaria infections and design better intervention strategies [4,5]. At the same time, it is need of the hour to look for new vaccine candidates and design vaccines using novel strategies for malaria elimination [6-8]. It has been noted as the species, rendering dreadful malarial syndromes, has developed resistance to every antimalarial compound available. The use of antimalarial drugs like chloroquine, sulfadioxine and pyrimethamine has been extensively implemented in the past, and therefore, been misused. The parasites have developed resistance under selective pressure due to the preparation of antimalarial drugs on a broad scale [9,10]. When the parasites developed resistance to these antimalarial drugs in Southeast Asia, the P. falciparum endemic regions, mefloquine replaced other drugs, but soon resistance developed for this compound [11].

ARTEMISININ (ART) AND ARTEMISININ-BASED COMBINATION THERAPY (ACT)

Except one notable drug – Artemisinin (ART), resistance has been developed to all known antimalarial drugs. For centuries, Artemisinins have been used as traditional Chinese herbal medicine, derived from the plant, Artemisia annua and proved its efficacy against the life-threatening disease [12,13]. To prevent the emergence of resistance, artemisinins in combination with partner drugs, where other antimalarial drugs like chloroquine, sulfadioxine-pyrimethamine etc. are used as partners, are widely used. Due to the high potency of ART with slow-acting and less potent partner drugs, ACTs are known for higher parasite killing rate, lack of side effects and absence of resistance [14-16].

The ARTs being highly active against the asexual cycle of P. falciparum are capable of reducing the biomass of the malarial parasite along with short half-life (<1h) of ARTs in plasma, which necessitates the use of long-lasting partner drugs [17]. However, in spite of the significant usage, clinical ART resistance has not yet been demonstrated. Interestingly, the mode of action and inhibition of parasite growth regarding Artemisinin remains a curious case and a mystery to-date [18,19].

Derivatives of artemisinin that include dihydroartemisinin, artemether, arteether and artesunate and many others are called the first generation derivatives of ART and thus synthesized and used in treating malaria [20]. These derivatives are sesquiterpene lactones known for their high activity and rapid elimination of malarial parasites almost at all stages of development [16]. The suggested mechanism for ART activation is a Fe-heam mediated process cleaving the endoperoxide moiety of the ARTs and forming the Reactive Oxygen Species (ROS), which targets the nucleophilic groups in parasitic proteins and lipids. The artemisinin is known to covalently bind to 124 parasitic proteins, most of which are involved in biological metabolism essential for survival.

As recommended by WHO in 2001, Artemisinin-Based Combination Therapies (ACTs) are widely used as first-line multidrug-treatment resistant to P. falciparum [21,22]. In Southeastern Asia, artesunate-mefloquine has been effectively used for uncomplicated malaria caused by P. falciparum. The ACT being widely used and recommended is the dihydroartemisininpiperquine in the Southeastern countries due to its promising efficacy [15]. White and others suggested that ART derivatives used along with antimalarial partner drugs could rapidly decrease the parasite density to a minimum, whereas keeping the optimum levels of the by longer activation of the drug components [23]. High efficacy of ACTs has been shown in the past in treating uncomplicated malaria in Asia and Africa; local data is not available in spite of the clear determination of antimalarial inefficiency during recent years [24-28].

The efficacy of ACTs was demonstrated in Afghanistan by one of the recent studies while conducting clinical trials utilizing a combination therapy of an ART derivative - Artesunate (AS) and Sulfadoxine-Pyrimethamine (SP). This study indicated the presence of drug resistant alleles which did not develop resistance against ACT treatment and hence, proved efficient for the intervention of malaria caused by P. falciparum [29].

EMERGENCE OF ART RESISTANCE

ART resistance phenotypes

Since a few years, ART resistance has emerged as a rising concern. The first report of ART resistance dates back to early 2000s near the

Thai-Cambodian border for which the results are still ambiguous. The signs of inefficacy of ACTs and artesunate monotherapy were clearly indicated in western Cambodian artesu nate-resistant parasitic isolates [30]. According to WHO, the emergence of piperaquine resistance in association to ART resistance and its aid in the selection of piperaquine-resistant parasites are contemporarily unclear. It is, however, suggested that the piperaquine resistance may have independently emerged due to the long life of piperaquine and its prior use as monotherapy [31].

