Emergence of drug resistance in *Plasmodium falciparum*: Reasons of its dispersal and transmission in different climatic regions of the world: a review

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Abstract

In the present time emergence, dispersal and transmission of drug resistant malaria parasite *P. falciparum* has become a serious health problem in human being throughout the globe. From various surveys it has been proved that intensity of drug resistance and pathogenesis of dreadful parasite is increasing day by day due to arousal of point mutations in dhfr and dhps genes mainly drug binding regions of *P. falciparum* genome. However, single or their multiple point mutations have altered the allele frequencies in *P. falciparum* clinical isolates collected from various regions of the world that resulted in emergence of drug resistance and lead to complete failures of anti-malarial drugs. Further it has increased dispersal and transmission of drug resistant *P. falciparum* throughout Africa and Asia. Genetic reasons of drug failures the intensity of parasite survival and its resistance to various drugs seems to be widely influenced due to climatic and demographic reasons mainly rapid and active breeding of disease transmission vectors, poor health hygienic conditions, use of substandard diagnostic facilities and low grade treatments provided to the patients. In addition, human migration and poor rehabilitation have enhanced the severity and complications of malaria and its seasonal outbreaks. Therefore, for fast control of malaria, high quality diagnostic and treatment facilities are required for better therapeutic results to fight against deadly *P. falciparum* outbreaks.

Introduction

Malaria is a dreadful infectious disease and has become a major impediment to socio-economic development in Africa, Asia and other poor nations of the world. Today it has become a global burden as an estimated 359 million cases are reported every year and 1.5-2.0 million deaths annually globally. Most of these deaths are largely concerned to the African countries [1]. Recent emergence of resistance to both old and new anti-malarial and its subsequent spread to non-infecting areas undoubtedly make the situation more terrible. Intervention by WHO and other malaria controlling agencies/institutions, it still exists as endemic diseases in densely populated South-East Asian and Sub Saharan African countries. In both the regions malaria became highly problematic due to eruption of multi-drug resistant *P. falciparum* mutants. Few countries, like Bangladesh, Myanmar, Philippines, Thailand, Cambodia, Eastern India, Indo-Nepal border, and Myanmar-China border become the breeding ground of multi-drug resistant *Plasmodium falciparum*. Recent detection of ACT resistance in *P. falciparum* has made the situation more alarming. However, due to long term over and repetitive use of antibiotics, malaria parasites have become resistant to most of them. It has further reduced the drug efficacy and increased the drug dose/level mainly IC₅₀ values manifold. Subsequently, it has resulted in an increased rapid dispersal and transmission of drug resistant falciparum malaria [2]. Their drug-resistant pfdhps haplotypes are circulating in West Africa and many Asian countries. Therefore, after seeing the rapid spread of multi-drug resistant *P. falciparum* mostly in poor countries of the world, it become mandatory to design new anti-malarial drugs with new viable strategies to check the emergence and spread of future drug resistance. All new alternative drugs need to be tested for their efficacy [3] to control high infection rate acquired by malaria parasite [4]. Besides this, there is a vast difference in drug sensitivity of parasites in many regions and it varies from region to region [5]. In some of the pockets same drug is thought to be effective but again it has no effect in other neighboring country. All it is due to increased drug pressure that has induced genomic changes mainly in dhfr and dhps genes at regional level. Therefore, there is an urgent need to collect molecular epidemiological information from different countries for quick analysis of data to know all possible reasons of origin and spread of drug resistant malaria [6].

