Review Article

The Drug Resistance of *Plasmodium falciparum* and *P. vivax* in Iran: A Review Article

Alihsan Heidari 1, *Hossein Keshavarz 2,3

1. Department of Medical Parasitology, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran
2. Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
3. Research Center for Endemic Parasites of Iran, Tehran University of Medical Sciences, Tehran, Iran

Received 13 Jul 2020
Accepted 06 Oct 2020

Abstract

**Background:** One of the main obstacles to malaria control in the world has been the emergence of resistance in *Plasmodium falciparum* to chloroquine and other antimalarial drugs. This study aimed to review studies in Iran on resistance in *P. falciparum* and *P. vivax* to drugs, and to reveal the mechanisms and molecular markers of resistance of these two species.

**Methods:** The databases of PubMed, Scopus, Google Scholar, Magiran, and reputable Iranian journals were searched to find published studies on the resistance in *P. falciparum* and *P. vivax* to antimalarial drugs in Iran.

**Results:** There is a significant relationship between resistance to chloroquine in *P. falciparum* and the emergence of K76T mutation in the *P. falciparum* chloroquine-resistance transporter gene in Iran. Resistance to sulfadoxine-pyrimethamine (SP) in *P. falciparum* is also significantly associated with the development of mutations in the dihydrofolate reductase and dihydropteroate synthase genes. Resistance to chloroquine in *P. vivax* has not been reported in Iran and it is used as a first-line treatment for *P. vivax* malaria.

**Conclusion:** *P. falciparum* has become resistant to chloroquine in different regions of Iran and is not currently used to treat malaria. Besides, cases have emerged of *P. falciparum* resistance to SP in different parts of southern Iran, and SP is not administered alone for treating *P. falciparum*.
Introduction

Drug resistance is a serious concern for the control and elimination of malaria in the world (1). Malaria has existed since ancient times, was first recorded by Hippocrates four centuries BCE, and Avicenna described it as an intermittent fever in the Canon of Medicine circa 1000 CE (2). However, as one of the most life-threatening infectious diseases in the world, malaria is still prevalent (3). Malaria infected about 219 million people and killed 409 thousand cases throughout the world in 2019 (4). Five species of *Plasmodium falciparum*, *P. vivax*, *P. malaria*, *P. ovale*, and *P. knowlesi* cause malaria in human (5). Two of these species, *P. vivax* and *P. falciparum*, are common in Iran with *P. vivax* accounting for more than 90% of malaria cases. Iran’s southern provinces including Sistan-Baluchestan, Hormozgan, and southern parts of Kerman are affected by indigenous cases of malaria (Fig. 1) with most cases found in Sistan and Baluchistan Province (6).

Extensive measures taken in Iran have brought malaria on the verge of elimination.

Accordingly, no cases of indigenous transmission were reported in 2018, 2019, and 2020 (4).
Iran’s neighboring Pakistan and Afghanistan with the highest rates of malaria cases in the Eastern Mediterranean Region after Sudan, and the resulting border contacts, migration and travel of citizens of these countries to Iran have made imported malaria particularly important (4, 7, 8). Some studies on the genetic diversity of P. falciparum and P. vivax genes in Iran indicate excessive diversity of their genes, which is not commensurate with the malaria situation in Iran and its low transmission rate, confirming the exchange of Plasmodium strains between neighboring countries and Iran (9-14).

One of the reasons for the persistence of malaria in the world is the decrease in the sensitivity of Plasmodium to anti-malarial drugs and the emergence of drug resistance in this protozoan, especially in P. falciparum (15). Although the highest malaria mortality is due to P. falciparum, severe and even more fatal cases can occur due to P. vivax (5, 16). This article aimed to review studies on the drug resistance in P. falciparum and P. vivax in Iran and to reveal the mechanisms and molecular markers of resistance in these Plasmodium species.

Methods

The databases of PubMed, Scopus, Google Scholar, Magiran and reputable Iranian journals were searched to find published studies on the resistance in P. falciparum and P. vivax to anti-malarial drugs and studies on molecular markers associated with anti-malarial drug resistance. All articles related to resistance to antimalarial drugs were selected and analyzed. Duplicate and insignificant articles were not reviewed.

Definition of drug resistance in malaria parasites

Drug resistance in Plasmodium is defined as its ability to survive or reproduce although the drug reaches the infected parasite or red blood cell sufficiently and that the drug is administered and absorbed in doses equal to or even higher than those tolerable for humans (17). Accordingly, the treatment failure which may be due to insufficient dose of the drug or insufficient drug delivery to the parasite is not considered Plasmodium resistance to anti-malarial drug (18, 19). Besides, drug resistance must be distinguished from recrudescence.

