REVIEW ARTICLE

Molecular markers of Chloroquine resistance in India and Southeast Asia

Rakshita Maskeri[1], Animesh Jain[2], Sheetal Dinkar Ullal[3]

[1]Tutor, Department of Pharmacology, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, India; [2]Professor, Department of Community Medicine, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, India, [3]Professor and Head, Department of Pharmacology, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, India

Corresponding Author

Dr Animesh Jain, Professor, Department of Community Medicine, Kasturba Medical College, Light House Hill Road, Mangalore, Karnataka, India 575001
E Mail ID: animesh_j@yahoo.com

Citation

Maskeri R, Jain A, Ullal SD. Molecular markers of Chloroquine resistance in India and Southeast Asia. Indian J Comm Health. 2021;33(4):553-558.https://doi.org/10.47203/IJCH.2021.v33i04.003

Source of Funding: Nil **Conflict of Interest:** None declared

Article Cycle

Received: 09/09/2021; **Revision:** 05/11/2021; **Accepted:**14/12/2021; **Published:**31/12/2021

Abstract

Malaria is a major global public health problem mainly in the tropics and subtropics. Malaria control and elimination strategies mainly rely on efficacious antimalarial drugs. At present the major hurdle faced by malaria control programs is the drug resistance to antimalarials. Molecular surveillance using genetic markers associated with resistance provides a valuable tool for detecting and tracking resistance as well as providing an in-depth understanding of the development and spread of resistance. Despite numerous published literatures there are limited review articles on molecular markers of drug resistance. Hence a review was planned. An exhaustive literature search was performed on PUBMED using “malaria”, “resistance”, “molecular”, “antimalarial”, “Chloroquine”, “Pfcrt”, “Pfmdr”, “Pvcrt”, “Pvmdr”as key words. Data pertaining to India and Southeast Asia were included. This review showed the widespread presence of molecular markers of drug resistance in Plasmodium falciparum and development of resistance in Plasmodium vivax over the years in Southeast Asia and India. This can have implications on malaria elimination and treatment guidelines in this region.

Keywords

Malaria; Antimalarials; Drug Resistance; Genes; Plasmodium falciparum; Plasmodium vivax; India; Southeast Asia

Introduction

Malaria remains a major threat to most of the countries across the globe. In 2019, an estimated 229 million cases of malaria occurred worldwide, and deaths were 4,09,000. In India and Southeast Asian region, malaria is caused by two most common species Plasmodium vivax and Plasmodium falciparum.P. falciparumis considered to be more severe as there is a higher probability of developing complications and fatality.(1)

Chloroquine is the first-line treatment for P. vivax infections in most countries the world over. Artemisinin combination therapy (ACT) is indicated for treatment of all cases of uncomplicated P. falciparum.(2)

Individual drug response might vary, and this is associated with polymorphisms in gene encoding for drug metabolizing enzymes and transporters.(3)Drug pressure and gene mutations are a few factors that contribute to the development of drug resistance. Due to mutations in its enzymes and proteins P. vivax and. falciparum are developing resistance to drugs. These mutations are used as molecular markers to detect drug resistant parasites.(4)

Aims & Objectives

1. To gather data available on molecular markers of chloroquine resistance.
2. To determine the extent of development of chloroquine resistance in P. falciparum and P. vivax over the years in Southeast Asia and India.

Material & Methods

An exhaustive literature search was performed on PUBMED using “malaria”, “resistance”, “molecular”, “antimalarial”, “Pfcrt”, “Pfmdr”, “Pvcrt”, “Pvmdr”, “Chloroquine” as key words. Articles published on Indian and Southeast Asian data in the English language were included. Selection criteria were: (a) articles published

between 2012 and 2019 representing data of samples collected after 2010 (b) studies comprising analysis of one or more molecular marker(s)of drug resistant malaria, (c) original articles but no review articles.