Various recent clinical, in vitro, transcriptomics and genomic studies in Southeast Asia have outlined the in vivo and in vitro ART-resistant phenotypes, determined its genetic basis, and have studied its clinical impact. Partial resistance is offered by the slow parasite clearance rates expressed only in the early-ring stages of the parasite [16]. A productive insight was provided by Duru and others in demonstrating the failure of ACTs, particularly dihydroartemisinin-piperquine in Cambodian isolates. All of the parasites in this study indicated the selection of parasite that were already resistant to artemisinin [32].

Clinical ART resistance

Clinical ART resistance can be defined as heightened half-life clearance of the parasite or the presence of detectable parasites on the 3rd day of ACT intervention. The parasitic half-life is highly associated with the in vitro and ex vivo Ring-Stage Assays (RSAs), which evaluate the endurance of the initial ring-stage parasites exposed to the 700nM dosage for 6 hours of the active metabolite of ART – the DHA (dihydroartemisinin) [17,33]. Nonetheless, the definition of clinical resistance is affected by various factors like host immunity, drug concentration in blood or activity of partner drug in the Artemisinin-Based Combination Therapy (ACT) [34]. No matter how impressive the gains of the ARTs and ACTs, the emergence of ART resistance has been noted in Greater Mekong Subregion (GMS) (Laos, Cambodia, Thailand, Vietnam, and Mayanmar), which can lead to disastrous effects of malariaand an eventual spread to the African sub-continent.On the other hand, the risk of ART resistance in the malarial isolates is a greater problem as compared to the failure of the chloroquine and sulphadioxinepyremethamine resistance in various parts of the worlddue to its emerging resistance to falciparum malaria where other drugs have failed [17,22,35,36].

PARASITE CLEARANCE RATES

In order to elaborate the parasite clearance rates in Upper Mayanmar despite the presence of ART resistance, another study revealed the therapeutic effect of another ART derivative - Dihydroartemisinin-Piperquine (DP). Tunet al. evaluated the median half-life of the parasite and determined it to be less than 5 h (4.7 h) due to the frequent evaluation of parasitaemia indicating an intermediate resistance as compared to other types of mutations in the relevant region. Also, the importance of relation of site with the delayed parasite clearance rate [33,37]. In a study by Amaratunga, et al. [38] the clearance of parasites took longer time than usual with a half-life of 11.28 h, indicating the widespread presence of ART-resistant phenotype outside Palin, Cambodia. It was also noted that some host factors accounted for the greater half-life while 40% half-life variation was due to parasite genetics. The identification of parasitic genetic clusterwas found corroborated with the genetic basis for the ART resistance phenotype.

The delayed parasite clearance rate and resistance has also been noted due to the presence of resistant parasitic hypnozoite reservoirs

as observed in P. falciparum and P. vivax [39]. Other studies relate that the parasitic clearance half-life depends upon the susceptibility of the parasite to ART as well as on the developmental stage during ART treatment [17,36].

However, ART resistance remains undetected due to the inefficacy of resistance phenotypes in drug susceptibility assays in vitro. Some success has been achieved using advanced in vitro assays providing an insight into the parasitic susceptibility at developmental ring stages in the erythrocytes. Moreover, artemisinin-resistant phenotypes have been reported with reduced susceptibility to ART in a T_0 [3H] hypoxanthine assay during the development of ring stage, prolonged resistant (ring) stage and reduced trophozoite stage during development. The extended resistant stages and temporary compression of the most susceptible developmental stage are observed to be highly associated with ART resistance. The altered pattern of development in the parasitic cell cycle is due to the increasing practicality of the ART-resistant parasites during ART exposure at ring stages. Such phenotype in the assayed samples indicates shortened asexual life cycle of the parasite. These novel phenotypes provide an opportunity to detect the function of mutations linked to ART resistance and determining molecular markers linked to ART clinical resistance [17].