WHO has defined the antimalarial treatment failure in P. falciparum in human as follows (20):

- Early Treatment Failure (ETF): The onset of clinical symptoms and/or the presence of parasitemia during the first three days of follow-up.
- Late Clinical Failure (LCF): The recurrence of symptoms in the presence of parasitemia within 4 to 28 d after starting the treatment.
- Late Parasitological Failure (LPF): Parasitemia from day 7 to 28 with axillary temperature less than 37.5 °C.

Meanwhile, the anti-malarial treatment failure in Plasmodium was previously defined at three levels of R1, R2, and R3 (21).

P. falciparum resistance to antimalarial drugs

Quinine, extracted from the leaves of the cinchona tree, was used as an important drug to treat malaria from the 17th century until the 1920s (22). During World War II, synthetic chloroquine was developed due to insufficient access to quinine. The German scientist Hans Andersag, who worked for the German pharmaceutical company Bayer AG, synthesized chloroquine as an antimalarial drug in 1934 (Fig. 2).
It was used as 7-chloro-4-quinoline in the 1940s to treat malaria (23-25). By adding the hydroxyl group to chloroquine, the hydroxychloroquine compound was also used as an alternative for chloroquine. Since 1946, both chloroquine and hydroxychloroquine have been used as effective inexpensive drugs with fewer side effects as a first-line treatment for malaria worldwide (23-25) However, the effectiveness of chloroquine decreased with the emergence and spread of chloroquine-resistant *P. falciparum* isolates, and in 1959, almost a decade after its use, the first cases of *P. falciparum* resistance to chloroquine were reported in Colombia and Southeast Asia. Currently, resistance in *P. falciparum* has been reported almost from all over the world except in small remote areas (17, 24, 26). *Plasmodium* feeds on hemoglobin such that young trophozoites consume up to 75% of the hemoglobin in red blood cells (27). In *P. falciparum*, hemoglobin is transferred to the digestive vacuole at a pH of approximately 5.2. In the digestive vacuole, *P. falciparum* breaks down the hemoglobin to obtain amino acids (28). *P. falciparum* prevents the accumulation of free toxic heme in the digestive vacuole and, by polymerizing heme, converts it to a crystalline substance called hemozoin or pigment (23). Chloroquine prevents the conversion of heme to hemozoin in the digestive vacuole (Fig. 3).
In other words, proteolysis of hemoglobin releases heme, which is soluble and is a form that can inhibit the activity of various Plasmodium enzymes (23, 24). K76T mutations in the P.falciparum chloroquine resistance transporter (PfCRT) have been associated with P.falciparum resistance to chloroquine in various global studies. This mutation converts K (lysine) to T (Threonine). The PfCRT gene encodes an integral protein membrane that is concentrated in the digestive vacuole (29). This mutation generally prevents the entry of chloroquine, a weak alkaline drug 4-aminoquinoline, into the acidic organelle of the digestive vacuole of P.falciparum or causes it to leave the digestive vacuole quickly, preventing chloroquine from binding to heme and preventing its detoxification. Finally, chloroquine will not be able to prevent the conversion heme to either hemozoin (27, 30).

Another factor that plays a role in resistance to antimalarial drugs that target hemoglobin is the P-glycoprotein homologue of P.falciparum multidrug resistance (Pfmdr) 1, a protein encoded by the Pfmdr1 transporter gene. Pfmdr1, like PfCRT, is located on the membrane of the digestive vacuole (27). Adequate drug delivery is essential for maximum drug activity. Mutations in Pfmdr1 probably prevent the transfer of the antimalarial drug from the cytosol into the digestive vacuole where it reduces the concentration of drugs that target hemoglobin, such as chloroquine and amodiaquine (31). Mutations in codon 86 (N86Y) of the Pfmdr1 gene are commonly involved in chloroquine resistance, and there are few studies on the polymorphism of other codons (32).

Iran has witnessed great successes in controlling malaria in many parts of the country since 1958 when the malaria eradication pro-
program began, and chloroquine has been used since the 1950s as a first-line treatment for malaria caused by *P. falciparum* and *P. vivax* (33). However, this program did not lead to the eradication of malaria in Iran for various reasons, including parasite resistance to antimalarial drugs and vector resistance to insecticides (33).

An in vivo study conducted in Hormozgan Province in southern Iran in 1968 reported susceptibility of *P. falciparum* to chloroquine (34). Another study on 45 patients with malaria in the same province also showed that *P. falciparum* was still susceptible to chloroquine and no case of resistance was reported (35).

In Iranshahr, a city in Sistan and Baluchistan Province, *P. falciparum* resistance to chloroquine in 1983 was reported at 5.7% at R1 level. However, its resistance to chloroquine gradually increased to 52.1% in Iranshahr at three levels of R1, R2, and R3 (2).

