Resistant Malaria: Current Scenario

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Abstract

One of the greatest challenges facing malaria control today in India and other part of the world is antimalarial drug resistance. Drug resistance has been implicated in the spread of malaria to new areas and re-emergence of malaria in areas where the disease had been eradicated. This may be the reason of the occurrence and severity of epidemics in some parts of the world. Factors like population movement have introduced resistant parasites to areas previously free of drug resistance. The purpose of this review is to describe the state of knowledge regarding drug- resistant malaria and to outline the current thinking in this part of the world.

Introduction

Malaria is one of the major public health problems in the developing countries. Recent estimates indicate that between 300-500 million clinical cases and between 1.5-2.7 million deaths due to it, occur world wide annually, 90% of which occur in tropical Africa. It is estimated that 1.2 billion people out of the 1.4 billion people of SE Region live in Malarious areas. About two thirds of reported cases in the Region are from India which reported 1.5 million confirmed cases in 2008 down from 2 million in 2000[1]. Of the confirmed reported cases, 60% came from five states namely, Orissa, Jharkhand, Chhattisgarh, Madhya Pradesh and West Bengal. A decline in the number of reported cases of between 31% and 66% were observed in all these states except Jharkhand which saw an increase of 60% which was associated with an increase in annual blood examination rate of 8.2%.

There was a complete failure to eradicate malaria in many countries due to technical, operational and socio-economical difficulties, which led to resurgence of malaria in this part of the world, even after National Malaria Control and Eradication Programmes initiated in 1950s, which had initial impressive results. The control programme has been hampered by the spread of drug resistance in parasite and insecticide resistance in mosquito vectors[2].

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Definition

Literally speaking, drug resistant malaria means malaria caused by a plasmodium resistant to usual antimalarial drugs. Antimalarial drug resistance has been defined as the "ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject". This definition was later modified to specify that the drug in question must "gain access to the parasite or the infected red blood cell for the duration of the time necessary for its normal action"[3].

Antimalarial drug resistance is a major public health problem which hinders the control of malaria. Resistance of Plasmodium falciparum to choloroquine, the cheapest and the most used drug is spreading in almost all the endemic countries. Resistance to the combination of sulfadoxine-pyrimethamine which was already present in South America and in South-East Asia is now emerging in East Africa.

Indian Scenario of Drug Resistant Malaria

- Of the two plasmodia which cause malaria in India, incidence of drug resistance is more common with P. falciparum.
- Occasionally, P. vivax may also be drug resistant and this occurs specially as a result of improper

treatment and inadequate dosage.

- Originally, both the Plasmodia - vivax and falciparum - were sensitive to chloroquine, but, in recent years, more and more P. falciparum are developing resistance against chloroquine.
- To overcome this problem of chloroquine resistance, Sulfadoxine + Pyrimethamine combination was used. But, very soon, some strains of falciparum developed resistance to this combination also.
- P. falciparum resistant to traditional drugs like Quinine have also been reported.
- Incidence in India will be difficult to know because in many cases it may not be recorded.
- In India, the first confirmed report of chloroquine resistance in P. falciparum was reported in Diphu area of Karbianglong district of Assam in 1973[4].
- A study carried out by the Clinical Pharmacological and Research Services Unit, in KEM hospital in Mumbai, confirmed the existence of chloroquine resistance in P. falciparum cases in Mumbai, incidence being 5% to full dose chloroquine.
- Resurgence of P. falciparum resistant to chloroquine has been noticed in several regions of India. Earlier reports indicated chloroquine resistance to P. falciparum in North Eastern parts of the country with new foci of drug resistance being added.