IDENTIFICATION OF K13 MUTATIONS AS A MOLECULAR MARKEROF ART RESISTANCE

In order to locate the gene responsible for artemisinin resistance, Genome-Wide Association Studies (GWAS) were carried out. The association of delayed clearance parasite rates with P. falciparum in Southeast Asia was indicated. After a Single-Nucleotide Polymorphism (SNP) assay, Takala-Harrison, et al. [40] via linkage-disequilibrium windows used as a marker of the decelerated clearance rate, recognized that four SNPs on chromosomes 10, 13, and 14 were related closely to the delayed parasite clearance. The SNPs on chromosome 10 and 13 indicated association with the genes involved in a DNA damage-tolerance pathway. These SNPs were later linked to the genes PF3D7_1343700 (Kelch 13) and PF3D7_1459600 (ENTH domain containing protein involved in clathrin mediated endocytosis) through an approach based on population genetics.The GWAS analysis has been utilized to highlight the heritable traits of clinical ART resistance and positive selection in geographical regions of ART resistance.

In recent studies, the K13-propeller mutations have been associated with artemisinin resistance in vivo and in vitro in Southeast Asia [36]. In relevance with the previous research, clinical resistance to ARTs have shown delayed parasite clearance rate, parasite clearance half-life of >5h, presence of heritable Kelch propeller mutations in the Pf3D7_1343700 domain, and its rapid spread, as noted in Southeast Asia [22,41]. It has now been proved via screening of the ART-resistant P. falciparum with K13 mutations that the presence of mutated P.falciparum has passed beyond the classical western Thai-Cambodia border [42].

As simple as it seems, the genetic basis for ART resistance is challenged by the introduction of molecular markers, which revealed a complex mystery that remains unsolved. With the objective to identify the genetic basis of ACT traits for adaptation, Cheeseman et al. [43] utilized a two-stage strategy to determine the genetic basis for underlying gene selection by comparing three geographical regions (Cambodia, Thailand and Laos). Screening and genotyping of 91

parasite clones determined Single-Nucleotide Polymorphisms (SNPs) and Copy Number Variations (CNVs). As a result, geographical differentiation and haplotype structure at 6969 SNPs determined a region of strong selection on chromosome 13 which corresponds to the decelerated parasite clearance rates.

Airey, et al. [36] conducted an expeditious study to analyze mutations in laboratory-adapted parasite clones selected for survival while receiving high ART dosage in vitro. The information yielded by such analysis can guide polymorphism analysis in ART-resistant parasitic samples from Cambodia. Sequencing an ART-sensitive F-32 Tanzania parasite line indicated artemisinin resistant K13 propeller mutations during DHA treatment in the RSA. The presence of linked-disequilibrium around genes indicated four K13 propeller mutations (Y493H, R539T, I543T, and C580Y) in the naturally-occurring parasites in Cambodia. These mutations were found associated with long parasite clearance half-life and higher frequency of survival rates in the RSA $^{0-3h}$. It was also found that different levels of K13 mutations present varying levels of ART resistance, which indicate the genetic background of the parasite influencing these levels. The need for a molecular marker persists to detect and control the widespread ART resistance. Thus, it was concluded that the prevalent mutations can be utilized as markers to determine the decelerated parasite clearance rates in malarial patients receiving ART treatment. Similar studies were conducted by Ye, et al. [14] who identified the K13-propeller region of the P. falciparum gene as a molecular marker to detect artemisinin-resistant parasites in vitro. K13-propeller mutations are also able to identify parasitic clearance half-life (>5h) with 98.1% ART sensitivity and 88.4% host specificity.