*P. falciparum* resistance to chloroquine was also explored by in vivo studies in other areas, including Hormozgan Province and Kahnooj in Kerman Province. During 1997-2000, the resistance reached 68% at the R1 level and 84% at the R2 level in the mentioned areas (2, 36). Since most of the reported cases of chloroquine resistance were detected in people who were either Afghan nationals or had a history of traveling outside Iran, chloroquine-resistant strains might have been imported from other countries (17).

Chloroquine efficacy was investigated in compliance with the 28-day WHO standard protocol from 2002 to 2004 in 5 locations in Sistan and Baluchistan, Hormozgan, and Kerman provinces in southern Iran. On day 28 of the follow-up, 78.5% of cases were found to be resistant to chloroquine, with 17.4% having ETF, 34.7% LCF, and 26.4% LPF (37). Based on this study and previous reports, the use of chloroquine was discontinued for the treatment of malaria caused by *P. falciparum* in Iran.

### P. falciparum resistance to chloroquine and genetic markers in Iran

Some studies have addressed the resistance of *P. falciparum* to chloroquine and its relationship with *Pfcrt* and *Pfmdr* genetic markers in Iran. A study reported 49 isolates with K76T mutation out of 50 isolates of *P. falciparum* in Sistan and Baluchistan Province (38). In another in vivo study conducted in the same province, 23 out of 25 patients with *P. falciparum* malaria treated with chloroquine showed resistance to chloroquine during the 28-day follow-up period. A correlation was reported between 76T mutant allele and resistance to chloroquine (39).

Furthermore, K76T mutation was observed in 60 isolates (93.75% of the participants) out of 64 patients with *P. falciparum* malaria in Sistan and Baluchistan Province using nested PCR. Only four *P. falciparum* isolates carried the wild K76 codon. Response to chloroquine was evaluated in 28 of these patients in vivo for 28 days. The response of *P. falciparum* isolates to treatment was as follows: ETF = 17.9%, LTF = 60.7%, and sensitive to chloroquine = 21.4%. In other words, 22 out of 28 patients (78.6%) showed resistance to chloroquine in vivo. All of these patients carried the 76T mutant allele (40).

In another study in Sistan and Baluchistan Province on 206 *P. falciparum* isolates, the 76T mutant allele in the *Pfcrt* gene was observed in 202 isolates (98%) while the 86 Y allele in the *Pfmdr1* gene was observed in 78 (37.8%) isolates (41). Finally, a study on 26 *P. falciparum* isolates that revealed treatment failure to chloroquine during the 28-day follow-up period showed that the N86Y mutation in the *Pfmdr1* gene was carried in six isolates (23.1%). No mutations were observed in the other four codons of this gene (42).

### P. falciparum resistance to sulfadoxine–pyrimethamine

Pyrimethamine, commercially known as Daraprim, was developed in the early 1950s by
Gertrude Elion and George Hitchings. The two researchers won the 1988 Nobel Prize in Medicine for developing pyrimethamine (24).

Sulfadoxine was developed in the early 1960s (24), but it was not used for a long time because the malaria parasite was resistant to it. Sulfadoxine-pyrimethamine (SP) was approved in 1981 for the treatment of malaria and was sold under the brand name Fansidar. Both drugs specifically target the folate biosynthesis pathway. Pyrimethamine inhibits the dihydrofolate reductase (dhfr) and sulfadoxine inhibits the dihydropteroate synthase (dhps) (24). SP is used as a single dose.

Following *P. falciparum* resistance to chloroquine in Iran, SP was used to treat *P. falciparum* malaria. However, *P. falciparum* resistance to SP was reported very quickly due to mutations in *dhfr* and *dhps* genes (Figs. 4 and 5). Since 2007, the Center for Infectious Diseases Management modified the treatment regimen and SP-artesunate replaced the previous treatment. An in vivo and in vitro study on the response of *P. falciparum* to SP showed that out of 26 chloroquine-resistant isolates that used amodiaquin+standard dose of SP, three cases were in R1 and three cases were resistant to SP at the R2 level. Another study administered a standard dose of SP for the treatment of 43 chloroquine-resistant *P. falciparum* isolates in three provinces of Sistan and Baluchistan, Hormozgan, and Kerman, and reported 11.6% of the isolates were resistant to SP (43).
Mutations in P. falciparum dhfr and dhps genes

In a study of 35 P. falciparum malaria patients in Hormozgan Province receiving SP for the treatment of malaria, two patients failed treatment, one of the two had a Glu 540 mutation in the dhps and Asn-108 and Arg 59 mutations in the dhfr gene (44).

