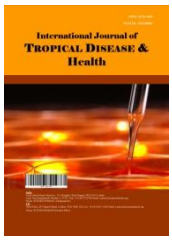

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Burden of Drug Resistance in vivax Malaria in India - A Brief Update

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Authors' contributions

This work was carried out in collaboration between both authors. Author PS did the literature search and wrote the first draft of the manuscript. Author NR guided the manuscript writing and designed the presentation of the content. Both authors read and approved the final manuscript.

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ABSTRACT

India contributes significantly to the global *Plasmodium vivax* burden. Treatment of malaria is more challenging than before owing to the rise of antimalarial drug resistance. The commonly used antimalarial drugs for treatment are chloroquine, antifolates like sulfadoxine-pyrimethamine, and artemisinin-based derivatives. Antimalarial resistance is studied by in vitro and in vivo methods. Study on mutations in the drug targets in the parasite is a widely used tool to help foresee likely resistance and relate to the clinical picture. The majority of studies on antimalarial resistance from the Southeast Asian region come from countries like Thailand and Myanmar. Though therapeutic failure with these antimalarial agents has not been reported in India, there have been reports of reduced clinical efficacy in the presence of mutations in their molecular targets.

Keywords: Antimalarials; Plasmodium vivax; drug resistance; disease burden; India.

ABBREVIATIONS

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UTs : *Union territories*
ACT : *Artemisinin based combination therapy*
NVBDCP : *National Vector Borne Disease Control Programme*
CQ : *Chloroquine*
SP : *Sulfadoxine-pyrimethamine*

1. INTRODUCTION

1.1 Malaria Burden in India

According to the WHO world malaria report 2018, India stood fourth in the global malaria burden, contributing to around 82% of all deaths due to malaria in the WHO South-East Asia Region [1]. At present malaria has been made notifiable in 31 states/UTs (Andhra Pradesh, Arunachal Pradesh, Assam, Chhattisgarh, Goa, Gujarat, Haryana, Himachal Pradesh, Jammu & Kashmir, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Manipur, Mizoram, Nagaland, Odisha, Punjab, Rajasthan, Sikkim, Tamil Nadu, Telangana, Tripura Uttar Pradesh, Uttarakhand, West Bengal, Puducherry Chandigarh, Daman & Diu, D&N Haveli ,and Lakshadweep) [2]. India has shown good progress in reducing its malaria burden, being the only high endemic country to have shown a significant decline in malaria cases (17.6%) from 2018 to 2019 [2]. India currently contributes to 2% of the malaria cases and 2% of deaths globally. However, it contributes to 47% of the global *P. vivax* malaria burden, which is very significant [2]. A map showing the indigenous malaria cases across countries from 2000-2020 is given in Fig. 1.

1.2 Clinical Features of Malaria

The hallmark feature of malaria in infections with all *Plasmodium* species is fever [3]. The fever is usually irregular and then becomes periodic depending on the synchronized schizogony. The classical malaria paroxysm has three stages: a cold stage, a hot stage, and a sweating stage. In the cold stage the patient feels extremely cold and lasts for about 10-30 minutes. In the hot stage skin becomes hot and dry, the face flushes, the person feels hot, and the stage lasts 2-6 hours. In the sweating stage, the temperature decreases and there is sudden profuse sweating, starting at the temples and then all over the body. The patient feels extremely tired and sleepy at this stage which lasts for about 2 to 3 hours. Thus one entire paroxysm lasts in total around 6–10 hours [3]. A diagrammatic sketch of the febrile paroxysms in *P.vivax* malaria is given in Fig. 2.

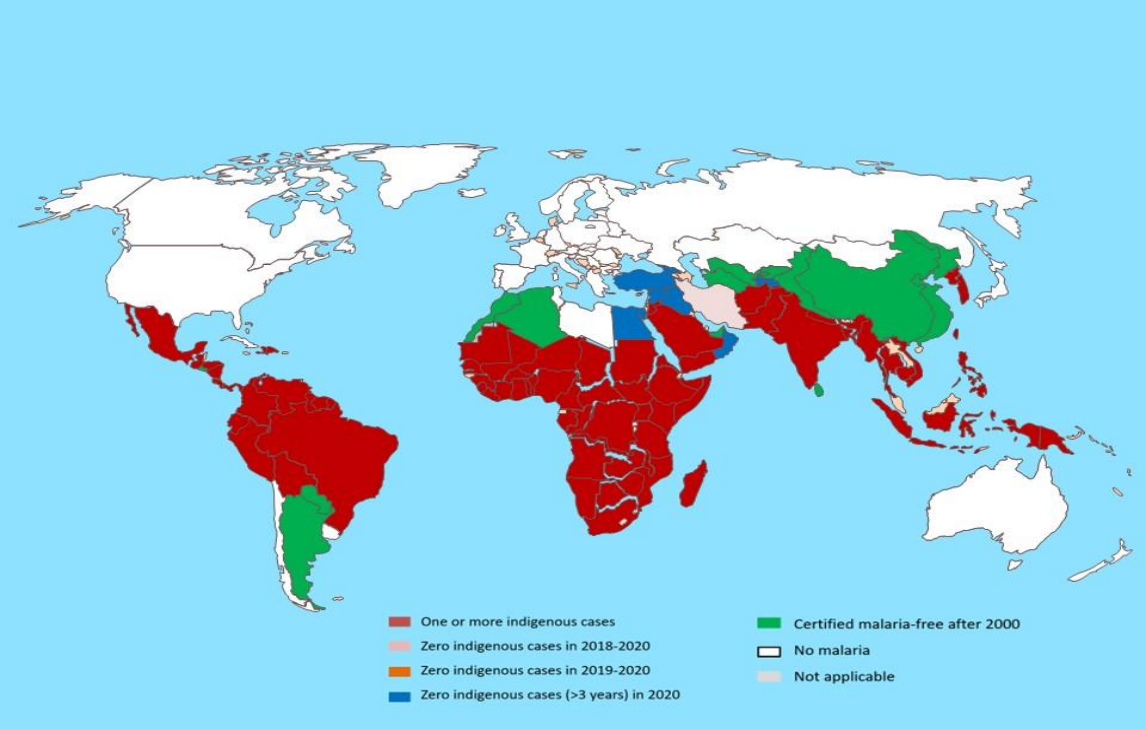


Fig. 1. Indigenous malaria cases across countries from 2000-2020

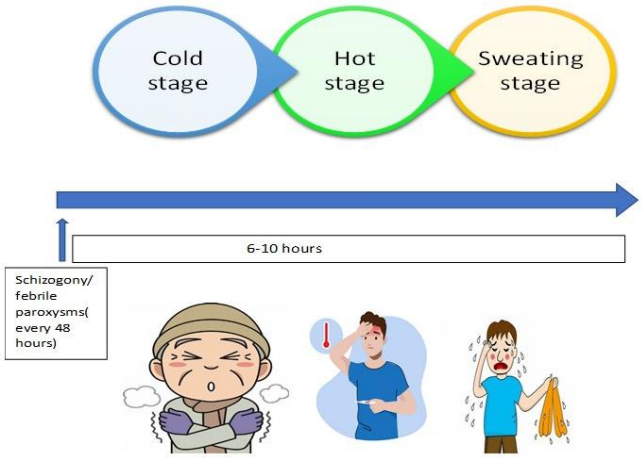


Fig. 2. Febrile paroxysms in *Plasmodium vivax* malaria

In *P. vivax*, schizogony and hence the febrile paroxysm occurs every 48 hours which is described as the third day or tertian fever [3]. Between febrile paroxysms, the patient remains afebrile. In *P. vivax* and *P. ovale* infection, the hypnozoites that are dormant in the liver may turn to hepatic schizont and release the merozoites into the blood which results in a relapse. Relapses can happen weeks or months or sometimes years, after the initial infection [3].

Severe malaria is usually seen in infections with *P. falciparum* and can involve central nervous system, respiratory system, renal system or hematopoietic system. Such malaria is characterized by one or more of the following features: "Impaired consciousness/coma, repeated generalized convulsions, renal failure (Serum creatinine >3 mg/dl) , jaundice (Serum bilirubin >3 mg/dl), severe anaemia (Hb <5 g/dl) , pulmonary oedema/acute respiratory distress syndrome, hypoglycaemia (Plasma glucose <40 mg/dl) , metabolic acidosis, circulatory collapse/shock (Systolic BP <80 mm Hg, <50 mm Hg in children), abnormal bleeding and Disseminated intravascular coagulation (DIC), haemoglobinuria, hyperpyrexia (Temperature >106°F or >42°C) and hyper parasitaemia (>5% parasitized RBCs)." [4] (p.9,10).