Results

Chloroquine resistance (CQR): Chloroquine previously was the most widely used antimalarial in India.(5)Substantialuse in the last few decades due to its safety, effectiveness and low cost gradually led to development of resistance in P. falciparum.

Molecular markers for chloroquine resistance in Plasmodium falciparum:

Plasmodium falciparum chloroquine resistance transporter (pfcrt) K76T mutations:The K76T mutation is the primary determinant of chloroquine resistance and susceptibility. K76T mutation is in the first transmembrane domain of pfcrt protein, where the positively charged lysine residue is replaced by neutrally charged threonine residue at 76th position. This leads to efflux of chloroquine by active transport out of digestive vacuole.

Pfmdr1N86Ymutations: The Plasmodium falciparum multidrug resistance gene 1(Pfmdr1) is located on chromosome 5 and has one exon. It encodes for P-glycoportein homolog1 protein. Polymorphism in the pfmdr gene is known to be involved in resistance to antimalarials. Results obtained from pfmdr1 Single Nucleotide Polymorphisms (SNPs)studies indicate a significant role for the N86Y mutation in contributing to resistance to chloroquine.Among the various genetic polymorphisms, point mutations in pfmdr1 gene at codon 86(from asparagine to tyrosine,N86Y) has been extensively studied worldwide. (6,7)

Of the total 16 studies included in this review for pfcrtand pfmdrgenotypesof drug resistance, 10 studies were carried out in India and 6 were from Southeast Asia. (Table 1) summarizes molecular markers of drug resistance in P. falciparum

Shrivastava SK et alconducted a study in malaria endemic states of Assam and Arunachal Pradesh in Northeast India.(8) A total of 115 blood samples were included out of which 100 (86.95%) showed presence of both drug resistantK76Tmutation in pfcrtgene and N86Y mutations in pfmdr gene. These results suggested that pfcrtK76T and pfmdrN86Y can be used as potentially useful markers of the assessment of in vitro chloroquine resistance in the Northeast India.

The study conducted by Antony HA et analyzed 60 samples, 30 samples from Puducherry and 30 from Odisha in India.(9)Of the 60 samples, 34 samples had pfcrtK76T resistant genes. Chloroquine resistant parasites were higher in Odisha (73.33%) whereas 40% of Puducherry samples had resistant genes. Pfmdr1 N86Y was found only in 6/60(10%) samples. This mutation resulting in resistant gene was higher in Puducherry samples (13.33%)compared to that in samples from Odisha (6.67%). This study showed astrong association of chloroquine resistance with pfcrt T76 but not with pfmdr1 Y86 mutation. The study also found a new pfcrt haplotype 'CVIKT' associated with chloroquine resistance which was found to be present in Indian strains of P. falciparum.

Chatterjee Met alconducted a study in Kolkata, West Bengal that included 89 samples in which pfcrtK76T mutations was present in all samples.(10)In this study Y184F mutation in the gene pfmdr1 was detected in all the samples but no mutations were detected at codon 86. The role of mutant Y184F in chloroquine resistance is debatable. The results of this study showed that there is no sign of regaining chloroquine sensitivity among the prevailing parasite population of the study area even after five years of its withdrawal as evidenced by 100% prevalence of mutantK76T in pfcrtgene.

Ramani S et al conducted a study of 26 cases of P.falciparum malaria in Puducherry in which pfcrt K76T mutations and pfmdr N86Y mutations was not detected.(11)As the sample size was small it was not able to detect any other known or unknown polymorphisms.

Sharma J et al conducted a study among 54 P.falciparum isolates in Assam and Arunachal Pradesh which found pfcrt K76Tmutations in 77.78% of cases.(12)Other mutations like M74I (61.11%), N75E (61.11%) and C72S (16.67%) were also found. Triple mutant allele M74I+N75E+K76T was found in 61.11% P. falciparum field isolates. Double mutant allele C72S+K76T was seen among 16.67% samples. This suggests high prevalence of chloroquine resistance in Northeast region of India.

