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Chemotherapy and drug resistance status of malaria parasite in northeast India

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ABSTRACT

India reports the highest number of malaria cases in Southeast Asia, of which *Plasmodium falciparum* contribute more than half of the cases every year. North eastern states of India contribute only 3.96% of country's population but account for >10% of total reported malaria cases, 11% of *Plasmodium falciparum* cases and 20% of malaria related deaths annually. In India, chloroquine resistance was reported for the first time from northeast region and since then chloroquine treatment failure is being reported from many parts of the region. Increased chloroquine treatment failure has led to change of the drug policy to artemisinin combination therapy as first line of malaria treatment in the region. However, replacing chloroquine to artemisinin combination therapy has not shown significant difference in the overall malaria incidence in the region. The present review addresses the current malaria situation of northeastern region of India in the light of antimalarials drug resistance.

1. Introduction

Malaria is one of the most important infectious diseases prevalent mostly in tropical and subtropical countries. Among the Southeast Asian countries, India reports maximum number of malaria cases. The entire Southeast Asian region reports >100 million malaria cases[1], whereas India records about 2 million malaria cases annually[2]. Among the four malaria paraswite species, *Plasmodium falciparum* (*P. falciparum*) has been reported to contribute about 50% of the total cases. Northeastern states of India are highly endemic to malaria and reports high number of malaria cases every year. As the region has uneven malaria distribution and frequent localized focal outbreaks, controlling has been a daunting task. Emerging antimalarial resistance is a major concern in malaria control programs[3]. Chloroquine resistance is wide spread contributing epidemic outbreaks regularly[4]. National vector borne disease control programme (NVBDCP) of India in 2008 changed the antimalarial drug policy in the region and replaced chloroquine by artemisinin combination therapy (ACT) for treatment of uncomplicated malaria. In the present article, available literature on malaria chemotherapy and drug sensitivity of malaria parasite in northeastern region has been reviewed and discussed. The literature was collected from the library of Defence Research Laboratory and internet using relevant key words.

2. Malaria situation in northeastern India

Northeastern part of India comprising of eight states is strategically important as it share international borders with China, Bangladesh, Bhutan and Myanmar. The international borders terrains in northeast India are mainly hills and foothills covered by thick forests. These areas lack communication and have poor health infrastructure. Due to congenial climate, difficult terrain, unstable

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population, human migration and tremendous *Plasmodium* parasite load, these states are highly endemic to malaria[5]. The population of north eastern region is only 4% of the country but records about 10% of total malaria cases in India annually. *P. falciparum* is the major infection throughout these states causing considerable mortality. About 11% of the *P. falciparum* cases of India are reported from these states[5]. Assam is the most populated state in the northeastern region and contributes majority of the malaria cases (~50%) followed by Arunachal Pradesh, Meghalaya and Tripura[2].

Malaria cases are reported across the region, however, the cases are relatively high in the foothills and areas close to the borders[6,7]. The available malaria data (source from NVBDCP) of seven northeastern states (excluding Sikkim) for the years 2007–2011 revealed that slide positivity rates have been higher consistently in Arunachal Pradesh, Meghalaya and Tripura (Figure 1). Percent falciparum cases were higher in Tripura, Meghalaya and Assam (Figure 2), whereas malaria attributable death rates were higher in Nagaland, Meghalaya and Mizoram respectively (Figure 3). Meghalaya is comparatively a small state sharing border with adjacent lowlands of Bangladesh. Malaria incidence is commonly reported from these lowland areas and consequently increases the malaria burden in Meghalaya state[8].
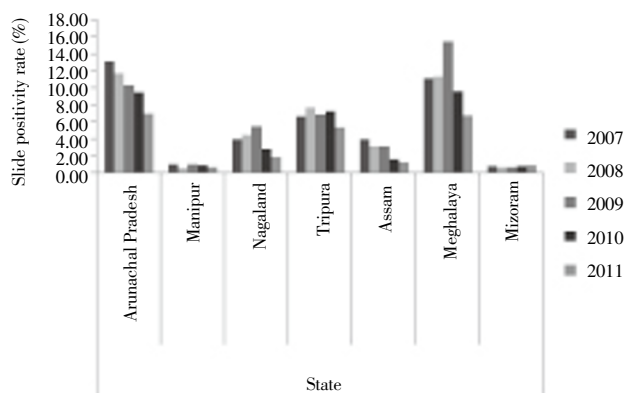