Continuous monitoring of the patient is required for signs of progression to severe malaria. Although *P. falciparum* is the common cause of severe malaria and deaths, there has been increasing evidence of severe malaria due to *Plasmodium vivax* as well. Studies from Indonesia and Papua New Guinea reported severe malaria due to *P. vivax* to be almost equal to or higher than those observed with *P. falciparum* [5]. Most common complications reported are seizures, circulatory collapse, jaundice, renal failure, severe anaemia, coma, and acute respiratory distress syndrome [6–9]. Traumatic or spontaneous rupture of the enlarged spleen causing fatal haemorrhage is one rare complication that has been reported with *P. vivax* [10].

2. TREATMENT GUIDELINES FOR *P. vivax* MALARIA IN INDIA

2.1 Uncomplicated Malaria

WHO guidelines recommend chloroquine combined with primaquine as the treatment of choice for chloroquine-sensitive infections and Artemisinin- based combination therapies (ACTs) in areas with chloroquine resistant *P. vivax,* along with at least a 14-day course of primaquine (0.25 – 0.5mg/kg/day). The treatment guidelines followed in India are adopted from the WHO and laid by the NVBDCP Programme. *P. vivax* malaria is treated with full dose of chloroquine 25mg/kg divided over three days and primaquine (0.25 mg/kg) under supervision for 14 days. Primaquine is given to prevent relapses due to the hypnozoites in the liver. However primaquine administration is avoided in pregnant women, infants and individuals with G6PD deficiency [4]. The NVBDCP treatment algorithm for treatment of malaria is given in Fig. 3.

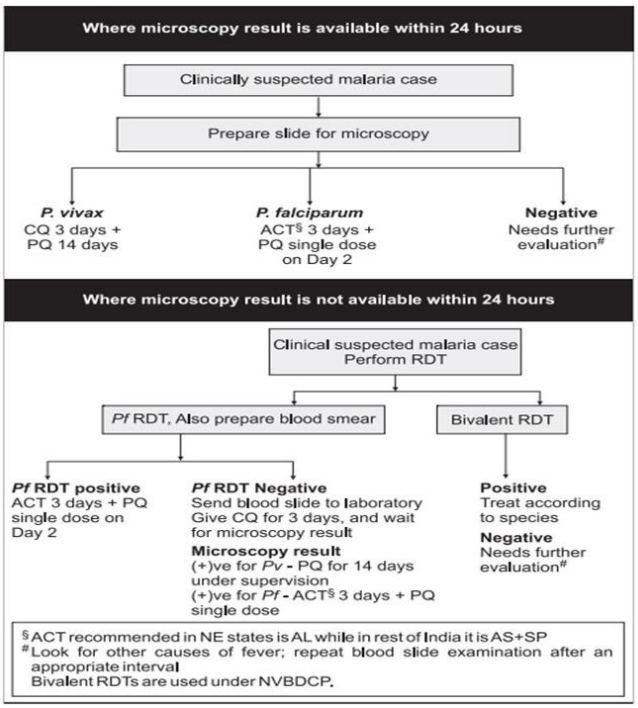


Fig. 3. NVBDCP algorithm for diagnosis and treatment of malaria
NVBDCP- National Vector Borne Disease Control Programme

2.2 Severe Malaria

In cases of severe malaria with *P.vivax*, treatment remains the same as for severe malaria by *P.falciparum*. However, primaquine should be given for 14 days for preventing relapse as per the guidelines after the patient recovers from acute illness and can tolerate primaquine [4]. Parenteral artemisinin derivatives or quinine is used irrespective of chloroquine sensitivity. Intravenous route is preferred over intramuscular.

2.3 In Pregnancy

In the first trimester, parenteral quinine is the drug of choice. However, if quinine is not available, artemisinin derivatives are given to save the life of mother. In second and third trimester, parenteral artemisinin derivatives are preferred. Doxycycline is contraindicated in pregnant women and children under 8 years of age and clindamycin is given instead [4]. A summary of the NVBDCP guidelines in the treatment of malaria in various scenarios is given in Table 1.

3. TREATMENT FAILURE IN MALARIA

Some patients may not respond to treatment which may be due to drug resistance or treatment failure, especially in falciparum malaria. After treatment the patient is considered cured if he/she does not have fever or parasitaemia till day 28 [4]. If patient does not respond or show signs of progression in clinical symptoms, he/she is given an alternative treatment.

3.1 Early Treatment Failure (ETF)

“Development of danger signs or severe malaria on day 1, 2 or 3, in the presence of parasitaemia; parasitaemia on day 2 higher than on day 0, irrespective of axillary temperature; parasitaemia on day 3 with axillary temperature >37.5°C; and parasitaemia on day 3, >25% of count on day 0.” [4](p.7,9)

3.2 Late Clinical Failure (LCF)

“Development of danger signs or severe malaria in the presence of parasitaemia on any day between day 4 and day 28 (day 42) in patients who did not previously meet any of the criteria of early treatment failure; and the presence of parasitaemia on any day between day 4 and day 28 (day 42) with axillary temperature >37°C in patients who did not previously meet any of the criteria of early treatment failure.” [4] (p.9).

Table 1. NVBDCP guidelines for treatment of malaria in various scenarios

Scenario	Treatment guidelines	Special considerations
Uncomplicated *P.vivax* malaria	Chloroquine (25mg/kg) over three days (10-10-5) plus primaquine (0.25mg/kg) oral for 14 days under supervision	Primaquine is contraindicated in G6PD deficient patients. Testing for G6PD deficiency to be done, if facilities are available, in areas with high prevalence before start of primaquine.
Complicated malaria	Parenteral quinine (20 mg/kg iv infusion on admission followed by 10mg/kg 8 hourly for 48 hours and 10mg/kg per oral three times a day for 5 days. Add on with doxycycline 3mg/kg per oral for 7 days OR Parenteral artemisinin derivatives- Artesunate 2.4 mg/kg i.v. or i.m. given on admission, then at 12 hours and 24 hours, then once a day. To be followed by a full course of oral ACT.	Quinine should never be given as a bolus dose. Loading dose of 20 mg/kg can be avoided in patients who have received quinine prior Intravenous preparations should be preferred over intramuscular preparations
Mixed infection with *P.falciparum*	Same as for *P.falciparum* infection. Artemisinin combination therapy (Artesunate plus sulfadoxine-pyrimethamine or Artemether- lumefantrine per oral for 3 days along with single dose primaquine on 1st day)	Artemether-lumefantrine preferred in Northeastern States
Pregnancy	1st trimester- Parenteral quinine 2nd and 3rd trimester- Parenteral artemisinin	Doxycycline is contraindicated. Clindamycin can be given instead
Children		Primaquine is contraindicated in infants and doxycycline in children <8 years of age

3.3 Late Parasitological Failure (LPF)

"Presence of parasitaemia on any day between day 7 and day 28 with axillary temperature <37.5°C in patients who did not previously meet any of the criteria of early treatment failure or late clinical failure. Such cases of falciparum malaria should be given alternative ACT or quinine with doxycycline. Therapeutic failure to chloroquine is a rare entity in India." [4](p.9)

4. MECHANISM OF ACTION OF ANTIMALARIAL DRUGS

4.1 Chloroquine

4.1.1 Mechanism of action

Chloroquine is the oldest drug in the treatment of malaria. It had successful treatment history until recently when drug resistance was noted especially in *Plasmodium falciparum* infections. Chloroquine belongs to quinoline containing group of drugs and it acts by accumulating in the food vacuole of the intraerythrocytic trophozoite. Once infected, the host haemoglobin is ingested through the cystosome of the parasite into its digestive vacuole [11]. Inside the digestive vacuole, it is degraded by the parasite's proteolytic enzymes into small peptides along with the release four molecules of heme (ferriprotoporphyrin IX, [Fe(III)PPIX] [12,13]. To evade the toxicity due to the free heme, most organisms use heme oxygenase to convert heme into its inert form [14]. Since *Plasmodium* lacks this enzyme, it utilises an alternate pathway where heme is crystallised into a nontoxic form, hemozoin (heme dimers bound by reciprocal iron carboxylate interactions and stabilised by hydrogen bonds) [14–16]. This finally leads to formation of an acidic digestive vacuole [17]. Chloroquine binds with heme and interferes with the formation of hemozoin [18]. Chloroquine that permeates the membrane of the digestive vacuole becomes protonated within the vacuole which prevents its exit from the vacuole. As a result, CQ accumulates in the vacuole which binds to heme [17]. Thus accumulation of chloroquine in the food vacuole prevents the formation of hemozoin formation from the free heme. The free heme that accumulates leads to the lysis of the membranes, formation of reactive oxygen intermediates which in turn becomes toxic to the parasite. Another recent finding about the cause of toxicity to the parasite with the drug is its inhibitory action on heme polymerase which is involved in polymerisation of the toxic free heme in the parasite's food vacuole [19,20]. This is known as the capping mechanism of heme polymerisation wherein chloroquine binds to the free heme as it is formed by the degradation of hemoglobin forming a complex. This complex binds (caps) to the growing heme polymer thus causing termination of the polymerisation and hence accumulation of free heme [20]. The mechanisms shown by the parasite to evade toxicity due to free heme is shown in Fig. 4.