A study conducted by Singh G et al in 22 P.falciparum samples in Mumbai found both pfcrt K76T and pfmdr N86Y mutations in 15 samples(68.18%).(13)

Kar NP et al conducted a study in P.falciparum malaria cases in endemic Odisha state in India.(14) Total of 229 cases of P.falciparum were included in the study. Pfcrt K76Tmutation was found in 51 out of 64 cases analyzed. Pfmdr N86Y mutation was found in 55 out of 73 cases analyzed. Results of this study found the presence of P.falciparumisolates resistant to chloroquine.

Kumar D et al conducted a study from July 2014 to June 2016 among 25 cases of P.falciparumnot responding to chloroquine therapy.(15)Pfmdr N86Y mutation was found in 16 samples. This study suggested strong association between pfmdr1 N86Y mutations and in vivo chloroquine resistance.

Patel P et al conducted a study in 180 samples of P. falciparum in Chhattisgarh.(16) A total of 143 samples were successfully analyzed for the pfcrt gene covering codons44–177. Out of these, 78%of the samples were mutant genotypes, while only 22% were wild type. In addition, codon K76T was found as a mutant position in all the mutant genotypes. Fifty-three percent of isolates were found to be a double mutant at positions C72S and K76T followed by 24% triple mutants at codons M74I,

N75E, K76T, and a single point mutation at K76T was observed in only 1% of samples. The pfmdr1gene was amplified and analyzed from 162 samples, and 59% were found mutant at N86Y. Only 18% of samples were found to be wild-type, and the majority (82%) were mutant genotypes for either pfcr to rpfmdr1 mutations. Additionally, 27% of the samples harbored a double mutant (SVMNT) pfcrt genotype with mutant pfmdr1, and 7% had a triple mutant (CVIET) genotype with mutant pfmdr1. This study showed a high level of chloroquine resistance genotypes.

Wedam J et al conducted a study on P. falciparum in Mangalore, South India.(17)Out of 276 patients with P.falciparum malaria138 cases were randomly selected of which 112 were amplified to check for pfmdr1 mutations.Pfmdr1 mutations N86Y, Y184F, D1246Y were analyzed. Wild type was not found. The prevalence of 86N-184F-1246Y mutation was 1.8% (2/112) and 86N-184F-1246D was 98.2% (110/112).

Huang F et al conducted a study on P.falciparumalong the China Myanmar border.(18) A total of 65 patients were included, of which 63 samples were amplified and mutations found in pfcrt codon 76 in all the isolates(100%). No mutations of pfmdr1 (codons 86 or 1246) were found. Although China has not used chloroquine to treat P. falciparum for over 30 years, the stable and high prevalence of K76Tmutation may be caused by the continued use of chloroquine as a first-line drug for P. vivax over several decades.

Saleh et al conducted a study on P.falciparum isolates in South Sumatera, Indonesia.(19) Since 2001 in Indonesia artemisinin combination therapy (ACT) has been used as the first line treatment of malaria. But in the study area chloroquine was still used for treatment of P.falciparum. This study was conducted from August to December 2012. A total of 25 samples were collected in which pfcrtK76T mutations was found in all the samples. Pfmdr N86Y mutation was detected in 20 samples and 5 samples did not give interpretable results. The results of this study strengthen the previous literature, which stated that resistance to chloroquine has spread to all malaria endemic areas in Indonesia, including South Sumatera.

Study conducted by Tan LL et al found pfcrt K76T in 70.9% of samples.(20)It showed that chloroquine resistance was still prevalent in Sabah, Malaysia despite discontinuation of chloroquine as first line treatment for P. falciparum since 1979.