Resistance to antimalarial drugs has been described for *P. falciparum* and *P. vivax* among the four species of malaria parasite that naturally infect humans. *P. falciparum* has developed resistance to nearly all antimalarials in current use. *P. vivax* infection acquired in some areas has been shown to be resistant to chloroquine and/or primaquine[5]. Chloroquine-resistant *P. falciparum* malaria has been described everywhere that *P. Falciparum* malaria is transmitted except for malarious areas of Central America (north-west of the Panama Canal), the island of Hispaniola, and limited areas of the Middle East and Central Asia. Sulfadoxine pyrimethamine (SP) resistance occurs frequently in South-East Asia and South America. SP resistance is becoming more prevalent in Africa as that drug is increasingly being relied upon as a replacement for chloroquine. Mefloquine resistance is frequent in some areas of South-East Asia and has been reported in the Amazon region of South America and sporadically in Africa[6]. Cross-resistance between halofantrine and mefloquine is suggested by reduced response to halofantrine when used to treat mefloquine failures[7].

Failure to clear malarial parasitaemia or resolve clinical disease following a treatment with an antimalarial drug may be treatment failure and not always antimalarial drug resistance. While drug resistance can cause treatment failure, not all treatment failure is due to drug resistance. Main factors which lead to treatment failure are incorrect dosing, non-compliance with duration of dosing regimen, poor drug quality, drug interactions, poor or erratic absorption, and misdiagnosis. Somehow these factors, apart from treatment failure may also contribute to the development of true drug resistance through increasing the likelihood of exposure of parasites to suboptimal drug levels.

Types of Drug Resistance

In defining criteria for resistance to the antimalarial drugs, the WHO has described three grades of resistance following treatment[8]

R1 (Low grade): Recrudescence of infection between 7 and 28 days of completing treatment following initial resolution of symptoms and parasite clearance.

R2 (High grade): Reduction of parasitaemia by > 75% at 48 hours but failure to clear parasites within 7 days.

R3: Parasitaemia does not fall by >75% within 48 hours.

Mechanisms of antimalarial resistance.

The possible mechanisms of development of resistance are as follows:

i. Parasite does not allow the entry of drug.

ii. After entry of drug, the malarial parasite does not retain it and throws it out.

iii. May be a combination of both.

In general it appears to occur through spontaneous mutations that confer reduced sensitivity to a given drug or class of drugs. For some drugs, only a single point mutation is required to confer resistance, while for other drugs, multiple mutations appear to be

required. Provided the mutations are not deleterious to the survival or reproduction of the parasite, drug pressure will remove susceptible parasites while resistant parasites survive.

It is believed that resistance of *P. falciparum* to chloroquine is related to an increased capacity for the parasite to expel chloroquine at a rate that does not allow chloroquine to reach levels required for inhibition of haem polymerization. It is unclear whether parasite resistance to other quinoline antimalarials (amodiaquine, mefloquine, halofantrine, and quinine) occurs via similar mechanisms[9]. This chloroquine efflux occurs at a rate of 40 to 50 times faster among resistant parasites than sensitive ones [10].

Current molecular studies of *P. falciparum* isolates suggest that few gene loci are associated with chloroquine resistance to *P. falciparum.* These genes have been named as *pfmdr*-1 & 2, *pfcrt. Pfmdr-1* gene located on chromosome-5 and coding for P-glycoprotein homologne-1 (*Pgh-1*) has generated interest in resistance to chloroquine and other antimalarial. Point mutation of aspartic acid to tyrocine in codon 86 (A-86 to T-86) is associated with chloroquine resistance[11]. Several other *pfmdr-1* polymorphisms— Phe 184, Cys 1034, Asp1042 and Tyr 1246 have been implicated to varying degrees in chloroquine resistance[12,13]. Another locus governing chloroquine resistance has been identified on chromosome 7 and encodes a transmembrane protein in a digestive vacuole of malaria parasites[14]. Sets of point mutations in *pfcrt* gene, a putative transmembrane transporter localized in the parasite digestive vacuole membrane have been found to be associated with *in vitro* chloroquine resistance in *P. falciparum* from Africa, South America and Southeast Asia[15]. This is also suggested that *pfmdr-1* mutation associated with chloroquine resistance may also account for reduced susceptibility to quinine[16]. However, the exact mechanism of resistance is not clear.

In case of Antifolate combination drugs, such as sulfadoxine + pyrimethamine, specific gene mutations encoding for resistance to both dihydropteroate synthase and dihydrofolate Reductase have been identified. Specific combinations of these mutations have been associated with varying degrees of resistance to antifolate combination drugs[17].