CQ acts by inhibiting the formation of hemozoin and by forming a complex with free heme that caps the growing heme polymer and terminates its formation and hence accumulating free heme.

4.1.2 Efficacy

Chloroquine with primaquine is the standard regimen for treating *Plasmodium vivax* malaria. Chloroquine is considered as the most apt drug for treating acute malaria in endemic areas. The low cost and its action against all *Plasmodium* species, the lesser number of doses, safety profile in pregnant women and small children and the few side effects are the highlights of this drug for use in malaria. Chloroquine brings down fever and parasitaemia caused by *P. vivax* within a maximum of 72 hours of the first dose. It is very rapidly absorbed and slowly eliminated with a half-life of about 50 hours, and therapeutic levels persisting in blood for about days 21 to 35 after the start of treatment [21].

4.2 Antifolates

4.2.1 Mechanism of action

Folates in their reduced state act as co-factors in many one-carbon transfer reactions in the synthesis of amino acids and nucleotides. The molecular structure of folate showing the binding sites is shown in Fig. 5. Since the parasite has a high rate of replication, there is a high need for nucleotides in the synthesis of DNA [19]. The antifolate drugs used against malaria are pyrimethamine, proguanil and the sulfa drugs like sulfonamide, sulfadoxine, sulfone, dapsone etc. Pyrimethamine targets the dihydrofolate reductase activity whereas the sulfa drugs target the dihydropteroate synthetase. The malaria parasite can synthesize folates de novo whereas the human host cannot synthesize folate and requires exogenous folates. Folate is synthesized from GTP, p-aminobenzoic acid (PABA), and glutamate. The sulfa drugs are structural analogs of PABA which disrupts folate synthesis and causes a depletion of the folate pool which results in disruption in the synthesis of DNA that requires thymidylate [19]. Thus antifolates prevent the formation of thymidylate

that arrests DNA synthesis in the parasite and causes parasite death.

4.2.2 Efficacy

Sulfadoxine-pyrimethamine (SP) is not recommended as a monotherapy in therapy of *P. vivax* malaria due to slow rate of clearing of parasites [23]. In fact it was considered that *P. vivax* is intrinsically resistant to SP but the present studies have confirmed acquired resistance than the organism being intrinsically

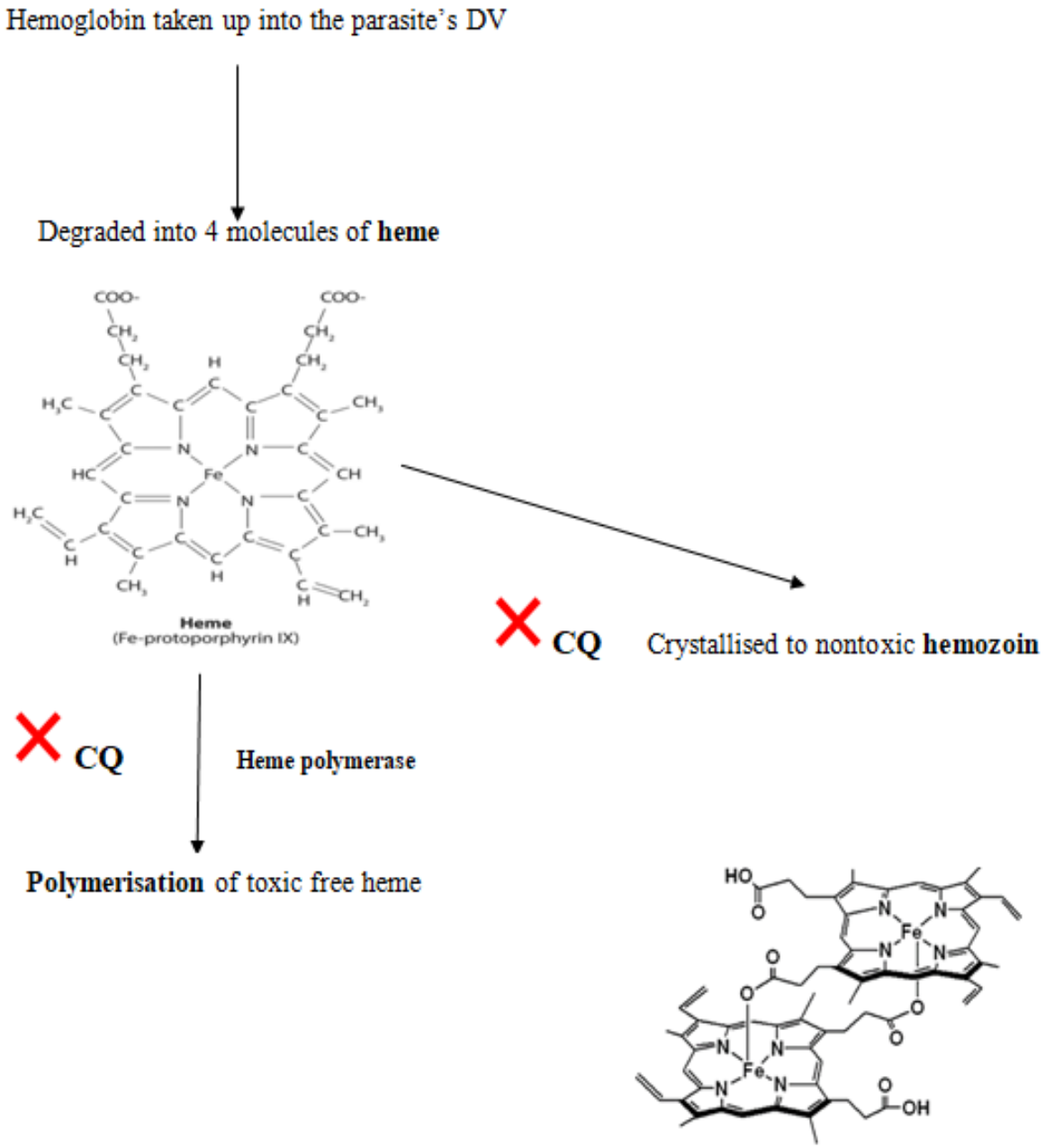
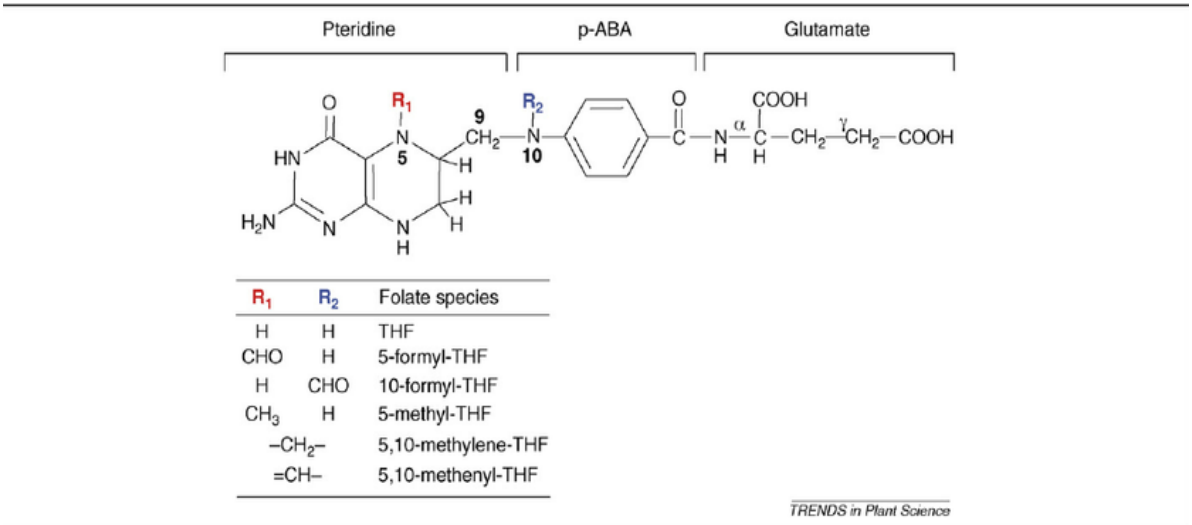


Fig. 4. Mechanisms by which parasite evades toxicity due to free heme

DV- Digestive(food) vacuole

Host hemoglobin taken up by the parasite's DV is degraded into heme. To evade toxicity due to the free heme, two mechanisms are followed.