Feng Ju et al conducted a study along China Myanmar border in 26 P.falciparum samples.(21)Sequencing of the pfcrt gene was successful in 9 isolates(34.6%, 9/26) that covered codons 74, 75, and 76. Of all three mutated codons, K76T was the most prevalent (23.1%, 6/26). The Pfmdr1 gene was sequenced in 3 isolates of all P.falciparum samples (11.5%, 3/26) that covered codon 86 and 184. Mutations at codon N86Y (11.5%, 3/26) were common. Even though China has not used chloroquine to treat P. falciparum infections for more than three decades, the stable and high prevalence of pfcrt K76T mutation may be a result of the continued use of chloroquine as a first-line drug for P. vivax infection over several decades. Chloroquine is also a first-line drug for P.vivax infection in Myanmar, especially in the Myanmar-Thailand border area, where a high prevalence of pfcrtK76T was found. This suggests the natural selection against chloroquine pressure has led to the maintenance of the pfcrt mutation in P.falciparumin this region.

A study was by conducted by Norahmad NA et al among 29 cases of P.falciparum in Sabah, Malaysia.(22) Mutations for pfcrtK76T and pfmdr1 N86Ywere found in only 2 cases (2/29 6.9%). This study demonstrated low prevalence of chloroquine resistance in Sabah area, in contrast to results obtained in previous study.

Retenget al conducted a study in 45 cases of P.falciparum isolates in North Sulawesi, Indonesia.(23) Among 45 cases, pfcrt K76Tand pfmdr N86Ymutations were found in all the samples. The results of this study suggest that the prevalence of the mutated genotypes remained dominant even 6 years after the withdrawal of chloroquine from this region.

Molecular markers for chloroquine resistance in Plasmodium vivax:

Potential molecular markers include mutations in the multidrug resistance 1 (mdr1) and P. vivax chloroquine resistance transporter (pvcrt) genes, orthologous to pfmdr1 and pfcrt in P. falciparum, respectively. K10 insertion ('AAG' insert) in first exon at tenth position of pvcrt-O is a known chloroquine resistance marker. Y976F and F1076L, which are non-synonymous amino acid mutations of the pvmdr-1, have been reported to correlate with chloroquine resistance.(24, 25)

According to (Table 2), four studies were included; of these three studies were from India and one was from Southeast Asia. Joy S et al conducted a study in Southwestern coastal region of India.(26)They collected 140 blood samples out of which sequencing was carried out for 54(38.5%) and 85(60.7%) isolates for pvcrt and pvmdr1 genes. Out of 54 samples, 3 samples showed K-10 insertion. Out of 85 isolates 84 P.vivax isolates had pvmdr mutant alleles. Seven non-synonymous mutations (I946V, T958M, Y976F, F979S, M980V, Y1028C and F1076L) were observed. Among these, I946V and Y1028C mutations were observed for the first time from this region. Prevalence of the Y976F mutation was found to be 7.1% whileF1076L mutation was 54.5%. The most prevalent and dominant (90.6%, n = 77) mutation was T958M. This was the first study from India reporting K10 insertion in pvcrt-o. This study indicates a trend towards parasites acquiring chloroquine resistance in endemic Mangalore city, South India.

Anantabotla MV et al conducted a study in 240 P.vivax samples from four different regions of India namely, Puducherry(PDY), Mangalore (MAQ), Cuttack (CTC) and