Spread of multidrug resistant malaria in Asia and Africa

Due to demographic, eco-climatic and genetic reasons multidrug resistant malaria is widely spreading in Asia and Africa and rest of the world [7]. Mainly mutations occurred in dhfr and dhps genes conferred high levels of resistance in malaria parasite. It has increased density of malaria parasite in patients; hence, malaria treatment has become very difficult [8]. These genetically resistant infectious strains of *P. falciparum* malaria are reported from many countries of the world such as Mali [9], Sub-Saharan Africa [10], Somalia [11], Thailand [12], Mozambique [13], Rwanda [14], Swaziland [15], Solomon islands [16], Iran [17], Nigeria [18] and Kenya [19]. Besides this, Trimethoprim

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sulfamethoxazole resistance mediating dhfr 166L mutations have been detected in Ugandan population [20]. CQ mefloquine, quinine and SP/pyrimethamine susceptibility in Somalia [21], Sierra Leone [22], Venezuela [23], Nigeria [24], Zambia [25], Philippines [26], Zambia [27] and Thailand [28], while mefloquine [29], Proguanil/sulfamalaria CQ and pyrimethamine-sulfadoxine resistance was detected in Nigeria [30], CQ resistance in Kenya [31] and Diaoaloa area in Hainan province of China [32]. Chloroquine resistant *P. falciparum* was identified in indigenous residents of Cameroon [33], Kenya [34] and Nigeria [35]. Similarly, few old prescriptions like fansidar-sulphate and quinine and fansidar-HCl tetracycline [36], proguanil/sulfamethoxazole and sulfalene-pyrimethamine are also used to cure *P. falciparum* malaria in many African and Asian countries but these have totally failed to fight against drug resistant malaria parasite [37]. Non-artemisinin and artemisinin based combination therapies are used to cure uncomplicated falciparum malaria patients [38]. But, again combination therapies fail to provide good results due to emergence of resistance in *P. falciparum* [39]; hence, malaria control becomes very difficult and seems to be impossible [40] because of genetic and statistical complexity of the parasite mutation (Table 1) [41].

Molecular basis of resistance to a number of common anti-malarial drugs is well known, but epidemiological reasons of emergence and dispersal of drug resistant mutations in *P. falciparum* in many Asian and African countries are not fully known. Few strong reasons which have been identified for transmission of multi drug resistant malaria are human trafficking/traveling to malaria endemic to epidemic regions. Problem of human migration and instability created due to cross border tensions, paved way for the establishment of large refugee camps devoid of sanitation, diagnostic and treatment facilities. These refugee camps become the epicenter of drug resistant *P. falciparum* strains and work as reservoir of parasites. Such regions are mainly present in eastern Afghanistan where refugees crossed into the federally administered tribal areas of northwestern Pakistan. When these camps were monitored they have shown very high malaria incidence as 100.4 cases/1,000 person-years (Table 1). Hence, proper diagnosis and better treatment is required for fast control of malaria in such regions [42]. Similar cases of malaria were detected in asymptomatic children in malaria endemic sites in Western Kenya [43]. It happens due to human travel that enhances prevalence, genetic variability and rate of gene mutations in *Plasmodium falciparum*. Blood bites made by local female mosquito vectors on non-resident mainly infected travelers and tourists further enhance the transmission rate of drug resistant malaria in non-drug resistant population. It is also responsible for shaping current parasite population structure having multiple mutations [44].

**Reasons of spread of drug resistant malaria in India**

In India after launch of the National Malaria Control Programme in 1953, the number of malaria cases reported in India has sharply declined. After 1965 number has been enormously increased due to spread of drug resistant malaria in different parts of the country. In spite of sound efforts made by ICMR and malaria control board, no initial success was achieved against resurgence of malaria since 1960 and every year thousands of people died due to malaria mainly infants below age of 5 years. In India first time chloroquine resistance in *Plasmodium falciparum* was reported in 1973. Since then, infectivity has increased manifold with time due to rapid urbanization, human migration and poor housing establishments in the vicinity of water reservoirs having active breeding of malaria vector. Low control of vector, poor diagnosis and faulty medication lead to increase in transmission rate of resistant *P. falciparum* and almost every year outbreak of malaria epidemics occurred in many parts of India [45] like Orissa, Assam, West Bengal and other north eastern states of the country [46]. Similar resurgence of malaria and chloroquine resistance in *P. falciparum* and *P. vivax* was reported from Bombay, India [47], all along Indo-Nepal border [48] and western Myanmar [49]. Hence, for good therapeutic outcomes [50] regular consultation, proper diagnosis and appropriate prescription [51] of the anti-malarial drugs are essentially required [52] to cure regional and imported malaria cases [53]. Besides this, spectrum of new anti-malarial drugs must be evaluated from time to time.