1. *Conversion of heme into a non-toxic crystalline hemozoin form (heme dimer)*
2. *Polymerisation of the free heme mediated by heme polymerase*



R_1	R_2	Folate species
H	H	THF
CHO	H	5-formyl-THF
H	CHO	10-formyl-THF
CH_3	H	5-methyl-THF
$-CH_2-$		5,10-methylene-THF
=CH–		5,10-methenyl-THF

Fig. 5. Molecular structure of folate showing the binding sites [22]

The folate molecule consists of pteridine, paraaminobenzoic acid and glutamate moieties. pteridine ring of folates can exist in tetrahydro, dihydro, or fully oxidized forms. The one carbon groups bind at positions N5 and/or N10 which is indicated as R1 and R2 respectively. Few of the naturally occurring combinations of R1 and R2 and the corresponding folate species is shown in the list

resistant to the drug. Among the DHFR inhibitors, pyrimethamine is the most widely used. They have a high affinity for binding with the parasite's DHFR. Similarly, there were attempts in the past to use the DHPS inhibitors (sulfa drugs) as monotherapy in malaria. Due to the toxicity and low efficacy, it has led to their discontinuation as a monotherapy. DHPS and DHFR inhibitors are now used in combination for a synergistic effect and to slow the development of drug resistance [24]. Pyrimethamine is a drug with a long half-life extending more than 80 hours [25]. Though dapsone is the most potent among the DHPS inhibitors, it has a relatively short half-life of about 24 hours [26] Thus the efficacy of the combination with DHFR inhibitors decreases significantly from the second day of the treatment and hence dapsone is not the preferred agent for the combination therapy. Sulfadoxine and sulfalene have longer half-lives similar to that of pyrimethamine and hence their combination is in use. Though both of these drugs combination with pyrimethamine have almost the same efficacy, sulfadoxine- pyrimethamine is the widely used DHPS-DHFR combination [24]. Another potential antifolate that can be used in treatment of malaria is methotrexate. Though it is primarily an anticancer agent, its use as an antimalarial has been established for over forty years through many in vitro studies [27]. But studies in murine models have failed to show its antimalarial action [28]. Few clinical trials in small groups have demonstrated that low doses of methotrexate given over a short period of 3-5 days is effective in the treatment of malaria due to both *Plasmodium vivax* and *P. falciparum* [29,30]. Methotrexate has also shown good therapeutic action against the *Plasmodium vivax* isolates having *dhfr* mutations that contribute to pyrimethamine resistance in some ex vivo studies [31]. However there are concerns of toxicity with methotrexate which limits its use [32].

4.3 Artemisinin

4.3.1 Mechanism of action

Artemisinin compounds are chemically sesquiterpene lactone compounds from a plant *Artemisia annua* [18]. It is used in treatment of severe malaria which is commoner with *P.falciparum*. Though artemisinin is a potent agent, it has a low bioavailability and a short half-life which limits its role in monotherapy. Hence they are given along with other anti-malarials as an artemisinin-based combination therapy (ACT). This increases the efficacy and reduces the risk of developing artemisinin resistance. Common artemisinin derivatives are artesunate, artemether, arteether, dihydroartemisinin and artemotil. These have endoperoxide bridges that are needed for antimalarial activity [33]. Artemisinin acts in a two-step mechanism. At first artemisinin is metabolized to its active form, dihydroartemisinin (DHA). The intraparasitic

heme-iron catalyzes the cleavage of the endoperoxide bridges in the artemisinin group of drugs. This results in formation of a free radical intermediate which causes alkylation of one or more of the essential malarial proteins that leads to killing of the parasite [26]. These free radicals can inhibit protein and nucleic acid synthesis in the parasite in all of its stages within an RBC [34].

The mechanism of action of various antimalarial drugs and the mechanism of resistance in the parasite to these drugs are given in **Table 2**.

4.3.2 Efficacy

Phosphatidylinositol-3-kinase is considered as the potential target for artemisinin [35]. Artemisinin slows parasite growth, reduces uptake of haemoglobin and causes an increased oxidative damage to the parasite proteins [36]. They rapidly clear parasites from the bloodstream which contributes to its high efficacy [35]. Artemisinin based combination therapies (ACTs) are frontline, fast acting drugs that have been known to reduce the disease burden and mortality due to malarial infections.

5. ANTIMALARIAL RESISTANCE IN *Plasmodium vivax*

Antimalarial resistance in *P.vivax* is said to have a slower course compared with *P.falciparum*. One challenge in ascertaining drug resistance in *P.vivax* is the factor that it is known to cause relapses because of the hypnozoite forms. There have been various in vitro and in vivo methods that have been employed to distinguish a relapse and a case of clinical resistance.

The World-wide Antimalarial Resistance Network (WWARN), a part of Infectious Diseases Data Observatory (IDDO), is a platform that provides resources and reliable information on the malaria trends and about the antimalarial resistance. The WWARN provides “The Vivax Surveyor” facility which is an interactive map that gives data on the clinical trials done in antimalarial resistance to *Plasmodium vivax* all over the world. The vivax surveyor page of the WWARN is given in **Figure 6**. [37] This tool gives upto date information on international, regional and national monitoring strategies. Since chloroquine remains the principal drug in India for the treatment of *P.vivax*, the WWARN have categorised regions in India based on their sensitivity to chloroquine. They are categorised as:

- “**CQR Category 1** - >10% recurrences by day 28, the lower 95% confidence interval on this estimate being >5%, irrespective of confirmation of adequate blood chloroquine concentration. Occasional breakthrough recurrences do occur within 28 days of chloroquine treatment, but a risk greater than 10% in a large enough sample is highly suggestive of resistance.
- **CQR Category 2** - Confirmation of recurrences within 28 days in the presence of whole blood concentrations greater than 100nM. Parasite growth in the presence of high blood concentrations of the drug confirms chloroquine resistance.
- **CQR Category 3** - >5% recurrences by day 28, with the lower 95% confidence of this estimate lying below 5%, irrespective of confirmation of adequate blood chloroquine concentration. This represents potential evidence of CQR but may reflect other factors such as poor drug absorption or quality.
- **Chloroquine Sensitive CQS** - confirmation of sensitivity requires all of the following: patients enrolled following a symptomatic clinical illness, less than 5% recurrences by day 28, no administration of primaquine before day 28, and a sample size of at least 10 patients.” [38].
  There is one category 3 region in India in Daltonganj of Jharkhand and one category 3 region in Pansora of Gujarat. Other regions in India are either categorised as CQS or yet to be categorised [37].

5.1 Chloroquine

In the past five years, there has been increasing evidence of chloroquine resistant *Plasmodium vivax* infections in countries of the Southeast Asian region including India. Resistance to CQ was first reported from Papua, New Guinea in 1989 [39]. Inappropriate use of chloroquine, sub-therapeutic dosing in case of presumptive treatment at the peripheral level, non-compliance by the patient can lead to faster development of resistance though at present there is not much clinical evidence of resistance to chloroquine [40].