Recent studies elaborate that the frequency of K13 mutations increase with ART usage, along with the purified selection working on the propeller region of the K-13 gene. The population of parasites unexposed to ACTs provide the fundamental information about K13-propeller gene behaving as a molecular marker of the ART resistance and elaborate a case of positive selection in the untreated propeller domain. Moreover, many contrasted K13-propeller mutations occur under ART pressure [44]. In vitro assays have determined decline in the P. falciparum susceptibility to ART, but no proof has yet been discovered. Feng and others accessed five mutations with three being recent. F446I mutation was predominant among the samples collected from China-Mayanmar border indicating the risks for emerging resistance in the Greater Mekong Subregion (GMS) [45]. Another molecular marker A578S was determined by Hawkes and associates in order to elaborate the genetic basis for the spread of malaria in the Ugandan Children. Being the severe case of malaria in Uganda, the nonsynonymous SNP A578S in the K13 gene may be another acknowledged marker, although not being directly associated with ART resistance, determining a delayed response and parasitic clearance rate to ART derivative [46]. Using the PCR method, DNA templates were derived from frozen samples for examination and measurement of the Plasmodium parasite by Tripura, et al. [39]. The authors related the decreased ART component drug sensitivity with the K13 propeller mutant gene (C580Y) in an attempt to prevent, treat and eliminate infections caused by P. falciparum and P. vivax.

Another breakthrough regarding the K13-propeller mutation (C580Y) has raised a concern among various studies conferring to its widespread resistance to artemisinin [39,47-51]. The C580Y is more prevalent than other ART resistant molecularmarkers, although it has not been observed as a consistent marker for ART resistance [33,36,37]. It is nonetheless a confirmed molecular marker of the K13

gene predominating along the Thailand-Mayanmar and Cambodia-Thailand border, while the F446I predominates along the Mayanmar-India and China-Mayanmar border [16]. Sequencing and genotyping of the PfK13 of 98 P. falciparum isolates in Guyana by Chenetet et al. [49] determined the presence of K13 mutation (C580Y) in strong association with drug resistance. Recently, a successful study expressed K13mutation (C580Y) in the genetically engineered clones of P. falciparum using the CRISPR-Cas9 system and demonstrated slow parasitic clearance rate. Through this study, direct link was established between K13 mutation and ART resistance [52].

Consequently, in a research conducted by Straimeret, et al. [17] P. falciparum K13 locus were genetically modified using zinc-finger nuclease and the ring-survival rates were evaluated after drug exposure in vitro. These studies suggested the decrease in the parasitic survival rates after removal of K13 mutations from ART resistant Cambodian isolates. In contrast to relevant observations, Straimer and others detected higher resistance in some K13 mutations (M4761,R493H, I543T) than other K13 mutations (Y493H and C580Y), indicating the modest resistance of C580Y being predominant mutant allele in Cambodia. Also, it suggested that additional factors were involved in augmenting K13-mediated resistance in the Cambodian isolates.

K13 POLYMORPHISM ACROSS THE GLOBE

Since the definite ART resistance phenotype is uncommon, the association of polymorphisms in marker genes is hard to relate with efficient results [53]. According to Mitaet, et al. 60 non-synonymous mutations have been identified in the K13 gene [44], while Fairhurst and Dondrop indicate that only 20 of 124 nonsynonymous K13 mutations can be associated with ART resistance (P441L, F446I, G449A, N458Y, C469Y, A481V, Y493H, S522C, G538V, R539T, I543T, P553L, R561H, V568G, P574L, C580Y, D584V, F673I, A675V, and H719N). However, only four of these have been validated in vivo and in vitro: Y493H, R539T, I543T, and C580Y [16].

Recently, Arieyet, et al. exposed the association of K13-propeller polymorphisms with ART resistance. They showed the strong relation between ART resistance and four K13-propeller polymorphisms, i.eC580Y, Y493H, R539T, and M476L [36]. Similarly, many SNPs have been reported in K13-propeller gene along with multiple origins of the K13-propeller polymorphisms across the world, including Africa as well as Southeast Asia [53,54]. As indicated by Tanabe et al., numerous SNPs along with haplotype (3D7 sequence) exist in the four continents being geographically distinct and continent specific [55]. The fundamental information about K13-propeller gene behaving as a molecular marker of the ART resistance provided a case of positive selection in the untreated propeller domain [44].