A study in Chabahar of Sistan and Baluchistan Province, on 206 P. falciparum isolates revealed that all isolates in the dhfr gene carried 108N mutations and 98.5% of the isolates had both 108N and 59R mutations. In total, 20.4% of the isolates had 59R/108N simultaneous mutations in the dhfr gene and 437G in the dhps gene (41).

To investigate the association between molecular markers of P. falciparum resistance to SP and in vivo P. falciparum resistance, 53 P. falciparum isolates were examined, in which 11.3% of the isolates (6 patients) were resistant to SP (45), indicating a higher level of resistance than the rate (5.77%) reported by Eskandarian (44). Mutant codons of Asn108 in 100% and Arg59 in 81.1% of isolates were observed in the dhfr gene. Besides, 85% of the isolates in the dhps gene carried the 436Ala/Phe mutant codon. All SP-resistant cases had at least three mutations in both genes (45). This study showed that the effect of SP in Iran was decreasing. The prevalence of Gly437 mutant codon was 32%, a significant increase when compared with 17% in the previous study (46).

Another study conducted in Hormozgan Province on 16 P. falciparum isolates revealed that all isolates had double mutations in codons 108 and 59 (allele 59R /108N) in dhfr gene (47). These mutations indicate possible P. falciparum resistance to pyrimethamine.

A study conducted on 107 isolates after the introduction of SP and artesunate in Iran as the first-line therapy for P. falciparum malaria showed that the mutant codons 108N, 59R, and 51I were 100%, 95.9%, and 4.1% in dhfr gene of the isolates, respectively. The 437G mutant codon was observed in dhps gene in 26.9% of isolates, a reduction compared with 55.5% before the introduction of the SP and artesunate combination (48). Cumulative mutations 59R, 108N in dhfr, and 437G in dhps were observed in 42.4% of isolates; meanwhile, the same cumulative mutations decreased to 38% (48).

Artemisinin and its derivatives

Artemisinin was first isolated from Artemisia annua in 1971 by Tu Youyou. This plant has been widely used in traditional Chinese medicine to treat malaria. Tu Youyou received the Nobel Prize in Medicine in 2015 for discovering a new treatment for malaria (24). Artemisinin is effective against multidrug-resistant P. falciparum strains and has become the first-line malaria treatment in the world (27). Artesunate is a semi-synthetic derivative of artemisinin, used in Iran together with SP to treat falciparum malaria (49).

Artemisinin-based combination therapies (ACTs) are the current strategy to combat falciparum malaria. ACTs have significantly reduced the burden of malaria and its mortality in the world (50). Despite the efficiency of ACTs, the first case of partial artemisinin-resistant P. falciparum was reported in Western Cambodia in 2008 (51). Artemisinin-resistant P. falciparum was again reported in Western Cambodia, western Thailand, southern Myanmar, and southern Vietnam (18, 52). In another study in 2018, 30 cases of artemisinin-resistant P. falciparum were reported in Southeast Asia (53).

There is no consensus over the artemisinin mechanism of action. One theory explains that the drug molecules are activated by heme to produce free radicals, which destroy the proteins needed for the parasite survival (24). Artemisinin acts as an effective inhibitor of phosphatidylinositol-3-kinase (54). Artemisinin-resistant mutations affect one of the B
sheets of the Kelch domain (52). Upregulation-resistant K13 mutant parasites have shown oxidative stress (50).

An in vivo study in Iran examined the susceptibility of *P. falciparum* to SP/artesunate in 38 symptomatic malaria patients and reported full treatment of all 38 patients, and no reduction in susceptibility to this drug during the 28-day follow-up period (55).

**P. vivax resistance to antimalarial drugs**

Annually, *P. vivax* causes about 14.3 million malaria cases with clinical signs worldwide (56). Chloroquine has been used to treat *P. vivax* malaria for over 60 years. *P. vivax* resistance to chloroquine was first reported in Australian soldiers returning from Papua New Guinea (16, 57). There have been reports of *P. vivax* resistance to chloroquine in many endemic areas (16).

A study on 270 symptomatic *P. vivax* malaria patients in Sistan and Baluchistan Province, Iran, showed that chloroquine was still effective against *P. vivax* at the usual therapeutic dose of 25 mg/kg body weight. Besides, no case of resistance was reported in vivo during a 28-day follow-up (58). Previous studies have also confirmed the susceptibility of *P. vivax* to chloroquine (59-61). An in vivo study with a 28-day follow-up period in Sistan and Baluchistan Province, Iran, on 170 patients with *P. vivax* malaria showed that *P. vivax* was still susceptible to chloroquine and there were no reports of treatment failure (55). The first case of *P. vivax* resistance to chloroquine was reported in a 26-year-old pregnant woman in Pakistan: administration of standard doses of chloroquine did not result in parasite clearance (62).