**Genetic analysis of *P. falciparum* parasite**

For determining the level of resistance and transmission, genetic and molecular analysis of malaria parasite is important. Few important tools like micro-satellite methods are used to show the presence of multiple lineages for the mutant dhfr genotype (Table 1) [54]. However, on the basis of number of mutations occurred in parasite, the level of drug resistance can be predicted in clinical isolates. Further, drug use

| Drug/Drug combination | Country | Allele | Frequency (%) |
|-----------------------|---------|--------|---------------|
| Chloroquinine-Sulfadoxine/Pyrimethamine | Malo Island | pfcr | 95.4 |
| Sulfadoxine | Cambodia | pfcr | 7.9 |
| Sulfadoxine/Pyrimethamine | Costal Kenya | dhfr | 41 |
| Chloroquinine-Sulfadoxine/Pyrimethamine | Bangladesh | pfcr | 2.39 |
| Sulfadoxine/Pyrimethamine | Myanmar | dhfr | 49.25 |
| Chloroquinine | Bangui (Central Africa) | pfcr | 0.6 |
| Sulfadoxine/Pyrimethamine | Peruivian Amazon | dhfr | 21.6 |
| Sulfadoxine/Pyrimethamine | Tanzania | dhfr | 89.5 |
| sulfadoxine-pyrimethamine | Africa | pfdhfr | 75 |
| sulfadoxine-pyrimethamine | India | dhfr | 17.52 |
| sulfadoxine-pyrimethamine | West Africa | pfdhps | 11 |
| sulfadoxine-pyrimethamine | Sudan | dhfr | 45 |
| Sulfonamide/Pyrimethamine | Thailand | pfdfhr | 3.89 |
| Sulfonamide/Pyrimethamine | Pakistan | dhfr | 28.5 |
| Tetracycline | Southern Mozambique | dhfr | 37.8 |
| Chlorotrimoxazole | Uganda | dhfr | 72.1 |
| Amiodarcone | Uganda | pfmdr1 | 72 |
| Sulfonamide/Pyrimethamine | Rwanda | pfdfhr | 61.4 |
| Chloroquinine | Zambia | pfcr | 58.3 |
| Sulfadoxine/Pyrimethamine | Zambia | dhfr | 26.4 |
| Chloroquinine | Senegal | dhps | 89.7 |
| Mefloquine-Sulfadoxine-Pyrimethamine | Nigeria | pfdfhr | 29.1 |
| Chloroquinine-Sulfadoxine/Pyrimethamine | Philippines | pfcr | 87.5 |
and its efficacy (ID50 value) are directly connected with mutations. If there is a single mutation then drug may be active or not, it cannot be decided by considering single factor in mind, but if any clinical isolate shows two or more mutations, it means there may be higher resistance is present in *P. falciparum* against multiple anti-malarial drug. There is a possibility that moderately mutated parasite with one mutation in DHPS genes may provide moderate asymptomatic pathological failures of drugs in malaria patients [55]. Active immunity in malaria patients also work against *P. falciparum* infection. There are few malaria hot spots where mutant allele frequencies in pfdhfr are very high and malaria is out of control because patients have used multiple drugs against which parasite has already acquired resistance. Therefore, it is very hard to establish relationship between parasite genetics and *in vivo* treatment failure rates. Parasite floating in community has succeeded to establish genetic changes at regional level according to environmental conditions and both factors do influence genetic changes that is the reason why clinical and community samples collected from above sites have shown nearly similar allele and haplotype frequencies. Hence, predictions about success rates of anti-malarial drugs and clinical outcomes cannot be easily done. In order to determine drug efficacy and monitoring of drug resistance, high quality molecular markers must be required to make more appropriate decision about potential alternative of present anti-malarials [56]. All indicators based on molecular data need to be considered with caution and interpreted in the local context rather than as a large area. In addition to it, community data may also be affected by prior drug usage and level of pre-existing immunity in patients. It is achieved by a time different recombination rates among parasites which contribute drug selection used by various population groups that influence gene frequencies and drug resistance in malaria parasite [57]. However, low level of parasitemia is an indication of drug resistance and presence of mixed infection [58]. It is also concerned to *P. falciparum* chromosomal mutations [59] and polymorphism occurred in pfcrt, dhfr and dhps genes [60]. Ineffective low dose treatment combination resulted in origin of mixed parasite with sensitive DHFR genotype sensitive isolates [62] while longer use of high drug dose level establishes more resistance and non-sensitive DHFR genotype (Table 1) [62].