Table 2. Antimalarial agents and the mechanism of resistance

Drug	Mechanism of action	Mechanism(s) of resistance	Associated gene mutations
Chloroquine	**Accumulation in the food vacuole** of the trophozoite form within the host RBC and preventing formation of hemozoin. **Accumulation of free heme** and lysis of membrane of the parasite and formation of reactive oxygen species and parasite death	1. **Reduced accumulation of the drug** in the food vacuole 2. **Energy dependent efflux** of the accumulated drug via certain transmembrane protein pumps 3. **Increase in vacuolar pH** which that affects accumulation of drug	*Plasmodium vivax* multidrug resistance protein 1 gene (***pvmdr1*)** Mutations in the ***pvcrt-o*** gene
Antifolates	Pyrimethamine targets the dihydrofolate reductase activity whereas the sulfa drugs target the dihydropteroate synthetase Prevent the formation of thymidylate that arrests DNA synthesis in the parasite and causes parasite death.	Stepwise acquisition of point mutations in the binding sites for these drugs	Mutations in the ***pvdhfr*** (pyrimethamine) and ***pvdhps*** (sulfadoxine) genes
Artemisinin	Formation of free radical intermediates by cleavage of the endoperoxide bridges within the drug by the parasite heme-iron and killing of the parasite	Enhanced stress response within the parasite involving the protein degradation and ubiquitination pathways	Mutations in the kelch gene (***pvk12***)

Fig. 6. WWARN Vivax surveyor page

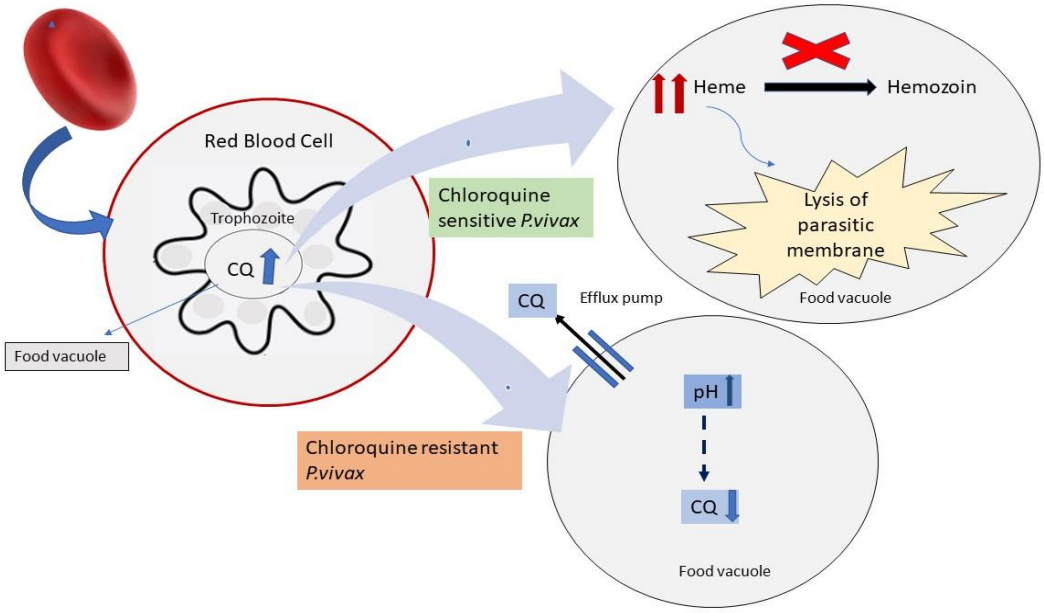


Fig. 7. Mechanism of resistance to chloroquine in *P. vivax*

CQ- Chloroquine. In a wild type Plasmodium vivax infected RBC, chloroquine prevents the detoxification of the host RBC's heme to hemozoin within the parasite's food vacuole. The accumulated heme causes the lysis of the parasitic membrane. But in the presence of chloroquine resistance due to mutation in the pvmdr1 gene which codes for a transmembrane protein pump, there is decreased influx of chloroquine along with the action of efflux pumps causing a reduced concentration of the drug within the parasite's digestive vacuole

The proposed mechanisms of resistance to the drug are reduced accumulation of the drug in the food vacuole, energy dependent efflux of the accumulated drug via certain transmembrane protein pumps, increase in vacuolar pH which affects accumulation of drug within the food vacuole. Chloroquine resistance arises when CQ cannot accumulate at its active site (within the

digestive vacuole of the parasite) to disrupt the parasite's haemoglobin degradation cycle. A diagram showing the mechanism of resistance to chloroquine in *P. vivax* is shown in Fig. 7. Two different transporters (CRT and MDR1) which are located in the parasite's digestive vacuole, have been implicated in resistance [41]. The *pvmdr1* gene encodes the p-glycoprotein transmembrane pump multidrug resistance protein 1. It functions as a transporter of antimalarial drugs and other solutes into the DV [42]. The *pvmdr1* gene is involved in coupling ATP hydrolysis to translocation of solutes across cell membranes. Mutations *in pvmdr-1* prevent influx of chloroquine from cytoplasm into the digestive vacuole thus making it impossible for the drug to act [17]. The *pvcrt* gene is expressed at all infected erythrocyte stages with maximal expression at the trophozoite stage. Mutations in the gene cause hydrogen ions to be transported out of the food vacuole. As a result CQ which accumulates and exert its action at an acidic pH is not able to act which is manifested as chloroquine resistance [17].

P. vivax parasitaemia recurring 28 days after start of recommended chloroquine therapy is counted as resistance to the drug. If the recurrence appears before day 16, it is taken as a case of recrudescence. Recurrence seen from day 17 and 28 is considered as either recrudescence or relapse by chloroquine-resistant *P. vivax*. Recurrences beyond day 28 are considered as relapse by chloroquine-sensitive *P. vivax* [42].

Antimalarial drug efficacy is monitored through therapeutic efficacy studies (TES), which track clinical and parasitological outcomes among patients receiving antimalarial treatment [43]. Six East Asian nations have reported data on CQ resistant *P.vivax*; Indonesia, Malaysia, Myanmar, Thailand, Vietnam and Philippines [44]. Very few cases of clinical CQ resistant *P.vivax* have been reported from India where the clinical response and the plasma concentrations of the drug were compared [45–47].

The molecular markers for chloroquine resistance are mutations in the *pvcrt-o* and *pvmdr1* genes. Insertion of lysine (AAG) at the 10th amino acid position (K 10 insertion) of exon1 of *Pvcrt-o* is the commonly reported mutation of the *pvcrt-o* gene and has been reported from India as well [48,49]. However, most of the K10 insertion that has been reported so far was from Myanmar (46-72%) and Thailand (56-89%) [50–52]. The multicentric study done in 2015-2016 reported K10 insertion in the *pvcrt-o* gene in three of the four centres where the study was conducted. Pondicherry reported 18.8% of this mutation [53].

The known mutations in the *pvmdr1* gene are at codons 958, 976, 1076 and 1028. At codon 958, threonine replaced by methionine (T958M), is the most widely reported mutation in almost all isolates studied across the world. This is known as the single mutant *pvmdr1*. The next commonly reported one is the double mutant with mutations at codon 958 and codon 1076. In this variant, besides the T958M mutation, at codon 1076, phenylalanine is replaced by leucine (F1076L). In a study conducted in Malaysia by Cheong et.al, 81.8% of the isolates had this double mutant [52]. Another study in China done in 2017-2019 also revealed high prevalence of this double mutant in 49/58 (84.48%) isolates [54]. Studies in India have also reported this double mutant. A study conducted in Chandigarh also had similar findings with 117/118 isolates (99%) having this double mutant [49]. A multicentric study done by Vamsi et al from different regions of India, such as, Puducherry, Mangaluru, Cuttack and Jodhpur revealed 91.6% of the isolates with mutations, T958M and F1076L [53]. In this study, 11 out of the 15 isolates analysed (73%) from Puducherry had the double mutant. All 15 isolates from Puducherry and the total 60 from all the centres combined carried the T958M mutation [53]. In a further study conducted in a tertiary care setting in Puducherry, South India, 14 out of 27 isolates (52%) carried the T958M F1076L double mutant *pvmdr1* gene [76]. Another documented double mutation is at codon 976 which have also been reported in India. Here at codon 976, tyrosine is replaced by phenylalanine (Y976F). However, this double mutant is relatively rare. The study by Tantiamornkul et.al showed 3.3% isolates with this mutant [55]. Y976F mutation in *pvmdr1* gene has been associated with a reduced CQ sensitivity in studies from South-east Asia. Polymorphism at Y976F is said to be associated with a 1.7-fold increase in resistance to the drug [56]. The triple mutants carrying the Y976F mutation along with T958M and F1076L mutations have been reported from India though the double mutant T958M Y976F is not much reported [48,49]. Other mutations which are reported in the *pvmdr1* gene are very rarely. They are at codons 946 (I946V) and 1028 (Y1028C) where isoleucine is replaced by Valine and tyrosine is replaced by cysteine respectively [48,49]. A study in Delhi by Matlani et.al has

shown two novel mutations that have been exclusively reported. One at codon 861 where alanine is replaced by glutamic acid (A861E) and another at codon 898 where tyrosine is replaced by glutamic acid (T898E) [57].

5.2 Antifolates

The molecular basis of *Plasmodium* resistance to sulfadoxine pyrimethamine has been attributed to point mutations in their genes. The dihydrofolate reductase (dhfr) acts as a binding site for pyrimethamine and dihydropteroate synthetase (dhps) for sulfadoxine. Different combinations of mutations in each gene results in varied resistance levels to SP [58]. Resistance to SP is linked to the stepwise acquisition of specific point mutations in the *dhfr* and *dhps* codons [59]. One proposed reason for *P.vivax* developing resistance to CQ is because of the use of SP to treat CQ resistant *P. falciparum* when there is a mixed infection with *P.vivax* as well [60].