The K13 propeller polymorphisms are known to exist widely around the world. According to a report by Edwards et al., the dispersion of malarial infection becomes widespread due to the shifting populations of higher transmission areas to lower transmission areas, which in turn retard the control and elimination of the dreadful disease by importing the infection and spreading drug resistance. Such has been demonstrated in the cross Cambodian border, the French Island of Mayaotte, and China, where the malarial infections are mainly imported from other regions or transmitted locally [45,56,57].

Reports of independent global emergence of K13 mutation in a variety of locations like Mayotte(N490H, F495L, N554H/K, and

E596G) and Guyana(C580Y) have been received recently, although no information on the clinical or phenotypic resistance has been noted in these isolates [49,57].To mark a standard for the spread of K13 polymorphisms, a novel study sequenced and genotyped 581 P. falciparum K13-propeller isolates from Asia, Africa, Maleneia and South America collected before and after ACT intervention. The population of isolates exposed to drugs showed higher frequencies of mutations, nucleotide and haplotype diversity as compared to the unexposed parasite population. Further indications included the prevalence of C580Y mutations earlier than that of the first report of ART resistance in 2007 [2,21,44].

A global analysis was conducted by Menard et al to map the K13-propeller polymorphisms. The authors utilized 14,037 samples from 59 countries and sequenced K13-propeller polymorphisms to evaluate the emergence and dissemination of mutations by haplotyping neighboring loci. Isolates having a similar K13 mutation were related genetically by evaluating two adjacent loci. Such phenomenon revealed the emergence of events beside the spread of mutations for ART resistance. Also, the difference of mutations and haplotypes in the two resistance regions in Asia suggested selection pressure in the relative areas due to the usage of ACTsmainly. The ratio of heterogenous nonsynonymous K13 mutations in Asia ranged from fixed to high in western Cambodia, intermediate in Mayanmar and Vietnam, moderate in eastern Cambodia, Thailand, China and Laos, and low everywhere else. K13 mutations were reported uncommon in South America, Oceania and Africa except a few African nations [58]. It is however interesting to learn that the K13 polymorphisms associated with ART resistance in Southeast Asia (Y493H, R539T, I543Tand C580Y)have been absent in the sub-Saharan regions and in contrast to it, the other non-synonymous SNPs identified in sub-Saharan regions have not been observed in the Southeast Asian P. falciparum isolates. Thus, it is proposed that the K13 polymorphisms can vary geographically and determination of K13 propeller genetic studies can reveal and monitor the global emergence of resistance to ART [34,59,60].

In relevance to the previous studies, other K13 polymorphisms were studied by Tacoli et al. Two K13 polymorphisms (P574L and A675V) are ubiquitously present in Southeast Asia and associated with decelerated clearance rate. The relevant inquiry also reports that the K13 polymorphism P574L was observed for the first time in Rwanda, suggesting their unique presence with the inclusion of strains linked to ART resistance [61]. However, the low prevalence of K13 propeller in Africa was confirmed by Torrentino-Madamet et al while identifying K13 propeller polymorphisms in the P. falciparum isolates collected from 29 patients receiving AL treatment on the French Island of Mayotte in 2013-2014 [57]. A study by Duru and colleagues indicated the limited genetic diversity of the parasites showing the presence of K13 polymorphisms in almost every parasite isolate from Cambodia [32]. However, the ART resistance was noted to be confined to Southeast Asia and China. Since ART-resistant K13 mutation has not been prevalent in Africa, the abundant presence of K13-propeller polymorphism A578S and others have been reported by various studies which pose serious threat to K13 propeller functioning [58,62,63].

Though it is crucial to urgently develop and implement targeted interventions to contain and eliminate ART resistance to its current locations [42], what is more alarming is the independent emergence of K13 mutations in multiple geographic locations suggesting that efforts to eliminate artemisinin-resistant malarial parasites in one

region may have a limited impact on the emergence of resistance in neighboring regions.It further highlights the need to map K13 mutations throughout the malaria-endemic world. This report is consistent in the studies determining polymorphisms in Haiti [59], Uganda [46,64,65], Angola and Mozambique [34], Rwanda [61], Kenya [66,67], Ethiopia [68, 69], Senegal [70], Mayotte [57], Southeast Asia [40], Cambodia [36], Vietnam [71], Bangladesh [72], China [51][73].