### Origin of point mutations in drug resistant genes

Point mutations in dhps and dhfr genes are responsible for formation of various drug resistant mutant alleles of *P. falciparum* [63]. Allelic exchanges occurred at the endogenous genomic locus in *P. falciparum* caused genetic variabilities [64] that determine the drug resistance in a particular area [65]. To find every minute difference caused genetic variabilities [65] that determine the drug resistance in a particular area [65]. To find every minute difference caused genetic variabilities that determine the drug resistance in a particular area [65]. To find every minute difference caused genetic variabilities that determine the drug resistance in a particular area.

The SDX- pyrimethamine resistance is caused after single point mutation occurred within the enzyme active sites [89] mainly dihydroprotozoa synthase (dhps) locus. It has shown independent origin of drug resistant alleles flanking the dhps locus [90] that has generated resistance to SDX, in *P. falciparum* [91]. It is an extremely rare mutation that has spread over large geographical areas of the world. Further, its subsequent spread has affected epidemiology at regional level that is an alarm for future [92]. Such isolates with point mutations in the dhfr and dhps genes of *P. falciparum* associated with pyremethamine and sulfadoxine resistance were also identified in India from Bikaner [93]. Majority of these isolates showed double mutant alleles for dhfr only because abundance of symptomatic carriers, reduced effectiveness of the available anti-malarial drugs and transmission of infection by highly adapted and pesticide resistant local mosquito strains in endemic regions (Table 1) [73].

### Tetracycline resistance

Tetracyclines are used as first line treatment to cure malaria patient’s worldwide. But it has been discontinued because of high prevalence of resistance acquired by malaria parasite. Therefore, for regaining therapeutic status, new and more active antibiotics are to be developed to strike upon malaria cases. In this category Tigacycline, a third generation tetracycline possesses broader spectrum activity was found to be good alternative for the treatment of complicated infections. But, due to very high toxicity and rate of resistance shown by malaria parasite [74] this drug is also banned and its use is being made very limited [75] that resulted in low infertility of *P. falciparum* gametocytes to *Anopheles gambiae* [76] and enhances the rate of gametocyte carriage [77]. Due to accumulation and high prevalence of mutations most of drug treatments become totally failed and proved useless [78]. Chloroquine resistance in *P. falciparum* is reported from India [79] while Clotrimoxazole (anti-foliate) resistance among persons infected with human immunodeficiency virus was reported in Eastern Uganda [80]. Similarly, 1246Y allele was found common in all field isolates collected from Bangui, Central Africa Republic (Table 1) [81].

### Resistance to amodiaquine/sulphadoxine-pyrimethamine (AQ/SP)

Sulfadoxine-pyrimethamine or amodiaquine are commonly used in first line drug therapy to treat uncomplicated falciparum malaria cases [82]. But increasing therapeutic failures associated with the development of significant levels of resistance worldwide has forced to use alternative treatment regimens against malaria. But unfortunately malarial parasite has shown wider resistance to both Sulfadoxine-pyrimethine (SP) and chloroquine (CQ) drugs. It has been spread rapidly within Africa mainly in Kenya where large portion of population is infected with Sulfadoxine- pyrimethrine resistant *P. falciparum* malaria due to rapid emergence of in pfdhps gene mutations [83]. Hence, there is a need to determine the factors related to adherence of amodiaquine/sulfadoxine-pyrimethamine (AQ/SP) and resistance grown in *Plasmodium falciparum*. It is spread across the continents due to high transmission mainly in community people [84]. Pyrimethamine shows high mutation rate in comparison to cycloguanil [85]. It enhances the degree of genomic polymorphism leading to diversity of natural parasite population [86]. SP. resistance in *P. falciparum* also shows deleterious effects in vitro on gametocyte infertility prevalence [87] and drug resistant highly infectious parasites [88].