The known mutations in the *pvdhfr* gene are at codon positions 57(F57L/F57I), 58(S58R), 61(T61M), 99(H deletion), 117(S117N/S117T) and 173. Among these, the most commonly reported mutations are at codon positions 58 and 117 [40,49,55]. At codon 58 serine is replaced by arginine and at codon 117, serine is replaced by asparagine or less commonly by threonine. In a study conducted by Valecha et.al in three different geographical locations in the country, mutations were observed at amino acid residues 58 and 117; 83.3% of isolates had a mutation at codon 117 and 76.7% isolates had mutation at codon 58 [40]. Mutations at codons 117 and 58 of *pvdhfr* are considered equivalent to mutations at codons 108 and 59 of *pfdhfr* respectively, which is associated with pyrimethamine resistance [44]. In another study conducted in Chandigarh from 2013-2016, the double mutant *pvdhfr* with S58R and S117N mutations were identified in 21.1% of the isolates [49]. A study from Puducherry in 2022 reported 18 (67%) out of 27 isolates with the S58R S117N *pvdhfr* double mutant [76]. A study by Hastings et al. which measured the resistance of the alleles in vitro, has reported that this double mutant (S58R and S117N) is associated with increased resistance to pyrimethamine drug [61]. However, based on the clinical response in patients, the double mutant *pvdhfr* is not very alarming. Though they are said to have a reduced action in terms of poor binding , only quadruple-mutant *dhfr* alleles are said to be associated with high risk of therapeutic failure to SP according to a study done in Indonesia [61]. Two types of quadruple mutant *pvdhfr* have been reported; (I57R58M61T117) and (L57R58M61T117) [55]. In the quadruple mutant the serine at codon 117 is replaced by threonine instead of asparagine as in case of double mutant and this has been reported to have a high level of resistance [5]. Most reports of quadruple mutants come from Thailand and Myanmar. In a study conducted by Tantiamornkul et.al, the quadruple mutant (I57R58M61T117) was identified in 47.1% isolates and the (L57R58M61T117) quadruple mutant seen in 2.9% isolates [55]. There has been few reports of quadruple mutant L57R58M61T117 from Goa and Assam [62].

The known mutations in the *pvdhps* gene are at codons 382, 383, 512, 553, 580 and 585. Among these the commonly reported mutations are at codons 383 and 553. The double mutant *pvdhps* is the widely reported in which at codon positions 383 and 553, alanine is replaced by glycine. Similar to the double mutant *pvdhfr*, it is reported that the sulfadoxine has reduced binding to the double mutant *pvdhps* [63]. The A383G A553G *pvdhps* double mutant was reported in 16 out of 27(59%) isolates in a recent study from Puducherry [76]. One unique mutation reported in few studies from India is at codon 459 where aspartic acid is replaced by alanine (D459A). This mutation was significantly higher in patients who presented with complications [49,64]. It is said that the V585 of *pvdhps* may be responsible for innate resistance of *P. vivax* to sulfadoxine [65]. The reported triple mutant *pvdhps* (A382G383G553) have an additional mutation at codon 382 where serine is replaced by alanine [55]. The quadruple mutant with mutation at codon 512 from K to M i.e., lysine to methionine have been reported very rarely [55].

5.3 Artemisinin

Resistance to artemisinin compounds that is reflected as delayed parasite clearance, was first reported in Cambodia in 2008 [66]. The whole genome sequencing study done in Cambodia on *P.vivax* isolates in 2011-2012 was a major breakthrough in identifying mutations in the *kelch 13* gene involved in the cell's response to oxidative stress as the cause of artemisinin

Table 3. The reported mutations in the drug targets of the various antimalarial drugs and their clinical implication

Drug	Gene	Mutation	Clinical implication	Regions in India with the mutation reported
Chloroquine	*pvmdr1*	Single mutant (T958M)	No therapeutic failure or reduced action	
		Double mutant (T958M F1076L)	Reduced action of drug [56]	Chandigarh [49], Delhi, Jodhpur, Orissa, Mangaluru [48], Puducherry [53], Chennai [40]
		Double mutant (T958M Y976F)	The Y976F mutation carries a 1.7 fold high risk of resistance to chloroquine [56]	Mangaluru
		Triple mutant(T958M Y976F F1076L)	Therapeutic failure [73]	Mangaluru, Chandigarh
	pvcrt-0	K10 insertion		Mangaluru, Orissa, Pondicherry
Pyrimethamine	*pvdhfr*	Double mutant(S58R S117N)	400 times more resistant than the wild type to pyrimethamine drug causing a reduced action [61]	Chandigarh, Mumbai, Uttar Pradesh [40], Puducherry
		Quadruple mutant (F57L/I S58R T61M S117T)	Therapeutic failure [61]	Goa, Assam [62]
Sulfadoxine	*pvdhps*	Single mutant (D459A)	Associated with complications [49,64]	Chandigarh
		Double mutant (A383G A553G)	Reduced binding [63]	Chennai, Pondicherry
		Triple mutant(S382A A383G A553G)	Reduced sensitivity [74,75]	Not reported in India
Artemisinin	*pvk12*	Non-synonymous mutations M548I, K596R and P641L		Not reported yet in India

resistance [67]. The mutation reduces the kelch13 function that is required for the uptake of the host hemoglobin by the trophozoite forms into their digestive vacuole [68] In vitro and in vivo studies have shown that mutations in the PfKelch13 BTB/POZ and propeller domain (PfK13) are associated with delayed parasite clearance that contribute to an entity known as artemisinin partial resistance [69]. In artemisinin partial resistance there is a delay in the clearance of the parasite from the patient's bloodstream which usually happens with 3 days of ACT. It is called as partial resistance since the mechanisms of resistance to this drug seems to affect the ring stage of the parasite's life cycle in humans [69]. In the presence of an effective partner drug, artemisinin partial resistance is not something that is concerning. Therapeutic failure is noticed only in the presence of resistance to the partner drug. Artemisinin full resistance has not been observed yet [69]. Strong correlations between mutations in the gene coding for kelch protein and artemisinin resistance has been established using the parasite survival rates in vitro studies and parasite clearance rates in in vivo studies [67]. Though the exact mechanisms by which mutation in kelch protein causes artemisinin resistance is unclear, an enhanced stress response was noted in the artemisinin resistant parasites [70]. This enhanced stress response involves the protein degradation and ubiquitination pathways [18].

Synonymous and non-synonymous mutations have been known in *pvk12* gene. Synonymous mutations are point mutations, that only changes one base pair in the RNA copy of the DNA. Here the amino acid coded remains the same and hence is called as a synonymous mutation. The non-synonymous mutations known in *pvk12* gene are at codon positions 548(M548I), 596(K596R), and 641 (P641L). The synonymous mutations known are at codons 437,675 and 682 (F437, N675, C682). In the study done in Thailand, non-synonymous mutations M548I, K596R and P641L were identified though in a smaller number of cases [55]. There has not been any study in the past on detection of mutations in the *pvk12* gene in India. The few studies from other countries have shown limited polymorphisms in the *pvk12* gene [71,72].

The reported mutations in the gene targets of the corresponding antimalarial agent and their propensity to cause therapeutic failure is summarised in Table 3.