Although molecular markers have been used to determine the resistance of *P. falciparum* to chloroquine, such studies have failed to show a correlation between the molecular markers PvCrv and PvMDR1 and the response of *P. vivax* to chloroquine (26, 63).

Several molecular studies investigated the status of mutations in the codons of *P. vivax* dhfr and dhps genes related to *P. vivax* resistance to SP in Iran. These studies have reported mutations in codons 57L, 58R, 117N, 117T, 93H, 33L, 61N in dhfr gene and mutations in codon 421 of dhps gene in southern Iran (47, 64-66). However, these studies have focused on the mutations and have not evaluated their association with in vivo resistance to SP in *P. vivax* isolates. SP are not currently recommended as the first-line treatment for *P. vivax* anywhere in the world. *P. vivax* appears to be resistant to this drug much faster than *P. falciparum* (63).

ACTs have a greater effect on *P. vivax* than on *P. falciparum* (67). However, they do not affect hypnozoites (63) and they have no role in preventing relapses (68). In countries where *P. vivax* is highly resistant to chloroquine, ACTs are used as the first-line treatment for *P. vivax* malaria (16).

Some forms of *P. vivax* hypnozoites in the liver are used to treat 8-Aminoquinoline compounds including primaquine. There are reports of malaria relapse in *P. vivax* endemic regions of the world (63). Although many cases of *P. vivax* are due to relapses, studies on the efficacy of primaquine in preventing relapses have led to controversies over the research design, implementation, and analysis (63). Some cases of *P. vivax* malaria relapse have also been reported in southern Iran (68, 69). There have been no reports of *P. vivax* resistance to primaquine in Iran.

**Conclusion**

One of the main obstacles to malaria control in the world has been the emergence of resistance to chloroquine and other antimalarial drugs in *P. falciparum*. Studies in Iran have reported a gradual decrease in susceptibility and then no response to chloroquine and SP used for the treatment of *P. falciparum*. *P. falciparum* has become resistant to chloroquine in different regions of Iran and it is not currently used
to treat *P. falciparum*. Besides, cases of *P. falciparum* resistant to SP have emerged in different parts of southern Iran and it is not applied alone for treating *P. falciparum*. In line with other studies in other parts of the world, there is a significant relationship between *P. falciparum* resistance to chloroquine and the emergence of K76T mutation in the *PfCRT* gene in Iran. *P. falciparum* resistance to SP is also significantly associated with the development of mutations in the *Pfdhfr* and *Pfdhps* genes.

To date, there have been no reports of decreasing susceptibility in *P. falciparum* to ACTs, including artesunate, in Iran. Monotherapy is not recommended to prevent resistance to antimalarial drugs in *P. falciparum*.

An alternative approach is to use several compounds at the same time as used against other infectious diseases such as tuberculosis and AIDS. Molecules of several drugs used to fight malaria parasites with different functional mechanisms and different half-lives may reduce the likelihood of parasite survival. In this case, if the malaria parasite is less sensitive or semi-sensitive to a drug, it may be killed by the accompanying drug and resistance to the original drug may be delayed.

Chloroquine-resistant *P. vivax* has not been reported in Iran and this drug is the first-line treatment for *P. vivax* malaria. Reports of chloroquine-resistant *P. vivax* in Pakistan and other parts of Asia emphasize that ongoing studies should monitor susceptibility of *P. vivax* to chloroquine in Iran. There are several reports of relapse due to *P. vivax* hypnozoites in Iran, but so far no cases have been reported of their resistance to primaquine.

**Acknowledgements**

We would like to thank Prof. Edrissian for providing valuable scientific information on malaria in Iran and are grateful to Dr. Ahmad Raeisi for valuable guidance and editing of the article. This review did not have financial source.

**Conflict of interest**

The authors declare that there is no conflict of interest.

**References**

1. Myers-Hansen JL, Abuaku B, Oyebola MK, et al. Assessment of antimalarial drug resistant markers in asymptomatic *Plasmodium falciparum* infections after 4 years of indoor residual spraying in Northern Ghana. *PLoS One*. 2020;15(12):e0233478.

2. Edrissian, GH. Malaria in Iran: Past and present situation. Iran J Parasitol. 2006; 1(1): 1-14.

3. Slater I, Betson M, Ashraf S, et al. Current methods for the detection of antimalarial drug resistance in *Plasmodium* parasites infecting humans. *Acta Trop*. 2021;216:105828.

4. WHO. World malaria report 2020: 20 years of global progress and challenges. World Health Organization. 2020.

5. Baird JK. Evidence and implications of mortality associated with acute *Plasmodium vivax* malaria. *Clin Microbiol Rev*. 2013;26(1):36-57.