Resistance to atovaquone develops very rapidly when used alone, when combined with a second drug, such as proguanil or tetracycline; resistance develops more slowly[18]. Resistance is conferred by single-point mutations in the cytochrome-b gene.

There are recent concerns that the efficacy of Artemisinin-based combination therapies which are the recommended first-line treatments of falciparum malaria in all countries with endemic disease, has declined on the Thai–Cambodian border, historically a site of emerging antimalarial-drug resistance. The possible mechanism proposed are mutations or amplifications of the gene encoding a multi-drug resistance protein [*PfMDR1*] or mutations in the gene encoding sarco–endoplasmic reticulum calcium ATPase6 [*PfSERCA*])[19].

Determination of drug resistance

Drug resistance by malaria parasites has been defined as the ability of a parasite strain to survive or multiply despite the administration and absorption of a drug when given in doses equal to or higher than those normally recommended and within the limits of tolerance of the subject[20]. This definition may be applied to the response of the parasite to antimalarial drugs used as schizontocides, gametocytocides or sporontocides. In general, four basic methods have been routinely used to study or measure antimalarial drug resistance: *in vivo*, *in vitro*, animal model studies, and molecular characterization.

***In vivo* tests**

These are based on the observation of parasite response in the patients to a fixed dose of a drug within the limits of tolerability, one of the key characteristics of *in vivo* test in the interplay between host and parasite[21]. The assessment of *in vivo* drug response of *P. falciparum* to antimalarials requires prolonged periods of follow-up (28 days) and seclusion of patients in screened rooms to prevent the possibility of reinfection. In 1990, WHO introduced a modified protocol, involving shorter period of follow-up (7–14 days) without seclusion, under the assumption that reappearance of parasites in peripheral blood within 14 days of treatment is more likely due to recrudescence than reinfeciton[22]. Traditionally, response to treatment was categorized purely on

parasitological grounds as Sensitive, RI, RII, and RIII[23]. Later modifications are based on adequate clinical response, early and late treatment failure. The test procedure is based on a 14-day follow-up with clinical, parasitological, haematocrit and fever assessment on Day 0, 3, 7 and 14[24].

***In vitro* tests**

Two types of *in vitro* assays are commonly used, WHO schizont maturation assay and the isotopic micro test. These tests are based on the estimation of the parasite metabolic process in short- or long-term culture. The data derived from *in vitro* tests have to be interpreted in relation to the *in vivo* and pharmacological tests to determine individual susceptibility levels for the drug tested. This test avoids many of the confounding factors, which influence the *in vivo* test, by removing parasites from the host and placing them in a controlled experimental environment, thereby accurately reflecting the intrinsic antimalarial drug resistance[25]. The *in vitro* assays not only yield quantitative results, but also determine the phenotype of the parasite independently of the immune and physiopathological status of the host.

Animal model studies

This type of test is basically an *in vivo* test conducted in a non-human animal model and so influenced by many of the same extrinsic factors as *in vivo* tests. The influence of host immunity is minimized by using lab-reared animals or animal-parasite combinations unlikely to occur in nature. These tests allow for the testing of parasites which cannot be adapted to *in vitro* environments (provided a suitable animal host is available) and the testing of experimental drugs not yet approved for use in humans[26]. A significant disadvantage is that only parasites that can grow in, or are adaptable to, non-human primates can be investigated.

Molecular techniques

Molecular tests use polymerase chain reaction (PCR) to indicate the presence of mutations encoding biological resistance to antimalarial drugs[27]. Theoretically, the frequency of occurrence of specific gene mutations within a sample of parasites obtained from patients from a given area could provide an indication of the frequency of drug resistance in that area analogous to information derived from *in vitro* methods. Advantages include the need for only small amounts of genetic material as opposed to live parasites, independence from host and environmental factors, and the ability to conduct large numbers of tests in a relatively short period of time. Disadvantages include the obvious need for sophisticated equipment and training.