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in few cases. Recent surveys have revealed wide spread of a high-level pyrimethemine resistant lineage of \textit{Plasmodium falciparum}, of Asian origin, across Africa from where it has shown some distinct genetic characteristics [94]. Undoubtedly, this lineage plays an important role in clinical failure to SP in Africa [95]. Similarly, non-responses gradient to SP and CQ were also found along Myanmar and India international border [96], which is probably indicative of the direction of the movement of the drug-resistant \textit{P. falciparum} parasite (Table 1) [97].

Similar, cases of SP related uncomplicated \textit{P. falciparum} malaria were found in Columbia [98] with an observed diversity of double and triple mutant alleles of dhps of a single origin. However, it can assumed that these multilocus genotypes including unlinked microsatellites loci were originated due to genetic exchanges taken place between low density parasite population and new migrants having malaria infected people [99]. Malaria patients from Rwanda have shown the highest levels of antimalarial drug resistance due to multiple resistances in pfdhfr genes and pfdhps mutations occur 1164L regions [100]. Similarly, increased prevalence of pfdhfr/pfdhps mutants and drug resistance of \textit{P. falciparum} is also reported in Kenya [101]. However, in the beginning of treatment parasite shows very low frequency but later on it enhanced enormously due to mutations occurred in the \textit{P. falciparum} gene (dhfr) encoding dihydrofolate reductase 11507G [102]. Both activity and effectiveness of drug can be assumed by seeing the number of fever episodes and deaths prevented in children (Table 1) [103].

Genetic changes occurred in \textit{P. falciparum} were also detected by using polymorphic microsatellite markers and its analysis [104]. With the help of this technology can explore origin and pattern of spread of drug resistant \textit{P. falciparum} throughout world and can explore new independent lineages and routes of geographical spread of resistance. Further, comparison of molecular evolutionary analyses of samples collected from various endemic regions can identify existence of multilineage SP resistance in many endemic regions [105]. Therefore there is a need to collect molecular epidemiological information regarding dhfr and dhps genes for avoiding the widespread distribution of high levels of resistant parasite in non-infected human population [106]. Hence, an appropriate drug formula should be chosen to reduce the emergence and spread of future drug resistance [107]. Further, for studying the origin and evolution of drug resistance, microsatellite markers flanking the pfdhfr gene are to be mapped. Besides this, lactic dehydrogenase monitoring can be done in \textit{P. falciparum} for screening therapeutic responses to standard malarial drugs (Table 1) [108].