6. Ehtesham R, Fazaeli A, Raeesi A, et al. Detection of mixed-species infections of *Plasmodium falciparum* and *Plasmodium vivax* by nested PCR and rapid diagnostic tests in southeastern Iran. *Am J Trop Med Hyg*. 2015;93(1):181-5.

7. Raeesi A, Gouya MM, Nadim A, et al. Determination of malaria epidemiological status in Iran's malarious areas as baseline information for implementation of malaria elimination program in Iran. *Iran J Public Health*. 2013;42(3):326-333.

8. Vatandoost H, Raeesi A, Saghatipour A, et al. Malaria situation in Iran: 2002–2017. *Malar J*. 2019;18(1):200.

9. Heidari A, Keshavarz H, Rokni MB, et al. Genetic diversity in merozoite surface protein (MSP)-1 and MSP-2 genes of *Plasmodium falciparum* in a major endemic region of Iran. *Korean J Parasitol*. 2007;45(1):59-63.

10. Miahipour A, Keshavarz H, Heidari A, et al. Genetic variation of MSP-1 gene in *Plasmodium vivax* isolated from patients in Hormozgan Province, Iran using SSCP-PCR. *Iran J Parasitol*. 2012;7(4):1-7.

11. Mardani A, Keshavarz H, Heidari A, et al. Genetic diversity and natural selection at the domain I of apical membrane antigen-1 (AMA-1)
of *Plasmodium falciparum* in isolates from Iran. Exp Parasitol. 2012;130(4):456-62.

12. Heidari A, Keshavarz H, Hajjaran H, et al. Genetic variation and selection of domain I of the *Plasmodium vivax* apical membrane antigen-1 (AMA-1) gene in clinical isolates from Iran. Iran J Parasitol. 2013;8(4):536-544.

13. Moin Vaziri V, Heidari A, Farokhi Z, et al. PCR-RFLP analysis of *Plasmodium vivax* reticulocyte binding protein2c gene in field isolates of Iran. Trop Biomed. 2017;34(3):533-9.

14. Abolghazi A, Heidari A, Moin Vaziri V, et al. Genetic diversity in C-terminal of SERA5 gene in the blood stage of human isolates of *Plasmodium vivax* in Sistan and Baluchistan, Iran. Iran J Parasitol. 2018;13(3):440-447.

15. Gebreyohannes EA, Bhagwathula AS, Seid MA, et al. Anti-malarial treatment outcomes in Ethiopia: a systematic review and meta-analysis. Malar J. 2017;16(1):269.

16. Abdel Hameed AA. Antimalarial drug resistance in the Eastern Mediterranean Region. East Mediterr Health J. 2003;9(4):492-508.

17. Thu AM, Phyoo AP, Landier J, et al. Combating multidrug-resistant *Plasmodium falciparum* malaria. FEBS J. 2017;284(16):2569-78.

18. WHO. Guidelines for the treatment of malaria. World Health Organization;2015.

19. WHO. Guide Lines for the treatment of malaria. World Health Organization;2006.

20. Erah PO, Arienmughare G, Okhamafe AO. *Plasmodium falciparum* malaria resistance to chloroquine in five communities in Southern Nigeria. Afr J Biotechnol. 2003;2(10):384-9.

21. Achan J, Talisuna AO, Erhart A, et al. Quinine, an old anti-malarial drug in a modern world: role in the treatment of malaria. Malar J. 2011;10:144.

22. Slater AF. Chloroquine: mechanism of drug action and resistance in *Plasmodium falciparum*. Pharmac Ther. 1993;57(2-3):203-35.

23. Tse EG, Korsik M, Todd MH. The past, present and future of anti-malarial medicines. Malar J. 2019;18(1):93.

24. Lei ZN, Wu ZX, Dong S, et al. Chloroquine and Hydroxychloroquine in the Treatment of Malaria and Repurposing in Treating COVID-19. Pharmacol Ther. 2020;216:107672.

25. Mosawi SH, Dalami A, Safi N, et al. An unlabelled probe based real time PCR and modified semi nested PCR as molecular tools for analysis of chloroquine resistant in *Plasmodium vivax* isolates from Afghanistan. Malar J. 2020;19(1):253.

26. Wicht KJ, Mok S, Fidock DA. Molecular mechanisms of drug resistance in *Plasmodium falciparum* malaria. Anna Rev Microbiol. 2020;74:431-54.

27. Vander Jagt DL, Hunsaker LA, Campos NM. Characterization of a hemoglobin-degrading, low molecular weight protease from *Plasmodium falciparum*. Mol Biochem Parasitol. 1986;18(3):389-400.

28. Durand R, Jafari S, Vauzelle J, et al. Analysis of pfcr pt mutations and chloroquine susceptibility in isolates of *Plasmodium falciparum*. Mol Biochem Parasitol. 2001;114(1):95-102.