How to prevent drug resistance-

Prevention strategies can be divided into those aimed specifically at preventing malaria infection and reducing the likelihood of development of drug resistance. Reductions of overall malaria infection rates or transmission rates have an indirect impact on development of drug resistance by reducing the number of infections needing to be treated (and therefore, overall drug pressure) and by reducing the likelihood that resistant parasites are successfully transmitted to new hosts.

They include the use of insecticide-treated bed nets, indoor residual insecticide spraying, environmental control (mosquito breeding site or "source" reduction), other personal protection measures (e.g. use of repellent soap or screening windows) and chemoprophylaxis in defined populations (use of mass prophylaxis is typically not recommended). An effective and deliverable vaccine would also be greatly beneficial.

Interventions aimed at preventing drug resistance, per se, generally focus on reducing overall drug pressure through more selective use of drugs; improving the way drugs are used through improving prescribing, follow-up practices, and patient compliance; or using drugs or drug combinations which are inherently less likely to foster resistance or have properties that do not facilitate development or spread of resistant parasites.

1. Reducing overall drug pressure -

This approach has gained support in North America and Europe for fighting antibacterial drug resistance[28]. The greatest decrease in antimalarial drug use could be achieved through improving the diagnosis of malaria. Basing treatment on the results of a diagnostic test, such as microscopy or a rapid antigen test, however, would result in the greatest reduction of unnecessary malaria treatments and decrease the probability that parasites are exposed to sub therapeutic blood levels of drug.

2. Directly observed therapy (DOT) like programme-

A Very good example is treatment of tuberculosis. While this has not yet received serious consideration for malaria, the use of drugs with single-dose regimens (SP, mefloquine) could potentially make DOT possible. Another approach that has not been widely adopted is the close follow-up and re-treatment, if necessary, of patients. The success of this approach is dependant on availability of reliable microscopy (to diagnose the illness initially as well as to confirm treatment failure), and either an infrastructure infrastructure to locate patients in the community or a community willing to return on a given date, regardless of whether they feel ill or not. With this system, patients who fail initial treatment, for whatever reason, are identified quickly and re-treated until parasitologically cured, decreasing the potential for spread of resistant parasites[29].

3. Combination therapy-

Trial of combination of antimalarial drugs, such as mefloquine, SP, or amodiaquine, with an artemisinin derivative has been tried in some part of the world with success30. Artemisinin drugs are highly efficacious, rapidly active, and have action against a broader range of parasite developmental stages. This action apparently yields two notable results. First, artemisinin compounds, used in combination with a longer acting antimalarial, can rapidly reduce parasite densities to very low levels at a time when drug levels of the longer acting antimalarial drug are still maximal. This greatly reduces both the likelihood of parasites surviving initial treatment and the likelihood that parasites will be exposed to suboptimal levels of the longer acting drug[30]. Second, the use of artemisinins has been shown to reduce gametocytogenesis by 8- to 18-fold[29]. This reduces the likelihood that gametocytes carrying resistance genes are passed onwards and potentially may reduce malaria transmission rates.

In the future, antimalarial therapy may be expanded by combining chemotherapy with vaccines (or other drugs) specifically designed to inhibit transmission of malaria. These “transmission-blocking” vaccines or drugs could reduce the potential for onward transmission of gametocytes carrying resistance genes, even if a relatively large number of parasites survive initial treatment. This could work through using drugs or vaccines with a high degree of specific antigametocytocidal activity (such as primaquine and related drugs), drugs that nonspecifically reduce the likelihood of gametocytes developing (such as appears to be the case with the artemisinins), or drugs or vaccines that interfere with sexual reproduction and infection of the parasite within the mosquitos when taken up with a blood meal (although short acting, the combination of atovaquone and proguanil has this type of activity).

New development –

CPP-ZFN technology - it enormously increases the number of drug targets because it can utilize the vast sequence diversity among structurally and functionally conserved enzymes of human and *Plasmodium* proteins. Cell-penetrating peptides (CPP) - mediated protein delivery is an established method that can be used for delivery of the newly designed zinc finger nuclease (ZFN). CPP-ZFN promises to be a safe and sustainable drug for malaria intervention [31]. The resulting new drug will be effective even against resistant strains, providing ample alternatives for drug resistance management.

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