MECHANISM OF ART RESISTANCE AND K13 POLYMORPHISM

Since K13-propeller mutations are highly prognostic of resistance, the knowledge of underlying mechanisms that yields ART-resistantP. falciparum remains unknown [74]. It has been reported that the range of K13 mutations and development of ART resistance by single mutations point towards the declining functionality of the K13 protein. Previous research has indicated the function of human kelch-containing proteins as adapters bringing substrates into ubiquitination complexes [75]. Nonetheless, as compared to human kelch proteins (Keap 1), K13 belongs to the kelch super family of proteins, constituting of a particular Plasmodium domain and an N-terminal domain, a BTB/POZ domain and a six-blade C-terminal propeller domain made up of basic kelch motifs [76,77].

The propeller domain entertains many protein-protein sites and intercedes cellular functions like ubiquitin-regulated protein degradation and oxidative stress responses. It is suggested that the Fe-dependent generation of Reactive Oxygen Species (ROS) mediates the potential antimalarial effect of the ART and its derivatives, inducing alteration in the redox balance, and hence damages the cellular targets. It seems interesting that the toxicity of ART derivatives depends upon their pro-oxidant activity, in contrast to their involvement in the regulation of cytoprotective and protein degradation responses to outside stress [36]. However, this hypothesis further supports the evidence that K13 is highly homologous to the human Kelch protein (Keap 1), which is required in cell adaptation to oxidative stress [78]. The human kelch protein (Keap 1) is a negative regulator of the inducible cytoprotective response dependent on the nuclear erythroid 2-related factor 2 (Nrf2) [79]. The Nrf2 binds to the Antioxidant Response Element (ARE) present in the gene promoters involved in phase II detoxification and oxidative stress responses. The Nrf2 is degraded by the Keap 1, which targets it through the cullin 3 ligase complex for ubiquilination [80]. Therefore, it is presumed that the K13 propeller performs similar functions in the Plasmodium, i.e directing the transcription factors incorporated in anti-oxidant responses through ligase complex. No orthologues of Nrf2 have been determined in the parasitic genome [36]. On the contrary, many suggested hypotheses have elaborated the K13 polymorphism role in regulating artemisinin resistance in P. falciparum isolates.

The drug responses of Cambodian wild-type K13 and mutated samples of P. falciparum, Dogovski, et al. indicated the inducement of drug retardation and accumulation of ubiquinated proteins by the ART. This action contributes to cellular stress response. The decelerated protein ubiquitination and delayed early apoptosis after drug exposure is exhibited by the resistant parasite strains, which indicates higher levels of cellular stress response. Due to its similarity to substrate adapters for cullin3 ubiquitin ligases, the role of K13 is determined in reducing the level of ubiquitinated proteins [80,81].

Furthermore, Mbengueet, et al. recently reported that artemisinins are potent inhibitors of P. falciparum phosphatidylinositol-3-kinase

(PfPI3K). PfPI3K phosphorylates Phosphatidylinositol (PI) to produce Phosphatidylinositol 3-Phosphate (PI3P) which promotes cell signaling for parasite survival, such as inhibition of apoptosis. Hence, inhibition of PfPI3K activity by DHA causes a reduction in PI3P level and subsequently leads to parasite death. They further showed that PfPI3K interacts with K13 and the K13 mutationshinder this interaction resulting in reduced polyubiquitination of PfPI3K, leading to the accumulation of PfPI3K, as well as its lipid product Phosphatidylinositol-3-Phosphate (PI3P). Thereby the authors concluded that levels of PI3P can be used as an additional marker for prediction of artemisinin resistance. Buthow the elevated PI3P leads to resistance needs to be further evaluated [50,65].