6. CONCLUSION

Among the antimalarials, most reports on mutations in the drug targets in *Plasmodium vivax* is towards chloroquine. The T958M F1076L mutation is the most widely reported mutation in *pvmdr1* gene in India. The Y976F mutation which is known to be associated with a 1.7 fold increase in risk of resistance to chloroquine is however reported in very few isolates from Chandigarh and Mangaluru. The resistance to sulfadoxine and pyrimethamine can be explained due to the drug pressure created while these drugs are given in treatment of mixed infections with *Plasmodium falciparum*. For pyrimethamine, the S58R S117N mutation in the *pvdhfr* gene is the widely reported one which is known to cause a poor binding to the drug but isn't alarming still. Reports on quadruple mutant *pvdhfr* which is said to be associated with a high risk of therapeutic failure are very rare in India. For sulfadoxine, the double mutant *pvdhps* (A383G A553G) is the widely reported. The quadruple mutants or triple mutant of *pvdhps* has not been reported from India as from countries like China. Resistance to artemisinin in *Plasmodium vivax* in India is an area way forward to analyse. So far there are no reports of any mutant *Plasmodium vivax* strains, contributing to artemisinin resistance, circulating in India.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World malaria report 2018 [Internet]. [cited 2022 Jun 21]. Available:https://www.who.int/publications-detail-redirect/9789241565653
2. World malaria report 2020 [Internet]. [cited 2022 Jun 21]. Available:https://www.who.int/publications-detail-redirect/9789240015791

3. Bartoloni A, Zammarchi L. Clinical Aspects of Uncomplicated and Severe Malaria. Mediterr J Hematol Infect Dis. 2012;4:e2012026.
4. Malaria :: Guidelines for diagnosis and treatment of malaria 2014. National Center for Vector Borne Diseases Control (NCVBDC) [Internet]. [cited 2022 Jun 21]. Available:https://nvbdcp.gov.in/index1.php?lang=1&level=1&sublinkid=5784&lid=3689
5. Tjitra E, Anstey NM, Sugiarto P, Warikar N, Kenangalem E, Karyana M, et al. Multidrug-Resistant *Plasmodium vivax* Associated with Severe and Fatal Malaria: A Prospective Study in Papua, Indonesia. PLoS Med. 2008;5:e128.
6. Kochar DK, Saxena V, Singh N, Kochar SK, Kumar SV, Das A. *Plasmodium vivax* Malaria. Emerg Infect Dis. 2005;11:132–4.
7. Nadkar MY, Huchche AM, Singh R, Pazare AR. Clinical Profile of Severe *Plasmodium vivax* Malaria in a Tertiary Care Centre in Mumbai from June 2010- January 201. 2012;60:3.
8. Rizvi I, Tripathi DK, Chughtai AM, Beg M, Zaman S, Zaidi N. Complications associated with *Plasmodium vivax* malaria: A retrospective study from a tertiary care hospital based in western Uttar Pradesh, India. Ann Afr Med. 2013;12:155.
9. Singh J, Purohit B, Desai A, Savardekar L, Shanbag P, Kshirsagar N. Clinical manifestations, treatment, and outcome of hospitalized patients with *Plasmodium vivax* Malaria in Two Indian States: A Retrospective Study. Malar Res Treat. 2013;2013:341862.
10. Kim KM, Bae BK, Lee SB. Spontaneous splenic rupture in *Plasmodium vivax* malaria. Ann Surg Treat Res. 2014;87:44–6.
11. Goldberg DE, Slater AF, Cerami A, Henderson GB. Hemoglobin degradation in the malaria parasite Plasmodium falciparum: an ordered process in a unique organelle. Proc Natl Acad Sci. 1990;87:2931–5.
12. Hemozoin and antimalarial drug discovery - PMC [Internet]. [cited 2023 Mar 25]. Available:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4928194/
13. Egan TJ. Haemozoin formation. Mol Biochem Parasitol. 2008;157:127–36.
14. Heme oxygenase-1: function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury. American Journal of Respiratory Cell and Molecular Biology [Internet]. [cited 2023 Mar 25]. Available: https://www.atsjournals.org/doi/abs/10.1165/ajrcmb.15.1.8679227
15. Direct tests of enzymatic heme degradation by the malaria parasite *Plasmodium falciparum*. - Abstract - Europe PMC [Internet]. [cited 2023 Mar 25]. Available:https://europepmc.org/article/PMC/3488054
16. Structural and morphological characterization of hemozoin produced by Schistosoma mansoni and Rhodnius prolixus - Oliveira - 2005 - FEBS Letters - Wiley Online Library [Internet]. [cited 2023 Mar 25]. Available:https://febs.onlinelibrary.wiley.com/doi/full/10.1016/j.febslet.2005.09.035
17. Frontiers | Chloroquine and Sulfadoxine–Pyrimethamine Resistance in Sub-Saharan Africa—A Review [Internet]. [cited 2022 Jul 11]. Available:https://www.frontiersin.org/articles/10.3389/fgene.2021.668574/full
18. Cowell AN, Winzeler EA. The genomic architecture of antimalarial drug resistance. Brief Funct Genomics. 2019;18:314–28.
19. Mechanisms of drug action and resistance [Internet]. [cited 2022 Apr 27]. Available:http://www.tulane.edu/~wiser/protozoology/notes/drugs.html
20. Sullivan DJ, Matile H, Ridley RG, Goldberg DE. A Common Mechanism for Blockade of Heme Polymerization by Antimalarial Quinolines*. J Biol Chem. 1998;273:31103–7.
21. Baird JK. Resistance to Therapies for Infection by *Plasmodium vivax*. Clin Microbiol Rev. 2009;22:508–34.
22. Bekaert S, Storozhenko S, Mehrshahi P, Bennett M, Lambert W, Gregory J, et al. Folate biofortification in food plants. Trends Plant Sci. 2008;13:28–35.
23. Darlow B, Vrbova H, Gibney S, Jolley D, Stace J, Alpers M. Sulfadoxine-pyrimethamine for the treatment of acute malaria in children in papua New Guinea: I. *Plasmodium falciparum.* Am J Trop Med Hyg. 1982;31:1–9.
24. Nzila A. The past, present and future of antifolates in the treatment of Plasmodium falciparum infection. J Antimicrob Chemother. 2006;57:1043–54.

25. Watkins WM, Mosobo M. Treatment of Plasmodium falciparum malaria with pyrimethamine-sulfadoxine: selective pressure for resistance is a function of long elimination half-life. Trans R Soc Trop Med Hyg. 1993;87:75–8.
26. Chlorproguanil/dapsone for uncomplicated Plasmodium falciparum malaria in young children: pharmacokinetics and therapeutic range. Transactions of The Royal Society of Tropical Medicine and Hygiene | Oxford Academic [Internet]. [cited 2022 May 8]. Available:https://academic.oup.com/trstmh/article-abstract/91/3/322/1896288?redirectedFrom=fulltext&login=false
27. Nzila A, Okombo J, Becker RP, Chilengi R, Lang T, Niehues T. Anticancer agents against malaria: time to revisit? Trends Parasitol. 2010;26:125–9.
28. Irungu B, Kiboi D, Langat B, Rukunga G, Wittlin S, Nzila A. Methotrexate and aminopterin lack in vivo antimalarial activity against murine malaria species. Exp Parasitol. 2009;123:118–21.
29. Sheehy TW, Dempsey H. Methotrexate therapy for *Plasmodium vivax* malaria. JAMA. 1970;214:109–14.
30. Wildbolz A. [Methotrexate in the therapy of malaria]. Ther Umsch Rev Ther. 1973;30:218–22.
31. Imwong M, Russell B, Suwanarusk R, Nzila A, Leimanis ML, Sriprawat K, et al. Methotrexate Is Highly Potent against Pyrimethamine-Resistant *Plasmodium vivax*. J Infect Dis. 2011;203:207–10.
32. Laing AB. Methotrexate in malaria. Trans R Soc Trop Med Hyg. 1972;66:518–9.
33. Antimalarial Drugs: Modes of Action snd Mechanisms of Resistance [Internet]. Medscape. [cited 2022 Apr 28]. Available:http://www.medscape.com/viewarticle/734498
34. Artesunate [Internet]. [cited 2022 Jun 15]. Available:https://go.drugbank.com/drugs/DB09274
35. Mbengue A, Bhattacharjee S, Pandharkar T, Liu H, Estiu G, Stahelin RV, et al. A molecular mechanism of artemisinin resistance in *Plasmodium falcipar*um malaria. Nature. 2015;520:683–7.
36. Klonis N, Crespo-Ortiz MP, Bottova I, Abu-Bakar N, Kenny S, Rosenthal PJ, et al. Artemisinin activity against Plasmodium falciparum requires hemoglobin uptake and digestion. Proc Natl Acad Sci U S A. 2011;108:11405–10.
37. Vivax Surveyor [Internet]. [cited 2022 Jul 13]. Available:http://www.wwarn.org/vivax/surveyor/#0
38. Chloroquine resistant *Plasmodium vivax* review [Internet]. Worldwide Antimalarial Resistance Network. 2014 [cited 2022 Jul 11]. Available:https://www.wwarn.org/tools-resources/literature-reviews/chloroquine-resistant-plasmodium-vivax-review
39. Baird JK. Neglect of *Plasmodium vivax* malaria. Trends Parasitol. 2007;23:533–9.
40. Valecha N, Joshi H, Eapen A, Ravinderan J, Kumar A, Prajapati SK, et al. Therapeutic efficacy of chloroquine in *Plasmodium vivax* from areas with different epidemiological patterns in India and their Pvdhfr gene mutation pattern. Trans R Soc Trop Med Hyg. 2006;100:831–7.
41. Slater AFG. Chloroquine: Mechanism of drug action and resistance in plasmodium falciparum. Pharmacol Ther. 1993;57:203–35.
42. Rohrbach P, Sanchez CP, Hayton K, Friedrich O, Patel J, Sidhu ABS, et al. Genetic linkage of pfmdr1 with food vacuolar solute import in Plasmodium falciparum. EMBO J. 2006;25:3000–11.
43. World malaria report 2021 [Internet]. [cited 2022 Jun 21]. Available:https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2021
44. Baird JK. Chloroquine resistance in *Plasmodium vivax*. Antimicrob Agents Chemother. 2004;48:4075–83.
45. Davis TM, Syed DA, Ilett KF, Barrett PHR. Toxicity Related to Chloroquine Treatment of Resistant Vivax Malaria. Ann Pharmacother. 2003;37:526–9.
46. Dua VK, Kar PK, Sharma VP. Chloroquine resistant *Plasmodium vivax* malaria in India. Trop Med Int Health. 1996;1:816–9.
47. Vivax malaria resistant to chloroquine: case reports from Bombay | Transactions of The Royal Society of Tropical Medicine and Hygiene | Oxford Academic [Internet]. [cited 2022 Jul 11]. Available:https://academic.oup.com/trstmh/article-abstract/89/6/656/1915439?redirectedFrom=fulltext&login=false
48. Joy S, Mukhi B, Ghosh SK, Achur RN, Gowda DC, Surolia N. Drug resistance