**Chloroquine resistance**

For eradication of \textit{P. falciparum} malaria infection [109] both CQ and SP are predominant anti-malarial drugs of choice [110]. Both drugs showed high efficacy in patients and parasite clearance rate [111] that is why despite diminishing efficacy, chloroquine remains the primary anti-malarial agent in many endemic areas [112]. Emergence of CQ-resistance in \textit{P. falciparum} is associated with a significantly higher prevalence of post-treatment gametocytemia [113] and enhanced the lethality in malaria patients [114]. It is also widely concerned with accumulation of chloroquine versus pyrimethamine/sulfadoxine resistant mutants in uncomplicated \textit{P. falciparum} malaria cases [115]. In India chloroquine resistance in \textit{Plasmodium falciparum} was first reported in 1973. It is caused due to rapid urbanization and civilian migration from infected to uninfected areas. In addition, intermixing of malaria infected patients with normal population and their migration to large geographical area caused very high transmission of malaria across the country [116]. A high degree chloroquine resistant \textit{P. falciparum} was detected in Mandla districh (M.P.) India [117] and east Africa [118]. Similar, CQ-SP resistant Pfcrt alleles were detected in \textit{P. falciparum} isolates that are responsible for seasonal out breaks of malaria on Malo island of Republic of Vanuatu [119]. Due to very high rate of transmission of chloroquine resistant malaria (CQ) led to its withdrawal from use in most countries like Malawi. But after a long gap of its withdrawal there was observed a rapid reduction in the frequency of resistance to the point mutations and the same drug is now considered to be effective once again. Such isolates need to be carefully examined by genetic markers to investigate the CQ-resistance against \textit{Plasmodium falciparum} prior to the withdrawal of CQ. Hence, prior to an official ban poor molecular investigation of clinical isolates should be done properly and very carefully, because it may be a clinical fault [120] or may be due to immunity developed by patients in absence of drug [121]. To cure acute uncomplicated falciparum malaria extra care should be given [122]. Sometimes drug dose level [123] shows lesser efficacy due to irrelevant drug combination used against \textit{P. falciparum} [124]. Hence clinical effectiveness [125] or therapeutic efficacy of both first line (chloroquine and amodiaquine) and second line drugs (sulfadoxine and pyrimethamine) must be prescribed very carefully [126] for successful management of uncomplicated \textit{Plasmodium falciparum} infection occurred mainly in children [127] because multi-drug-resistant \textit{P. falciparum} causes hematological malignancies in children [128]. Similar cases of sulfadoxine resistant falciparum malaria were detected along Thailand-Comodia border. It is real place for origin of CQ-PM resistance from where resistant strains were spread to Asian and African countries [129]. Similar cases of Q and SP resistant \textit{P. falciparum} infection are reported in Solomon island (Table 1) [130].

**Antifolate drug resistance**

Due to increasing trends in chloroquine resistance the antifolate (Sulfadoxine+pyrimethamine combination) drugs are used to treat of falciparum malaria [131]. Antifolate drugs primarily act as DHFR inhibitors and target folate biosynthesis in malaria parasite \textit{P. falciparum} [132]. These drugs, (SP+PQ) in combination were found to be highly effective, safe and better tolerated to children and patients infected with drug resistant malaria [133] and showed superior efficacy than mono-therapies [134]. But due to long term anti-malarial monotherapy (MT) independent point mutations occurred in \textit{P. falciparum} and \textit{P. vivax} [135] both have attained antifolate resistance [136] and these novel pfdhps haplotypes are circulating in West Africa [137] and a mixture of wild-type and resistant pfdhfr and pfdhps alleles are also detected in tourist from this South-East Asian region [138]. Hence, there is an urgent need for the evaluation of alternative and affordable combination treatments (CT) for malaria patients [139]. In such cases both mefloquine [140] primaquine were found effective against \textit{P. falciparum} up to some extent at early infection stage [141]. Besides this, to overcome drug resistance falciparum malaria fixed oral dose of artemisinin-naphthoquine combinations (AQ, ARCO) can be used. These combination therapies provide safety, efficacy and tolerability to the patients. However, a single dose regimen of combination drug may be an effective treatment of uncomplicated \textit{P. falciparum} malaria if regularly prescribed for three days (10 mg/kg/day) to adults [142]. Similarly PG-Ds could provide an effective affordable therapeutic alternative in East Asia [143]. But again such anti-malarial treatments provided in combinations are no longer found effective against \textit{P. falciparum} [144].
Use of Artimisin-based combination therapies and resistance

Artimisin-derivative combination therapies (ACT) were found highly effective against multidrug resistant \textit{P. falciparum} malaria than any other therapy used [145]. ACT is considered as a highly successfully anti-malarial therapy that rapidly reduce both asexual and gametocyte stages of the \textit{P. falciparum} life cycle [146]. It also reduces gametocyte carriage and infection rate in patients [147] and is potentially used for treatment of multidrug-resistant malaria in Africa [148] and Cameroon [149]. Similarly, in Vietnam, use of artesinin derivatives provided initial high success in malaria control [150] but later on malaria parasite become highly resistant to them [151]. Similar cases of artimisin resistance concerned to sulfadoxine/pyrimethamine usage resulted in dhfr quadruple mutants i.e. pfcrt, pfmdr1, dhfr, and dhps in \textit{Plasmodium falciparum} which were identified in clinical isolates collected from Myanmar and Bangladesh border areas (Table 1) [152] and also from Cambodia [153], where it is used to treat uncomplicated malaria [154]. Similarly, Artemether-Lumefantrin (Coartem) and artesunate with sulfoxide-pyrimethamine therapy is also provided to uncomplicated malaria in Ethiopia that has also failed [155]. Hence, monotherapy for self-treatment should be avoided because inadequate treatment regimens favor emergence of drug resistance in malaria parasite [156].