To elaborate the mechanism of ART further, Mok, et al. [82] emphasized the comprehensive changes of the parasite transcriptional program altering its physiology as a reason for ART resistance. Later, the authors carried out the transcriptome analyses of 1043 P. falciparum isolates to uncover the underlying mechanism of artemisinin resistance. They found that ART resistance was highly correlated with up-regulated genes incorporated in protein process, and since these pathways participate in Unfolded Protein Response (UPR) involving the major Plasmodium Reactive Oxidative Stress Complex (PROSC) and TCP-1 ring complex (TRiC) chaperone complexes, they may serve as the major intermediate for ART resistance caused by K13 mutation in P. falciparumand mitigate protein damage caused by artemisinin. It has been proposed that the K13 mutations mediate ART resistance by limiting their effects on particular targets at ring stage. Recent studies have provided evidence that the phosphatidylinositol-3-kinase (PfPI3K) of the P. falciparum is targeted specifically by the artemisinins and its levels are increased with K13 mutations in parasites [50]. Thus screening of K13 and PI3K proteins in Plasmodium vivax may help us extrapolate our current knowledge of drug resistance to Vivax parasites [83-85]. Another report by Wang and others proposes that the parasites with K13-propeller mutations are able to overcome protein damage due to the drug modifications by activating the stress response; thus, they are selected as they have a higher capability to survive the drug treatment at the early ring stage, at which point drug activation and drug pressure are relatively low; thereby enriching these mutations in the parasitepopulation [33].

Other proteins have been conferred in mediating the ART resistance in the absence of K13 mutations. TRAC studies have revealed that nonsynonymous polymorphisms in multidrug-resistance protein 2, apicoplast ribosomal protein S10, chloroquine-resistance transporter (pfcrt), and ferredoxin determine the genetic background for the K13 mutations to arise [86]. It would also be important to decipher the K13-independent mechanisms of ART resistance [87-89]

The role of these proteins and pathways in artemisinin resistance is plausible, but needs further evaluation. It would be very interesting to delineate the normal function of K13 and the effect of various mutations found in the propeller domain of K13. Furthermore, it would also be interesting to decipher the identity of putative K13 targets and their association with ubiquitin ligase activity. K13-molecular targetswould give the critical insight forinterrogatingits role in the underlying mechanism of ART resistance.Currently K13-propeller polymorphisms appear to be the only useful molecular marker for chasing the emergence and spread of ART resistance in P. falciparum.

CONCLUSION AND FUTURE IMPLICATIONS

The spread of malaria and the threat to the efficacy of antimalarial drugs have raised a global concern. Artemisinin has been used as a potential anti-malarial in combination with less potent drugs, but it has confronted resistance in the malaria species. The supposed ART resistance is likely to be defined as the increased rate of decelerated parasite clearance phenotype or the K13-propeller mutations, while the confirmed ART resistance delivers the slow clearing parasite phenotype along with K13 mutations associated with ART resistance [16].

K13 polymorphism has proved to be the only crucial molecular marker available for tracking the ART resistance. It may be speculated that mutations in K13 may also come with a cost to parasite fitness, and might be lost rapidly in populations in the absence of artemisinin selection. Most critical in this direction would be to determine the exact physiological roles of K13 in the parasite and the effect of these polymorphisms on its function.Very interestingly, there have been some reports of slow parasite clearance rates even in the absence of K13 mutant alleles suggesting the role of additional molecules in development of ART resistance in P. falciparum. It would be crucial to identify additional genetic loci involved in ART resistance [14,21,86].Novel methodologies like GWAS [40,43], click chemistry [33], genetic tools [17,52], transcriptomics [82] and chemogenic profiling [90]can prove to be vital for solving this mystery of parasite clever escape from the currently usedantimalarialdrugs.Apart from understanding the current state and mechanisms of antimalarial drug resistance, it is also extremely essential to broaden understanding of this intelligent parasite [91-93] and at the same time to expand the current arsenal used against the parasite [94-97].

All in all, the artemisinin resistance still remains a gray area, about which not much is known. Strategies for regular monitoring and extensive surveillance of K13 prevalence should be implemented. The national drug policies should be viewed carefully and altered in a timely fashion according to the frequency of spreading resistance. The discovery and identification of infection phenotypes should be monitored in the malaria endemic regions and the research for the mechanism and intervention for the prevailing resistance needs to be urgently investigated.

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