genes: pvcrt-o and pvmdr-1 polymorphism in patients from malaria endemic South Western Coastal Region of India. Malar J. 2018;17:40.

49. Kaur H, Sehgal R, Kumar A, Bharti PK, Bansal D, Mohapatra PK, et al. Distribution pattern of amino acid mutations in chloroquine and antifolate drug resistance associated genes in complicated and uncomplicated *Plasmodium vivax* isolates from Chandigarh, North India. BMC Infect Dis. 2020;20:671.
50. Genetic polymorphism in pvmdr1 and pvcrt-o genes in relation to in vitro drug susceptibility of *Plasmodium vivax* isolates from malaria-endemic countries. Acta Trop. 2011;117:69–75.
51. Nyunt MH, Han JH, Wang B, Aye KM, Aye KH, Lee SK, et al. Clinical and molecular surveillance of drug resistant vivax malaria in Myanmar (2009–2016). Malar J. 2017;16:117.
52. Cheong FW, Dzul S, Fong MY, Lau YL, Ponnampalavanar S. *Plasmodium vivax* drug resistance markers: Genetic polymorphisms and mutation patterns in isolates from Malaysia. Acta Trop. 2020;206:105454.
53. Anantabotla VM, Antony HA, Parija SC, Rajkumari N, Kini JR, Manipura R, et al. Polymorphisms in genes associated with drug resistance of *Plasmodium vivax* in India. Parasitol Int. 2019;70:92–7.
54. Wang X, Ruan W, Zhou S, Feng X, Yan H, Huang F. Prevalence of molecular markers associated with drug resistance of *Plasmodium vivax* isolates in Western Yunnan Province, China. BMC Infect Dis. 2020;20:307.
55. Tantiamornkul K, Pumpaibool T, Piriyapongsa J, Culleton R, Lek-Uthai U. The prevalence of molecular markers of drug resistance in *Plasmodium vivax* from the border regions of Thailand in 2008 and 2014. Int J Parasitol Drugs Drug Resist. 2018;8:229–37.
56. Suwanarusk R, Russell B, Chavchich M, Chalfein F, Kenangalem E, Kosaisavee V, et al. Chloroquine resistant *Plasmodium vivax*: In Vitro characterisation and association with molecular polymorphisms. PLoS ONE. 2007;2:e1089.
57. Matlani M, Kumar A, Singh V. Assessing the in vitro sensitivity with associated drug resistance polymorphisms in *Plasmodium vivax* clinical isolates from Delhi, India. Exp Parasitol. 2021;220:108047.
58. Antifolate antimalarial resistance in southeast Africa: a population-based analysis - The Lancet [Internet]. [cited 2022 Jun 27]. Available:https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(03)12951-0/fulltext
59. Oyibo W, Agomo C. Scaling up of intermittent preventive treatment of malaria in pregnancy using sulphadoxine–pyrimethamine: prospects and challenges. Matern Child Health J; 2010.
60. Cui L, Escalante AA, Imwong M, Snounou G. The genetic diversity of *Plasmodium vivax* populations. Trends Parasitol. 2003;19:220–6.
61. Hastings MD, Porter KM, Maguire JD, Susanti I, Kania W, Bangs MJ, et al. Dihydrofolate reductase mutations in *Plasmodium vivax* from Indonesia and Therapeutic Response to Sulfadoxine plus Pyrimethamine. J Infect Dis. 2004;189:744–50.
62. Alam MT, Bora H, Bharti PK, Saifi MA, Das MK, Dev V, et al. Similar Trends of Pyrimethamine Resistance-Associated Mutations in *Plasmodium vivax* and P. falciparum. Antimicrob Agents Chemother. 2007;51:857–63.
63. Barnadas C, Kent D, Timinao L, Iga J, Gray LR, Siba P, et al. A new high-throughput method for simultaneous detection of drug resistance associated mutations in *Plasmodium vivax* dhfr, dhps and mdr1 genes. Malar J. 2011;10:282.
64. Garg S, Saxena V, Lumb V, Pakalapati D, Boopathi PA, Subudhi AK, et al. Novel mutations in the antifolate drug resistance marker genes among *Plasmodium vivax* isolates exhibiting severe manifestations. Exp Parasitol. 2012;132:410–6.
65. Sulfadoxine Resistance in *Plasmodium vivax* Is Associated with a Specific Amino Acid in Dihydropteroate Synthase at the Putative Sulfadoxine-Binding Site | Antimicrobial Agents and Chemotherapy [Internet]. [cited 2022 Jul 14]. Available:https://journals.asm.org/doi/10.1128/AAC.48.6.2214-2222.2004
66. Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM. Artemisinin resistance in Cambodia 1 (ARC1) study consortium. Evidence of artemisinin-resistant malaria in western Cambodia. N Engl J Med. 2008 Dec 11;359(24):2619-20.

67. Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, Khim N, et al. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. Nature. 2014;505:50–5.
68. Uwimana A, Legrand E, Stokes BH, Ndikumana JLM, Warsame M, Umulisa N, et al. Emergence and clonal expansion of in vitro artemisinin-resistant *Plasmodium falciparum* kelch13 R561H mutant parasites in Rwanda. Nat Med. 2020;26:1602–8.
69. Malaria: Artemisinin partial resistance [Internet]. [cited 2023 Mar 26]. Available:https://www.who.int/news-room/questions-and-answers/item/artemisinin-resistance
70. Dogovski C, Xie SC, Burgio G, Bridgford J, Mok S, McCaw JM, et al. Targeting the cell stress response of Plasmodium falciparum to overcome artemisinin resistance. PLoS Biol. 2015;13:e1002132.
71. Deng S, Ruan Y, Bai Y, Hu Y, Deng Z, He Y, et al. Genetic diversity of the Pvk12 gene in *Plasmodium vivax* from the China-Myanmar border area. Malar J. 2016;15:528.
72. Wang M, Siddiqui FA, Fan Q, Luo E, Cao Y, Cui L. Limited genetic diversity in the PvK12 Kelch protein in *Plasmodium vivax* isolates from Southeast Asia. Malar J. 2016;15:537.
73. Faway E, Musset L, Pelleau S, Volney B, Casteras J, Caro V, et al. *Plasmodium vivax* multidrug resistance-1 gene polymorphism in French Guiana. Malar J. 2016;15:540.
74. Afsharpad M, Zakeri S, Pirahmadi S, Djadid ND. Molecular assessment of dhfr/dhps mutations among *Plasmodium vivax* clinical isolates after introduction of sulfadoxine/pyrimethamine in combination with artesunate in Iran. Infect Genet Evol J Mol Epidemiol Evol Genet Infect Dis. 2012;12:38–44.
75. Rungsihirunrat K, Sibley CH, Mungthin M, Na-Bangchang K. Geographical distribution of amino acid mutations in *Plasmodium vivax* DHFR and DHPS from malaria endemic areas of Thailand. Am J Trop Med Hyg. 2008;78:462–7.
76. Stanley P, Rajkumari N, Sivaradjy M. Mutation patterns in the antimalarial drug resistance markers- pvdhfr, pvdhps, pvmdr1 and pvk12 genes, among *Plasmodium vivax* isolates from a tertiary care setting in Puducherry. (Unpublished data).

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