29. Summers RL, Martin RE. Functional characteristics of the malaria parasite’s chloroquine resistance transporter: implications for chemotherapy. Virulence. 2010;1(4):304-8.

30. Rohrbach P, Sanchez CP, Hayton K, et al. Genetic linkage of pfmdr1 with food vacuolar solute import in *Plasmodium falciparum*. EMBO J. 2006;25(13):3000-11.

31. Piccir S, Olliaro P, de Monbrison F, et al. A systematic review and meta-analysis of evidence for correlation between molecular markers of parasite resistance and treatment outcome in *falciparum* malaria. Malar J. 2009;8:89.

32. Zaim M. Malaria control in Iran—present and future. J Am Mosq Control Assoc. 1987;3(3):392-6.

33. Manouchehri AV, Motabar M, Alemo-hammad A. Assessment of the response of *Plasmodium falciparum* to chloroquine in southern Iran. Iran J Public Health. 1973;2(2):97-102.

34. Suroso T, Hamidi AN, Manouchehri AV. The activity of chloroquine against *Plasmodium falciparum* in Bandar Abbas, Southern Iran, 1976. Bull Soc Pathol Exot Filiales. 1978;71(2):164-71.

35. Edrssian GH, Afshar A, Kanani A, et al. The response of *Plasmodium falciparum* to chloroquine and mefloquine in Bandar-Abbas and Minab areas, Hormozgan Province, southern Iran. J Trop Med Hyg. 1989;92(2):75-9.

36. Racei A, Ringwald P, Safa O, et al. Monitoring of the therapeutic efficacy of chloroquine for the treatment of uncomplicated, *Plasmodium falcipa-
nous malaria in Iran. Ann Trop Med Parasitol. 2006;100(1):11-6
38. Jafari S, Le Bras J, Asmar M, et al. Molecular survey of Plasmodium falciparum resistance in south-eastern Iran. Ann Trop Med Parasitol. 2003;97(2):119-24.
39. Zakeri S, Afsharpad M, Kazemzadeh T, et al. Association of pfcrT but not pfmdr1 alleles with chloroquine resistance in Iranian isolates of Plasmodium falciparum. Am J Trop Med Hyg. 2008;78(4):633-40.
40. Esmaeili RA, Nateghpour M, ASMAR M, et al. Detection of K76T mutation in pfcrT gene as an applicable genetic marker for prediction of chloroquine resistant falciparum malaria in isolates from an endemic district of Iran. Iran J Parasitol. 2008;3(2):48-56.
41. Zakeri S, Afsharpad M, Raeisi A, et al. Prevalence of mutations associated with antimalarial drugs in Plasmodium falciparum isolates prior to the introduction of sulphadoxine-pyrimethamine as first-line treatment in Iran. Malar J. 2007;6:148.
42. Jalousian F, Dalimi A, Samiee SM, et al. Mutation in Pfmdr1 gene in chloroquine-resistant Plasmodium falciparum isolates, Southeast Iran. Int J Infect Dis. 2008;12(6):630-4.
43. Edrissian G, Afshar A, Sayedzadeh A, et al. Assessment of the response in vivo and in vitro of Plasmodium falciparum to sulphadoxine-pyrimethamine in the malarious areas of Iran. J Trop Med Hyg. 1993;96(4):237-40.
44. Esfandarian AA, Keshavarz H, Basco LK, et al. Do mutations in Plasmodium falciparum dihydropterotate synthase and dihydrofolate reductase confer resistance to sulphadoxine-pyrimethamine in Iran?. Trans R Soc Trop Med Hyg. 2002;96(1):96-8.
45. Heidari A, Dittrich S, Jelínek T, et al. Genotypes and in vivo resistance of Plasmodium falciparum isolates in an endemic region of Iran. Parasitol Res. 2007;100(3):589-92.
46. Zakeri S, Gil JP. High prevalence of double Plasmodium falciparum dhfr mutation at codons 108 and 59 in the Sistan–Baluchistan province, Iran. J Infect Dis. 2003;187(11):1828–1829.
47. Sharifi-Sarasiabi K, Haghighi A, Kazemi B, et al. Molecular surveillance of Plasmodium vivax and Plasmodium falciparum DHFR mutations in isolates from southern Iran. Rev Inst Med Trop Sao Paulo. 2016;58:16.
48. Afsharpad M, Zakeri S, Pirahmadi S, et al. Molecular monitoring of Plasmodium falciparum resistance to antimalarial drugs after adoption of sulphadoxine–pyrimethamine plus artemunate as the first line treatment in Iran. Acta trop. 2012;121(1):13-8.
49. Saebi E, Masoumi Asl H, Salehi M, et al. national malaria treatment guideline (Persian). Ministry of Health and Medical Education. 5th Edition 2020.
50. Suresh N, Haladar K. Mechanisms of artemisinin resistance in Plasmodium falciparum malaria. Curr Opin Pharmacol. 2018;42:46-54.
51. Noedl H, Se Y, Schaecher K, et al. Evidence of artemisinin-resistant malaria in western Cambodia. N Engl J Med. 2008;359(24):2619-20.
52. Duru V, Witkowski B, Menard D. Plasmodium falciparum resistance to artemisinin derivatives and piperaquine: a major challenge for malaria elimination in Cambodia. Am J Trop Med Hyg. 2016;95(6):1228-38.
53. Amato R, Pearson RD, Almagro-Garcia J, et al. Origins of the current outbreak of multidrug-resistant malaria in southeast Asia: a retrospective genetic study. Lancet Infect Dis. 2018;18(3):337-45.
54. Mbengue A, Bhattacharjee S, Pandharkar T, et al. A molecular mechanism of artemisinin resistance in Plasmodium falciparum malaria. Nature. 2015;520(7549):683-7.
55. Moghadm HA, Nateghpour M, Raeisi A, et al. Monitoring the Response of Plasmodium vivax to Chloroquine and Uncomplicated P. falciparum to Artesunate-fansidar Antimalarials in Southeastern Iran. Iran J Parasitol. 2018;13(1):31-38.
56. Battle KE, Lucas TC, Nguyen M, et al. Mapping the global endemicity and clinical burden of Plasmodium vivax. 2000–17: a spatial and temporal modelling study. Lancet. 2019;394(10195):332-43.
57. Rieckmann KH, Davis DR, Hutton DC. Plasmodium vivax resistance to chloroquine? Lancet. 1989;2(8765):1183-4.
58. Heidari A, Keshavarz H, Shojaee S, et al. In vivo susceptibility of Plasmodium vivax to chloroquine in Southeastern Iran. Iran J Parasitol. 2012;7(2):8-14.
59. Nateghpour M, Sayedzadeh SA, Edrissian GhH, et al. Evaluation of sensitivity of Plasmodium vivax to chloroquine. Iran J Public Health. 2007;36(3):60-3.

