International Journal of Academic Health and Medical Research (IJAHMR)
ISSN: 2643-9824
Vol. 6 Issue 2, February - 2022, Pages:54-59

Plasmodium Falciparum Resistance or Decreased Susceptibility to Artemisinin Combination Therapy; the Way Forward

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Abstract: *Plasmodium falciparum resistance to artemisinin-based combination therapy (ACT) is caused by polymorphism in the genes malaria parasites which convey resistance either to artemisinin or to the partner drugs which make up the ACTs. Mutations of pfk13 and pfatpase are responsible for hypersensitivity to artemisinin. With most of the current partner drugs sharing the same quinoline moiety with chloroquine, resistance due to P. falciparum resistance to the partner drugs could be caused by mutation of pfcrt, pfdhfr and pfdhp. On the other hand, mutation of pfmdr 1 could be responsible for resistance to both artemisinin and the partner drugs. High ACT resistance prevalence has been reported in areas where resistance to artemisinin and the partner drugs have been common. In order to control antimalarial drug resistance parasites, strategies aim at controlling the rate of malaria transmission and reducing the rate of development of antimalarial drug resistance parasites should be embarked upon.*

Keywords: artemisinin resistance, *Plasmodium falciparum*, mutation

Introduction

Malaria is one of the most dangerous infectious diseases in the world caused by protozoan parasite of the genus *Plasmodium* with *Plasomdium falciparum* being deadliest out of the five species of which cause malaria.[1,2] This infection has adverse effects on the medical, social and economic lives of the world population particularly in areas where it is endemic. Africa is the epicenter of this infection where most illnesses and deaths occur with children under five years and pregnant women mostly affected.[3-6] The World Health Organization has reported that over 400 000 people die of treatable malaria and most of them are children.[7] Approximately 11 million pregnant women in areas of moderate and high malaria transmission to the sub-Saharan Africa were infected with malaria.[8] This results to maternal anaemia, poor perinatal and neonatal growth, miscarriage, stillbirth, premature birth and intrauterine growth restriction.[9-11]

The WHO recommends artemisinin-based combination therapies [ACTs] for the treatment of uncomplicated falciparum malaria made up rapid-acting artemisinin component and slow-reacting partner drug. However, resistance to ACTs by *Plasmodium falciprum* has been great challenge to the control of malaria.[12] This ACT resistance is mediated by *Plasmodium falciparum* kelch [*Pf*K13] as well as resistance to the partner drug component of the ACTs resulting to delayed clearance of the parasite after treatment.[13,14,15]

Also, the mutation of *Plasmodium falciparum* multidrug resistance transporter 1 (*pfmdr1*) genes which were common in Chloroquin resistance have been significantly associated with hypersensitivity to ACTs.[16,17] The changing of *pfmdr1* to a drug regimen which contains ACT drugs leads to the reselection of *pfmdr1* wild-type alleles.[18] ACT resistance arises through the amplification of *pfmdr1* which contributes significantly to recrudescence.[19,20] This study was aimed at describing based on the available data, the emergence, spread and control of ACTs resistance or decrease susceptibility. *Plasmodium falciparum* endoplasmic reticulum calcium-ATPase calcium pump (*Pf*ATP6) is another artemisinin resistant marker that is greatly linked with reduced susceptibility or resistance to artemisinins.[21]

Emergence and spread of *Plasmodium falciparum* resistance or decreased susceptibility to artemisinin combination therapy (ACT)

Over the years, every monotherapy has led to quick resistance to *Plasmodium falciparum*. With the advent of combination therapies between artemisinin and molecules with long lasting action, hope was raised.[22] Artemisinin resistance was first reported in 2008 at the Greater Mekong Subregion (GMS) in South-East Asia after artesunate monotherapy.[23] However, a report from a retrospective study suggests that partial resistance to artemisinin may have come up in 2001 before the widespread in GMS. In the late 2013, there was a discovery of a new molecular marker as a result of mutations in the K13 propeller domain which was responsible for partial resistance in artemisinin at the GMS.[24]

ACT resistance due to K13 mutation has currently spread from South-East Asia to other parts of the world. In study conducted between 2012 and 2013 at Dakar in Senegal, K13 mutation *Plasmodium* was not recorded.[25] In a separate study conducted in the same study area between 2013 and 2014, three mutations of K13 (N554H, Q613H and V637I) were observed.[26]

Between 2012 and 2013 at a high malaria transmission area in Kenya, there were 4 new types of nonsynonymous and 5 of synonymous mutations of K13 from 539 blood samples.[27] In a study of 1212 *Plasmodium falciparum* positive samples from 12 countries in Sub-Saharan Africa, 22 distinctive mutation of K13 genes were discovered but none were similar to those earlier reported in South-East Asia.[28]