Jodhpur (JDH) respectively.(27)Sixty samples were collected from each region. Eighteen samples from each study site making a total of 72 samples were randomly selected for pvcrt and pvmdr sequence analysis. Out of 18 samples, 16 samples from each site were successfully sequenced. Only six isolates (6/64, 9.4%) had K10 insertion. The prevalence of K10 insertion in pvcrt-o gene was detected for 18.8% in PDY, 12.5% in MAQ and 6.3% in CTC P. vivax isolates, whereas no change in nucleotide was identified in P. vivax isolates collected from JDH region. This is the second report to detect CQ resistance in Indian P. vivax isolates and is the first study to report K10insertion in P. vivax isolates from PDY and CTC region. The sequence analysis shows that the CQ resistance in P. vivax isolates, based on K10 insertion in pvcrt-o gene, was observed more in Southern region of India as compared to North India. The pvmdr-1 gene product of 604 bp, targeting the Y976F andF1076L mutation, was sequenced successfully in 60 isolates. Out of 60 isolates sequenced for pvmdr-1 (15 from each site), most of the isolates showed drug resistant mutations for chloroquine with 91.6% having theF1076L mutation in the pvmdr-1 gene. All the P. vivax isolates sequenced from CTC and JDH showed drug resistant mutations for chloroquine based on the pvmdr-1 molecular marker, and the prevalence of resistance in MAQ isolates fall almost close to CTC and JDH. The prevalence of chloroquine resistance among the PDY isolates were 73.3%. The T958M non-synonymous mutation, resulting due to the nucleotide change at the position 2873from C to G, was present in all the P. vivax isolates collected from four different regions of India with 100% frequency. The results suggest that P. vivax isolates from four different study regions of India are susceptible to chloroquine; however, the low prevalence rate of both K10 insertion in pvcrt-o and Y976F mutation inpvmdr-1 gene in P. vivax field isolates indicates that CQ resistance in the studied geographical regions might a risein the near future.

Tacoli C et al conducted a study on P.vivax samples in Mangaluru, South India.(28)Total of 116 patients were included for molecular analysis. Pvmdr1 sequencing was successful for 108 isolates (108/116, 93.1%). Four synonymous (T529T, A970A, S1358S, and R1422R) and eight non-synonymous (S513R, T958M, Y976F, F1076L, Y1028C, L1393N, L1425R, and T1269S) point mutations were identified. Ten pvmdr1 haplotypes were observed with mutations T958M and F1076L occurring in all isolates, whereas the candidate chloroquine resistance marker Y976F was present in one isolate only. The candidate marker Y976F occurred only once (0.9%) in the present study, the figure was almost 8-fold higher in a previous report (Joy et al. 2018). The abundance of pvmdr1 F1076L in isolates from Mangaluru has been considered as an indication of emerging chloroquine resistance.

Noisang C et al conducted a study in 157 P.vivax isolates in Southern Thailand.(29)Two point-mutations at codons 976 and 1076 in thepvmdr1 gene were identified in 125 isolates from four provinces in Southern Thailand. The occurrence of double mutations (Y976F and F1076L) inpvmdr1 was observed in nine Chumphon isolates, seven Ranong isolates, and one Surat thani isolate. A single mutation at codon 976 (Y976F) was detected in only one Chumphon isolate, while another single mutation atcodon 1076 (F1076L) was discovered in 81 Yala isolates, four Chumphon isolates, three Surat thani isolates, andtwo Ranong isolates. Wildtypes alleles were observed in11 Chumphon isolates, four Ranong isolates, and two Surat thani isolates. The K10 insertion (addition of AAG) in pvcrt-o genes occurred in three of three Ranong isolates only. Results of this study calls for urgent drug resistance surveillance of P.vivax and P.falciparum in Southern Thailand.

Conclusion

Most studies showed the presence of K76T pfcrt and N86Y pfmdr mutations which are strongly associated with chloroquine resistance in P. falciparum. In India pfcrt and pfmdr mutations are still present after years of ACT implementation for the treatment of P.falciparum. We also found the presence of mutations in pvcrt-o and pvmdr genes, which indicates that P.vivax may acquire chloroquine resistance in future. To conclude we found that various studies across India and Southeast suggest the widespread resistance to chloroquine among P. falciparum and the emerging development to chloroquine resistance in P.vivax. This can have implications on malaria elimination and treatment guidelines in this region.