Available at: http://ijpa.tums.ac.ir
60. Hamedi Y, Nateghpour M, Tan-Ariya P, et al. *Plasmodium vivax* malaria in Southeast Iran in 1999-2001: establishing the response to chloroquine in vitro and in vivo. Southeast Asian J Trop Med Public Health. 2002;33(3):512-8.

61. Edrissian GhH, Nateghpour M, Afshar A, et al. Monitoring the response of *Plasmodium falciparum* and *Plasmodium vivax* to antimalarial drugs in the malarious areas in south-east Iran. Arch Iran Med. 1999;2(2):61-6.

62. Waheed AA, Ghanchi NK, Rehman KA, et al. *Vivax* malaria and chloroquine resistance: a neglected disease as an emerging threat. Malar J. 2015;14:146.

63. Ferreira MU, de Sousa TN, Rangel GW, et al. Monitoring *Plasmodium vivax* resistance to antimalarials: Persisting challenges and future directions. Int J Parasitol Drugs Drug Resist. 2021;15:9-24.

64. Maghsoodloorad S, Haghighi A, Sarasiabi KS. Genetic diversity of dihydropteroate synthetase gene (dhps) of *Plasmodium vivax* in Hormozgan province, Iran. Iran J parasitol. 2016;11(1):98-103.

65. Zaman J, Shahbazi A, Asgharzadeh M. *Plasmodium vivax* dhfr mutations among isolates from malarious areas of Iran. Korean J Parasitol. 2011;49(2):125-131.

66. Parsaei M, Raesi A, Spotin A. Molecular evaluation of pvdhfr and pvmdr-1 mutants in *Plasmodium vivax* isolates after treatment with sulfadoxine/pyrimethamine and chloroquine in Iran during 2001–2016. Infect Genet Evol. 2018;64:70-5.

67. Daher A, Aljayyoussi G, Pereira D, et al. Pharmacokinetics/pharmacodynamics of chloroquine and artemisinin-based combination therapy with primaquine. Malar J. 2019;18(1):325.

68. Miahipour A, Keshavarz H, Heidari A, et al. Assessment of the efficacy of 8 weeks of primaquine for the prevention of relapse in *vivax* malaria patients using SSCP-PCR and sequencing in south and south-east Iran, 2008–2011. Trans R Soc Trop Med Hyg. 2013;107(7):420-6.

69. Heidari A, Sheikhi S, Fallah P, et al. Relapse of a *Plasmodium vivax* infection in an Iranian patient: A case report. Jundishapur J Nat Pharm Prod. 2017;12(4):e14499.