In Lagos, South-West Nigeria, three mutant genotypes of K13 (A578S, D464N and Q613H) were reported in 92 out of 195 sequenced *Plasmodium falciparum* positive samples while the remaining genes were the wild types.[29] There was no K13 mutation according to a study in Rwanda in 2010. However in 2014 and 2015, there were reported prevalence of 2.5% and 4.5% K13 mutants respectively in the same area with two of the mutants (P574L and A675V) in 2015 similar to the mutated K13 genes in South-East Asia.[30] A study in Mali in both ACTs and pre-ACTs malaria infections reported K13 mutation.[31]

Polymorphism of *pfmdr1*-N86Y was linked with delayed recrudescence among African children with severe after treatment with artesunate monotherapy.[32] A study on children with severe malaria in Central (Garbon), West (Ghana) and East (Kenya) African children reveals that rates of mutation on *pfmdr1*-N86Y were 48%, 10% and 10% respectively.[33] In Uganda (a malaria endemic region) after the administration of Artemether-Lumefantrine, three polymorphisms in the *pfmdr1* gene were identified. These polymorphisms were not due to failure in clinical treatment, but were an indicator of the ability of this drug combination to selectively change the parasites to resistant strains.[34] At very low frequency, mutations of H243Y and A623E at *pfatp6* locus were reported and the rates of mutation of E431K at pfatp6 were 6%, 18% and 17% in Gabon, Ghana and Kenya respectively.[33]

Failure to treatment with ACTs could have rarely occurred if was largely dependent only on artemisinin resistance. However, resistance to ACT partner drug is the most significant contributed ACT treatment failure. Consequently, varieties of ACTs drugs have failed in areas where both artemisinin resistance and ACT partner drug resistance have been recorded.[24] This buttresses the report of a study among 200 *Plasmodium falciparum* positive travelers from eighteen African countries to Spain where there was mutation of *pfdhfr* and *pfdhps* genes in 76% of the travelers.[35] Also, 92.78% (167/180) mutant genes of *Pfdhfr* and 87.78% (158/180) mutant genes of *pfdhps* genes respectively were observed in a study conducted in Equatorial Guinea on 180 *Plasmodium falciparum* infected samples between 2013 to 2014. The increase in *pfdhfr* and *pfdhps* genes negatively affected the therapeutic efficacy of Sulfadoxine-pyrimethamine as those mutants convey resistance against sulfadoxine-pyrimethamine.[36]

With most of the current ACT partner drugs (amodiaquine, piperaquine, and pyronaridine) sharing the same quinoline moiety with chloroquine, a consideration on the potential role of *pfcrt* in ACT resistance is very important.[37,38] In an *in vitro* study of artemether-lumefantrine (CoArtem), it has been revealed that lumefantrine [an arylaminoalcohol related to mefloquine] is selective for wild type *pfcrt*[39] while amodiaquine, a partner drug in amodiaquine-artesunate (Coarsucam) is selective for mutant forms of *pfcrt* and *pfmdr1* in field isolates.[40] In the case of piperaquine, there is a documented evidence that parasites with mutant *pfcrt* Dd2 which conveys resistance for piperaquine were discovered to have a novel *pfcrt* mutation, C101F, together with a change in *pfmdr1* copy number and amplification of a novel locus on chromosome 5.[41]

From the standpoint of usage, effects and modes of action of drugs, there is a rising concern about cross resistance with compounds, namely: ozonides and trioxaquines, which have the same chemical structures and modes of action with artemisinin. A study has revealed that artemisinin-resistant strains with Cambodian synonymous resistance strains showed cross-resistance with trioxaquines (endoperoxide-based hybrid antimalarial molecules). In vitro study of trioxaquine drug pressure identified a novel lineage that was resistant to both trioxaquines and artemisinins which was similar to resistance conveyed by *pfk13* polymorphism.[42] The two promising antimalarial drugs of ozonide derivatives, OZ439 and OZ277 which are also respectively known as artefenomel and arterolane have an endoperoxide bridge (a chemical function with similar peroxide pharmacophore to artemisinins). Although during clinical trials, artefenomel (OZ439) had shown a good safety profile and rapid parasitemia clearance in species of *Plasmodium*,[43] later studies have further revealed that this is different with artemisinin-resistant strains in an in vitro experiment. It is reported that artemisinin-resistant strains can exhibit a reduction in in vitro susceptibility to the ozonide antimalarials depending on the time of exposure.[44] Furthermore, arterolane (OZ277) has been discovered to immensely show limited in vitro susceptibility against artemisinin-resistant parasites and while artefenomel has been more effective against majority of the mutated *pfk13* mediated resistant parasites except for those which have mutation in I543T.[45]

The available research data have raised concerns about the risks of parasite cross-resistance between artemisinins and other endoperoxide-based antimalarials, including ozonides. In addition, with the increasing rate and complexity of polymorphisms ACT resistance genes and their varying effects on antimalarial chemotherapy, there is a growing concern that further mutations which may always contribute to parasite resistance to the recommended therapies as well as possible candidate replacement compounds in the near future will always spring up.