Figure 1. Slide positivity rate of the seven northeastern states for the year 2007–2011 (source: NVBDCP).
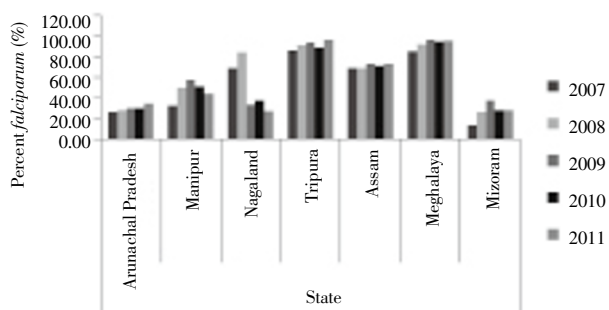

Figure 2. Percent *falciparum* in the seven northeastern states for the year 2007–2011 (source: NVBDCP).
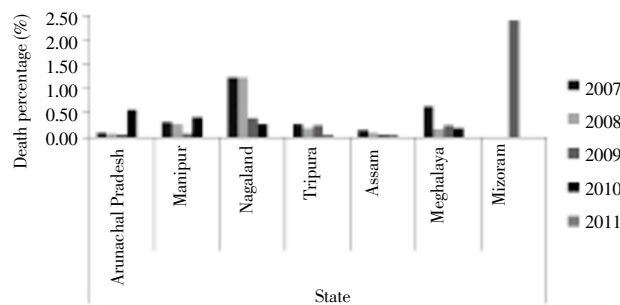

Figure 3. Percentage of death cases in seven northeastern states for the year 2007–2011 (source: NVBDCP).

In India, 58 species of *Anopheles* mosquitoes have been recorded, out of which six species, namely, *Anopheles culicifacies*, *An. dirus*, *An. minimus*, *An. fluviatilis*, *An. sundaicus* and *An. stephensi* are regarded as malaria vector of major importance[9]. In northeastern region, 45 species of *Anopheles* mosquitoes have been recorded so far, of which *An. minimus*, *An. baimai* and *An. fluviatilis* play a crucial role in malaria transmission in this region[9,10]. *An. minimus* is a perennial species, whereas *An. baimai* (formerly *An. dirus* D) is a monsoon species and *An. fluviatilis* is mostly recorded in the winter months[11]. These vectors are highly anthropophilic and hence highly efficient in malaria transmission in the region[11,12]. Although these species are susceptible to DDT and deltamethrin, which are used in the vector control program, they don't rest on the sprayed walls avoiding the killing action of these insecticides because of exophilic and exophagic behaviour[13].

3. Chemotherapy in malaria

Treatment of malaria has a long history from herbals to synthetic antimalarials since the time eternity. Most of the current synthetic antimalarials have been derived from the herbal materials that were used for malaria treatment for centuries. Currently available antimalarial compounds belong to three distinct groups, viz, aryl amino alcohol compounds (chloroquine, quinine, mefloquine, amodiaquine, halofentrine, lumefentrine, and primaquine); the antifolates (pyrimethamine, trimethoprime, and proguanil) and artimisinin derivatives (artemisinin, dihydroartemisinin, artesunate, and arteether).

Chloroquine and other quinolines like mefloquine and primaquine are widely used drugs for malaria treatment. Chloroquine has been the antimalarial of choice in many parts of the world including India due to its high efficacy and comparatively low toxicity. However, due to increased selection pressure and indiscriminate use, malaria parasite

resistance to these drugs has been emerged in various parts of malaria endemic regions. Quinine is very effective and is also one of the oldest antimalarial drug against malaria parasite. It has been used in first line of treatment of severe malaria cases in India and some African countries, however, its use has been limited due to toxicity and side effects. Artemisinin is a herbal derived drug traditionally used in China for treatment of fever. Its derivatives have been very effective but result in treatment failures due to its short half life period. Artemisinin based combination therapies (ACT) use combination of artemisinin or any of its derivatives with some partner drug having long half live period. The ACT approach helps in reducing selection pressure of a single drug and may be helpful in reducing resistance development to a particular antimalarial drug. Some of the ACTs recommended by world health organisations (WHO) are artesunate and mefloquine, artesunate and sulfadoxine–pyrimethamine, artesunate and amodiaquine, artemether–lumefantrine[14].

In India, NVBDCP has laid down the guidelines for treatment of malaria cases and provided new malaria drug policy for entire India, some of the guidelines of new malaria drug policy are as follows[14]:

(1) All the fever cases should be preferably investigated for malaria by microscopy or rapid detection test.

(2) The first line of treatment is chloroquine and the second line is ACT (artesunate+sulfadoxine/pyrimethamine) combination. In malaria cases resistant to ACT and treatment of severe and complicated malaria, treatment with quinine has been suggested.