Recommendation

Malaria and the growing role of drug resistance as a major impediment to its control are global issues. The identification and interpretation of molecular markers of antimalarial drug resistance are critical steps toward understanding and eventually controlling resistance. This review elucidates chloroquine resistance and recommends development of appropriate treatment guidelines based on the resistance pattern observed. Treatment protocols may be reviewed and further studies may be recommended for identification of resistance and mutation from time to time.

Limitation of the study

This study focuses exclusively on chloroquine resistance. It makes no mention of other important antimalarials.

Relevance of the study

There are only a few systematic reviews available on molecular markers of drug resistance. This study explains how drug resistance evolves over time.

Authors Contribution

The study's concept and design were developed in collaboration with RM, AJ & SU. Additionally, they defined intellectual content. They were tasked with conducting a literature search. Furthermore, they analysed the data. Besides this, they assisted in the preparation, editing, and review of manuscripts. RM assisted in the data collection process. AJ is the study's Guarantor. RM wrote the first draft. All authors participated in reviewing the draft, revising and finalizing it.

References

1. World Health Organization. 2020. World Malaria Report 2020. Geneva, Switzerland: WHO. Available on Available on https://www.who.int/publications/i/item/9789240015791 . [Accessed on 25/12/2021]
2. WHO guidelines for Malaria. World Health Organization, Geneva 2021.Available on https://www.who.int/publications/i/item/guidelines-for-malaria .[Accessed on 25/12/2021]
3. Shah RR. Pharmacogenetics in drug regulation: promise, potential and pitfalls. Philosophical Transactions of the Royal Society B: Biological Sciences. 2005;360(1460):1617-38.
4. Sharma YD. Molecular surveillance of drug-resistant malaria in India. Current Science. 2012 Mar 10:696-703.
5. Sharma VP. Battling the malaria iceberg with chloroquine in India. Malaria Journal. 2007;6(1):1-5.
6. Fidock DA, Nomura T, Talley AK, Cooper RA, Dzekunov SM, Ferdig MT, et al. Mutations in the P. falciparum digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. Molecular Cell. 2000;6(4):861-71.
7. Sidhu AB, Verdier-Pinard D, Fidock DA. Chloroquine resistance in Plasmodium falciparum malaria parasites conferred by pfcrt mutations. Science. 2002;298(5591):210-3.
8. Shrivastava SK, Gupta RK, Mahanta J, Dubey ML. Correlation of molecular markers, Pfmdr1-N86Y and Pfcrt-K76T, with in vitro chloroquine resistant Plasmodium falciparum, isolated in the malaria endemic states of Assam and Arunachal Pradesh, Northeast India. PLoS One. 2014;9(8):e103848.
9. Antony HA, Das S, Parija SC, Padhi S. Sequence analysis of pfcrt and pfmdr1 genes and its association with chloroquine resistance in Southeast Indian Plasmodium falciparum isolates. Genomics data. 2016;8:85-90.
10. Chatterjee M, Ganguly S, Saha P, Guha SK, Basu N, Bera DK, Maji AK. Polymorphisms in Pfcrt and Pfmdr-1 genes after five years withdrawal of chloroquine for the treatment of Plasmodium falciparum malaria in West Bengal, India. Infection, Genetics and Evolution. 2016;44:281-5.
11. Ramani S, Parija SC, Mandal J, Hamide A, Bhat V. Detection of chloroquine and artemisinin resistance molecular markers in Plasmodium falciparum: A hospital based study. Tropical Parasitology. 2016;6(1):69.
12. Sharma J, Soni M, Dutta P, Khan SA, Mahanta J. Mutational prevalence of chloroquine resistance transporter gene among Plasmodium falciparum field isolates in Assam and Arunachal Pradesh, India. Indian Journal of Medical Microbiology. 2016;34(2):193-7.