The way forward to antimalarial drug resistance

The journey into the future of combating the war against antimalaria drug resistance will always be an uphill task because no strategy at the present or in the future is sufficient to completely eradicating malaria transmission. This promotes the development of antimalarial drug resistance as long as antimalarial drugs will still be in long used in the future.[46] Also, the development of new antimalarial drugs is slower than the rate at which malaria parasites develop resistance against existing drugs. To worsen the situation especially in malaria endemic regions, affordability of quality antimalaria drugs has become a keep issue which has helped in the development of antimalaria drug resistance in those regions.[46]

In order to control the development and spread of antimalaria drug resistance parasites, strategies aim at controlling the rate of malaria transmission and reducing the rate of development of antimalarial drug resistance parasites should be embarked upon. In controlling malaria transmission, comprehensive vector and etiologic control strategies are the keys. These include the use of insecticide-treated nets; use of insecticide spraying; environmental control to either eliminate or reduce mosquito breeding sites; other personal protection measures like the use of repellent soap/creams and screening windows; the use of chemoprophylaxis in defined populations especially in pregnant women; and the administration of effective vaccines.[46]

Another key approach to malaria control is the availability of quick and effective malaria treatment. This is achievable through easy access to quality health care facilities; training and employment of adequately qualified health care workers who are effectively supervised and/or well motivated; and provision of affordable and easily accessed quality antimalaria drugs.[47] Decrease in the rates of malaria infection or its rates of transmission have an indirect impact in reducing the development of drug resistance through the reduction in the number of malaria cases to be treated consequently reducing the overall drug pressure and finally, decreasing the possibility of successfully transmitting resistant parasites new hosts.[48,49]

Interventions aim at preventing the development and the spread of drug resisttance are targeted towards reducing drug pressure; improving drug prescriptions and usage in a way that does not facilitate development or spread of resistant parasites. Development of drug resistance is generally attributed to drug pressure. A more restrictive approach aim at controlling drug prescribtions and usage will limit the development, spread and intensity of drug resistance.[47] This could be achieved through improved diagnosis of malaria using microscopy or a rapid antigen test in areas where miscrocopy is not readily available. There may also be a role for prophylaxis programmes and presumptive treatment or even mass drug administration in epidemic cases. Close follow-up and retreatment of patients either at a particular community or patients booked on appointment irrespective of whether the patient feels ill or not in order to rapidly identify patients who may failed initial treatment identified quickly and re-treat them until the parasites are cleared. This will decrease the possibility of spreading drug resistant parasites.[50]

Artemisinin-based combination therapy (ACT) promotes the use of a drug combination with grossly mismatched half-lives. Theoretically, in areas with low malaria transmission rates, this is of minimal concern because the likelihood of being infected by mosquito during the period when drug levels are suboptimal is very low. However in areas with high transmission rates are very high especially in Africa where inoculation rates can be as high as five infective bites per night, there is a high probability that malaria infection can still occur even when the drug levels are suboptimal. Therefore, the development of resistance parasites from the initial malaria treatment compared with new parasites being exposed to suboptimal drug levels is unknown.[46] In the future, triple artemisinin combination therapy may be developed to incorporate vaccines or other drugs solely designed to inhibit transmission of gametoacytes carrying antimalarial drug resistance genes or interfere with sexual reproduction and infection of the parasite within the mosquitos when blood meal is been taken up by the mosquitoes.[46]

Conclusion

There is an emergence of *Plasmodium falciparum* strains which are resistant to both artemisinins and partner drugs. Also, there is currently no short term effective antimalarial replacement for artemisinin due to cross-resistance with novel synthetic antimalarial drug which have similar endoperoxide bridge with artemisinin.

Recommendation

It is recommended that:

(i) Triple arteminin-based combination therapy being developed should be fast tract.
(ii) The robustness of strategy against self-medication aim at checking for the effectiveness of combination therapy should be imvestigated.
(iii) Investigate on the mechanisms of financing combination therapy strategies.
(iv) Strategies to improve acceptance and compliance to combination therapy regimen should be developed.
(v) Effectiveness of drug regulations should be improved.
(vi) Access to definitve diagnosis-based treatment should be improved.
(vii) Vector control with treatment should be supported.

Statement of competing interests and funding support:
Authors have declared that no competing interests exist.

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