(3) Microscopy confirmed *P. falciparum* cases are to be treated with chloroquine in therapeutic dose of 25 mg/kg body weight over 3 d and a single dose of primaquine 0.75 mg/kg body weight on the first day. The practice is to be followed at all levels including voluntary health workers like drug distribution centers, fever treatment depots and accredited social and health activist. *etc*.

Further, all *P. vivax*, undiagnosed fever and clinical malaria cases are to be treated with chloroquine in full therapeutic doses. ACT is the first line of antimalarial drug for treatment of *P. falciparum* in chloroquine resistant areas. According to NBVDCP guidelines[14], all the northeastern states have switched over to ACT from chloroquine as the first line of treatment since 2008.

4. Antimalarial drug resistance

One of the major challenges faced by malaria control programes is antimalarial drug resistance. Spread of malaria to new areas or re–emergence of malaria in areas where the disease has been eradicated can be attributed to antimalarial drug resistance. Human migration, population movement due to development and natural disaster have fueled the spread of drug resistance to new areas. Antimalarial drug resistance has been involved in the occurrence and severity of epidemics in many parts of different countries[15].

Out of four species of malaria parasite that naturally infect human, *P. falciparum* and *P. vivax* have developed resistance to antimalarials, however the geographical distribution of resistance to a single antimalarial drug varies in different region[3]. Resistance to all classes of antimalarials with the exception of artemisinin has been reported from different regions in recent past decades[16,17]. Antimalarial resistance to *P. vivax* has emerged comparatively later and reported mostly in Southeast Asian countries[17,18].

Northeastern India has very high prevalence of *P. falciparum* infection and recorded antimalarial resistance in last three decades. The first case of chloroquine resistance in India was reported in Karbi Anglong District of Assam in 1973[19,20]. Subsequently, it has been reported in different part of northeastern India, viz, Arunachal Pradesh, Nagaland, Mizoram and Meghalaya during 1979–1981[21]. Chloroquine resistant strains multiplied quickly in this region under the influence of several efficient *Anopheles* vectors. *P. falciparum* gradually developed multiple drug resistance particularly in border areas. Presently, entire northeastern region is considered to be nidus of spread of drug resistance[22]. Upsurge of malaria cases in 1970s and subsequent reports of antimalarial treatment failures led to review the entire situation and a new programme called *P. falciparum* Containment Programme (PfCP) was launched in 1978 with the help of Swedish International Development Agency (SIDA). With its main objective to control the spread of drug resistant *P. falciparum* malaria, the drug resistant foci were diluted in its ten years of operation[23]. Studies on antimalarial efficacy in the northeastern region have shown that chloroquine resistance is widespread. Sehgal *et al.* has reported the RI level of resistance in 52.5% and RII level in 22.5% of cases[19]. In other studies, Sehgal *et al.* reported 24% RI resistance[24], while Gogoi *et al.* has reported RI, RII and RIII resistance in some tea garden population of Assam[25]. Baruah *et al.* has shown RI and RII level of resistance in different age groups[26]. Altogether 37% of chloroquine resistance has been observed, but no resistance case has been reported for sulfadoxine–pyrimethamine treatment. Most of the studies are of localized nature and have involved a limited number of samples. However, in 1997, national level compilation of antimalarial drug resistance included 12 863 *P. falciparum* cases and revealed about 24% chloroquine resistance of varying level[4]. Overall resistance at RI level is common in many parts of India

except northeastern states, whereas spread of RII and RIII resistance was restricted to the selected areas only[4]. High level of resistance in northeastern states has been confined along Indo–Bangladesh and Indo–Myanmar international border areas[22]. Chloroquine resistance of varying level was reported from different areas of Tripura[6,7]. Dhiman *et al.* reported 35% treatment failure in chloroquine treatment from South Tripura[6], whereas Majumdar *et al.* reported 67.5% chloroquine resistance in Dhalai District of Tripura[27]. In one of the largest drug sensitivity study conducted in this region, Campbell *et al.* reported 95.8% treatment failure in chloroquine and sulfadoxine–pyrimethamine (57.1%)[28]. However, resistance to mefloquine and mefloquine artesunate combination was not very much prevalent[28]. Resistance to sulfadoxine–pyrimethamine has been reported in many forest fringe areas of northeastern region, however, resistance to artemisinin group drugs has not been reported from this region making it suitable for the treatment of uncomplicated malaria cases[28,29]. Studies have also reported multi drug resistance in few locales of this area[30,31].