13. Singh G, Singh R, Urhekar AD, Rane K. Gene sequence polymorphisms mutations in PFMDR-1 and PFCRT-O genes of Plasmodium falciparum. Int J CurrMicrobiol App Sci. 2016;5(10):451-8.
14. Kar NP, Chauhan K, Nanda N, Kumar A, Carlton JM, Das A. Comparative assessment on the prevalence of mutations in the Plasmodium falciparum drug-resistant genes in two different ecotypes of Odisha state, India. Infection, Genetics and Evolution. 2016;41:47-55.
15. Kumar D, Prakash V, Nath G. Prevalence of polymorphisms at position 86 of the Pfmdr1 gene in Plasmodium falciparum parasites. Parasitologists United Journal. 2016;9(2):103.
16. Patel P, Bharti PK, Bansal D, Ali NA, Raman RK, Mohapatra PK, Sehgal R, Mahanta J, Sultan AA, Singh N. Prevalence of mutations linked to antimalarial resistance in Plasmodium falciparum from Chhattisgarh, Central India: a malaria elimination point of view. Scientific Reports. 2017;7(1):1-8.
17. Wedam J, Tacoli C, Gai PP, Siegert K, Kulkarni SS, Rasalkar R, Boloor A, Jain A, Mahabala C, Baliga S, Shenoy D. Molecular evidence for Plasmodium falciparum resistance to sulfadoxine–pyrimethamine but absence of K13 mutations in Mangaluru, Southwestern India. The American Journal of Tropical Medicine and Hygiene. 2018; 99(6):1508.
18. Huang F, Tang L, Yang H, Zhou S, Sun X, Liu H. Therapeutic efficacy of artesunate in the treatment of uncomplicated Plasmodium falciparum malaria and anti-malarial, drug-resistance marker polymorphisms in populations near the China-Myanmar border. Malaria Journal. 2012; 11(1):1-8.
19. Saleh I, Handayani D, Anwar C. Polymorphisms in the pfcrt and pfmdr1 genes in Plasmodium falciparum isolates from South Sumatera, Indonesia. MajalahKedokteranSriwijaya. 2014.
20. Tan L, Lau T, Timothy W, Prabakaran D. Full-Length Sequence Analysis of Chloroquine Resistance Transporter Gene inPlasmodiumfalciparumIsolates from Sabah, Malaysia. ScientificWorldJournal, 2014; 2014:1-7.
21. Feng J, Zhou D, Lin Y, Xiao H, Yan H, Xia Z. Amplification ofpfmdr1,pfcrt,pvmdr1, and K13 Propeller Polymorphisms Associated with Plasmodium falciparum and Plasmodium vivax Isolates from the China-Myanmar Border. Antimicrobial Agents and Chemotherapy. 2015; 59(5):2554-2559.
22. Norahmad NA, Razak MR, Abdullah NR, Sastu UR, Imwong M, Muniandy PK, et al. Prevalence of Plasmodium falciparum molecular markers of antimalarial drug resistance in a residual malaria focus area in Sabah, Malaysia. PLoS One. 2016; 11.
23. Reteng P, Vrisca V, Sukarno I, Djarkoni I, Kalangi J, Jacobs G et al. Genetic polymorphisms in Plasmodium falciparum chloroquine resistance genes, pfcrt and pfmdr1, in North Sulawesi, Indonesia. BMC Research Notes. 2017; 10(1):1-8.
24. Brega S, Meslin B, de Monbrison F, Severini C, Gradoni L, Udomsangpetch R, et al. Identification of the Plasmodium vivax mdr-like gene (pvmdr1) and analysis of single-nucleotide polymorphisms among isolates from different areas of endemicity.Journal of Infectious Diseases. 2005; 191(2):272-7.
25. Sá JM, Yamamoto MM, Fernandez-Becerra C, de Azevedo MF, Papakrivos J, Naudé B, Wellems TE, Del Portillo HA. Expression and function of pvcrt-o, a Plasmodium vivax ortholog of pfcrt, in Plasmodium falciparum and Dictyosteliumdiscoideum. Molecular and Biochemical Parasitology. 2006; 150(2):219-28.
26. 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. Malaria Journal. 2018; 17(1):1-5.
27. 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. Parasitology International. 2019;70:92-7.
28. Tacoli C, Gai PP, Siegert K, Wedam J, Kulkarni SS, Rasalkar R, et al. Characterization of Plasmodium vivax pvmdr1 Polymorphisms in Isolates from Mangaluru, India. The American Journal of Tropical Medicine and Hygiene. 2019; 101(2):416-417.
29. Noisang C, Prosser C, Meyer W, Chemoh W, Ellis J, Sawangjaroen N, et al. Molecular detection of drug resistant malaria in Southern Thailand. Malaria Journal. 2019; 18(1):1-1.