Intermittent preventive treatment of malaria in infants

There is a serious problem to intermittent preventive treatment (IPTp) where drug combinations are provided to mother during pregnancy [157]. It is a promising malaria control strategy which is routinely used to cure the mother and her infant [158]. Use of (AQ/SP) combination raised many questions regarding high level of toxicity observed in clinical trials in context of family use. It shows a parasitological rebound effect due to an appropriate selection of drug and its clearance. But, recently, malaria parasite becomes resistant to IPTp and infections with mixed resistant and susceptible parasites get exacerbated [159]. During pregnancy severe malaria infection resulted in a low birth weight of infants [160]. If such mothers are not treated well, they usually pose high risk of miscarriages and also show weak prospective delivery [161] and high placental infection rates [162].

Such IPTp treated mothers contain high parasite diversity, increased level of parasitemia and severe inflammation in the placenta. It all happens due to changes in allele frequency at DHPS codon 581 in \textit{Plasmodium falciparum} during pregnancy. Hence, regular assessment and chemoprophylaxis of malaria during pregnancy is highly needful to know the severity of infection caused by \textit{Plasmodium falciparum} and physiological adverse effects imposed by the antibiotics on mother and her fetus [163]. For this purpose, routine screening of \textit{P. falciparum} infection must be done up to delivery [164]. Drug susceptibility can be predicted by determining the IC50 values of drug in vitro studies being used. Increased IC50 of a drug determined in clinical isolates represent an instant increase in number of mutations occurred in the malaria parasite, but it is not absolutely true, because sometimes a single mutation occurred in any isolate may responsible for treatment failure in case of a particular drug but do not against all drugs. Undoubtedly, if two or three mutations observed in clinical isolates have shown very high drug resistance level [165]. Meanwhile, few drugs like quinine, amodiquinine, chloroquine, pyronaridine and sulfadoxine/pyrimethamine have shown very high ID50 values i.e. 46, 480, 52, 150, 15, and 10(4) nmol/L [166] in vitro against \textit{P. falciparum}, hence rejected from use only after to confirmation of mutations and treatment failures occurred [167]. Hence, most of the drugs such as sulfadoxine-pyrimethamine (SP) have shown high IC50 values against \textit{P. falciparum} and are totally banned in Africa [168]. But, due to low IC50 values Ciprofloxacin and norfloxacin are prescribed by physicians to kill infections generated by \textit{P. falciparum} [169].

Origin of Gametocytaemia

Long term use of non-effective drugs has increased transmission rate [170] of drug resistant falciparum parasite [171] and its infection rate in vector mosquitoes [172]. However, post treatment with CQ alone [173] or SP and CQ both [174] have shown a significant increase in the density of gametocytes [175] that resulted in gametocytaemia [176] in patients mainly in children [177]. It also reduces genotype formation in malaria infected patients [178] and enhances severity of pathogenesis [179]. To check the acute malaria cases patients should be monitored at an early stage of infection for their proper diagnosis and treatment to reduce the risk of disease progression and gametocyte carriage [180]. In such cases placental infection modulates the appearance of drug resistance in \textit{P. falciparum} in pregnant women mainly in HIV positive women patients [181]. Contrary to this, few patients are able to clear genetically resistant \textit{P. falciparum} genotype [182] that depends on adjustment of endogenous folate level, age, and resistance conferring mutations [183]. In conditions of acute infections, mosquito bites should be avoided [184] because from infected persons mosquitoes lift gametocytes to generate sporozoites in sexual cycle and so they transmit it to new uninfection person. In endemic areas CQ-R resistant falciparum harbors in acute malaria patients which also work as large reservoirs of gametocytes. However, certain drugs like primaquine, artesinin and its derivatives in combination are used to lower down gametocyte carriage parasite density, which reduces the chances of re-infection in treated individuals [185]. Hence, well tested treatment strategies are to be used for successful combating the occurrence of gametocytaemia in patients [186].