5. Molecular epidemiology of antimalarial drug resistance

Antimalarial drug resistance is generally monitored using *in vivo* test as per the WHO guidelines. Though this is an expensive, time consuming and specialized study requiring much expertise, it provides better results for the treatment perspectives. Alternatively, *in vitro* method has also been used to determine the drug resistance status in many areas[32]. This is also an expensive method requiring good laboratory facilities, but it is practically less feasible as the parasite may not adapt to the *in vitro* culture many a times[32].

Attempts have been made to develop molecular markers which can give idea about drug resistance status of a parasite population[33–38]. *P. falciparum* chloroquine resistance transporter protein (pfcrt) has shown good correlation with chloroquine resistance[33–35]. Mutations at several amino acid positions of pfcrt protein have been reported in resistant strains. Mutation at 76th position where lysine is replaced by threonine has been found mostly in all the resistant cases, therefore, it is widely used in the *in vitro* chloroquine resistance monitoring studies[33]. However, sometimes the presence of this mutation is also observed in chloroquine susceptible cases. The studies have shown that the association of other mutations with K76T mutation in pfcrt has revealed varying level of resistance[36]. Three other mutations in the adjacent upstream of 76th position, i.e., 72nd, 74th and 75th position with the monomorphic

73rd position, have been reported to form different pfcrt haplotype[36]. Two major haplotypes, CVIET and SVMNT, have been found to be associated with chloroquine resistance in malaria endemic regions[36,37]. Four different chloroquine resistant haplotypes along with two pfcrt mother haplotypes CVIET and SVMNT have been reported from this area[38]. SVMNT haplotype has been reported in majority of isolates, whereas CVIET haplotype is more prevalent in high endemic areas including northeastern states of India. Association of this allele with high chloroquine resistance has been observed in many places. Chloroquine resistance isolates with single mutant allele were few and reported from Assam and Arunachal Pradesh only[38]. Chloroquine sensitive haplotype CVMNK at a lower frequency of 2%–3% has also been reported in northeastern states[38]. Evolutionary studies suggested that India seems to have received chloroquine resistant *P. falciparum* from Southeast Asian countries[36]. Carefully observing the distribution of different haplotypes, it is inferred that CVIET haplotype might have entered India through Myanmar from Southeast Asia. Further, SVMNT haplotype initially originated from Papua New Guinea seems to reach India through Southeast Asian via Andaman and Nicober Islands[36].

In addition to chloroquine, the antifolate antimalarials which are mainly used in chloroquine resistant areas as first line of treatment have also been monitored using molecular markers. The antifolate antimalarials markers are better defined than chloroquine resistance markers. Sulfadoxine and pyrimethamine molecular markers have also been reported in the form of mutations that occurred in the corresponding metabolic enzymes[39–47]. Point mutations at the 51, 59, 108 and 164 amino acid positions of *P. falciparum* dihydrofolate reductase enzyme involved in folate biosynthetic pathway show pyrimethamine resistance[39,48]. It has been shown that higher number of mutation gives rise to higher level of resistance[49]. In Northeastern states, frequency of triple mutation is present in higher number than that in North India which indicate higher level of resistance to pyrimethamine[50]. Further, mutations at sulfadoxine binding enzyme *P. falciparum* dihydropteroate synthetase (Pfdhps) reduce its binding capacity rendering it to become resistant[51]. Wild type alleles of pfdhps are mostly prevalent throughout India except some places like Andaman & Nicober Island, where five different mutations in amino acid position 436, 437, 540, 580 and 613 in pfdhps have been associated with the sulfadoxine resistance[51,52]. In Assam, four types of mutant alleles were reported, which indicated higher level of resistance. The isolates from Northeastern states have showed higher number of multi–locus mutation and thus could be having higher level of sulfadoxine pyrimethamine resistance than those from other

parts of India[49,52].

6. Conclusions

Upsurge of *P. falciparum* cases and spread of drug resistant malaria in northeast India has threatened the malaria control campaign in the region. As the region experiences frequent focal malaria outbreaks at local level, the control programme must include region specific intervention measures in order to check the disease proliferation. The resistance to chloroquine and other antimalarials is wide spread in this region. Increasing development activities and high human migration in this region may result in spread of resistant strains to new areas and can create new threat to malaria control. Vector control measures such as, insecticide treated bed nets and other personal protective measures need to be encouraged rather spraying DDT alone in indoor residual spray. Changes in the drug policy are much needed step as on today, however the effective implementation of the new drug regimen at ground level may take some time. Improving health infrastructure at peripheral level is of topmost importance for improvement in disease status in the endemic areas. Regular monitoring of drug resistance and associated molecular markers are needed to be studied at larger extent for proper inputs to the drug policy makers.

Conflict of interest statement

We declare we have no conflict of interest.

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