Tables

TABLE 1 SUMMARY OF KEY FINDINGS OF STUDIES THAT ANALYSED THE PFCRTK76T AND PFMDR1 N86Y MUTATIONS ASSOCIATED WITH CHLOROQUINE RESISTANCE

Sl no	Authors	Year of Publishing	Halpotypes Found	*Pfcrt* K76T mutations	*Pfmdr*1 N86Y mutations
1	Shrivastava SK *et al*[8]	2014	_____	100/115 (86.95%)	100/115
2	Antony HA *et al*[9]	2016	CVMNK, SVMNT, CVIET and CVIKT	34/60 (56.6%)	6/60 (10%)
3	Chatterjee M *et al*[10]	2016	SVMNT	89/89 (100%)	0/89
4	Ramani S*et al* [11]	2016		0/26	0/26
5	Sharma J *et al*[12]	2016	CVMNK, SVMNT and CVIET	77.78%	Not done for *pfmdr* genes
6	Singh G *et al*[13]	2016	_____	15/22 (68.18%)	15/22 (68.18%)
7	Kar NP *et al*[14]	2016	CVIET, CVMNK, SVMNT	51/64 (84%%)	55/73 (75.4%)
8	Kumar D*et al*[15]	2016	_____	Not checked for *pfcrt* mutations	9 (34.6%)
9	Patel P *et al*[16]	2017		143(78%)	96 /162 (59.26%)
10	Wedam J *et al*[17]	2018	_____	Not checked for *pfcrt* mutations	86N-184F- 1246Y - 1.8%(2/112) 86N-184F- 1246D – 98.2% (110/112)
11	Huang F *et al*[18]	2012	_____	63/63 (100%)	0
12	Saleh I *et al*[19]	2014	_____	25/25 (100%)	20/25
13	Tan LL *et al*[20]	2014	CVIET and SVMNT	22/ 31 (70.96%)	Not done for *pfmdr* gene
14	Feng J*et al*[21]	2015		6/26 (23.1%)	3/26 (11.5%)
15	Norahmad NA*et al*[22]	2016	K76T	2/29 (6.9%)	2/29 (6.9%)
16	Reteng P *et al*[23]	2017		45/45 (100%)	51/59 (88.2%)

TABLE 2 SUMMARY OF KEY FINDINGS OF STUDIES THAT ANALYSED THE PVCRT-O AND PVMDR-1 GENES ASSOCIATED WITH CHLOROQUINE RESISTANCE IN P.VIVAX

Sl. No	Authors	Year of publishing	*pvmdr* Mutant Y976F	*pvmdr* Mutant F1076L	*pvmdr* Mutant T958M	*pvcrt-o* K10 insertion
1	JoyS *et al*[26]	2018	6/85 (7.1%)	65/85 (76.5%)	77/85 (90.6%)	3/54 (5.5%)
2	Anantabotla VM *et al*[27]	2019	_____	55/60(91.6%)	60/60(100%)	6/72(8.3%)
3	Tacoli C *et al*[28]	2019	1/108(0.9%)	94/108 -87.00%		
4	Noisang C *et al*[29]	2019	1/125 (0.8%)	90/125 (7.2%)	_____	3/125 (2.4%)