Refugee camps are source of drug resistance alleles

Because of instability in eastern Afghanistan, new refugees crossed into the federally administered tribal areas of northwestern Pakistan in 2002. Investigators have identified an epidemic of \textit{Plasmodium falciparum} malaria in 1 of the camps. Incidence was 100.4 cases/1,000 person-years; in other nearby camps it was only 2.1/1,000 person-years. Anopheline mosquitoes were found despite even after spray campaigns. The main clinical failure identified was used of locally manufactured sulfadoxine-pyrimethamine for routine treatment. In vivo failure rate was 28.5% and PCR analysis of the \textit{P. falciparum} dihydrofolate reductase and dihydropyrimido synthase genes showed no mutations associated with clinical failure. Therefore, clinically standard drug regimens should be used at global to level decrease incidence rate and rising malarial epidemics. To check this threat, enhanced quality assurance of control interventions is essential [44]. Molecular monitoring of parasite resistance is more important to launch anti-malarial drug policies. Again there is a possibility that large number of refugees surging up in European countries from Syria, Iraq, Lebanon and Afghanistan will sit in camps and acquire drug resistant malarial strains in future. Hence, testing of community samples for molecular drug resistance new bio- markers should be explored to be using them complementary tool for decision-making for the best treatment options and appropriate potential alternatives [187]. In addition, indicators based on molecular data have to be considered with caution and interpreted in the local context, especially with regard to prior drug usage and level of pre-existing immunity. For finding different climate and drug induced changes large scale genotyping and genetic mapping.
be needed in Plasmodium parasites. There must be identification of reasons of clinical failures due to selection and prescription of anti-malarial drugs. It is also important to identify effects of patient treatment on non-resistance group. Here, it is suggested that current scientific challenge regarding drug resistance should be accepted and all different reasons related to patient treatment regimes, prevention of pathogen transmission and developing mutations in malaria parasite will be explored to achieve complete elimination of drug resistant malaria as fast as possible.

Conclusion

In the present time control of multidrug resistant *Plasmodium falciparum* malaria has become a very difficult task because endogenous allelic exchanges occurred in *P. falciparum* have increased the therapeutic failures and significantly increased the levels of resistance worldwide. Big question here is how formation of drug resistant mutant alleles stops, because resolution is unending process. It is an important issue that has many dimensions to study and most important are demographic, eco-climatic and eco-genetic issues. Demographic issues are manmade while origin of malaria is natural and widely concerned to eco-climatic conditions. Hence, it is a great challenge, how to check the movements of symptomatic carriers that are responsible for transmission and dispersal among the non-infected human population across the continents. Further, genomic adaptations generated in *P. falciparum* in such carriers are proved highly prone to new mutations and many more genetic exchanges are possible when such migrants mix with uninfected population. Highly adapted and pesticide resistant local mosquito strains in endemic regions invited new possible mutations with in them and *P. falciparum*. Hence, there is a need to determine the factors related to adherence of various drugs and resistance grown in *Plasmodium falciparum*. However, for quick analysis of genomic polymorphism or diversity of natural parasite population exists in *P. falciparum* polymorphic microsatellite markers are to be used. With the help of this technology one can explore origin, pattern and spread of drug resistant *P. falciparum* worldwide. It may also help to find occurrence of new independent lineages and routes of geographical spread of resistance. Further, comparison of molecular evolutionary analyses of samples collected from various endemic regions can help to explore existence of multi-lineage drug resistance. Therefore there is a need to collect molecular epidemiological information regarding changes occurring at genomic level for avoiding the widespread distribution of high levels of drug resistant malaria parasite to stop it from spreading among non-infected human population.

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