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Short Communication

Genetic Diversity of Dihydropteroate synthetase Gene (*dhps*) of *Plasmodium vivax* in Hormozgan Province, Iran

***Somayeh MAGHSOODLOORAD [1], Ali HAGHIGHI [2], Khojasteh SHARIFI SARASIABI [3], Niloofar TAGHIPOOR [2], Nahid HOSSEINZADEH [2], Latif GACHKAR [4], Ehsan NAZEMALHOSSEINI MOJARRAD [5], Elham MAGHSOODLOORAD [6]**

1. *Dept. of Parasitology and Mycology, School of Medicine, Golestan University of Medical Sciences, Gorgan, Iran*
2. *Dept. of Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
3. *Paramedical Faculty, Hormozgan University of Medical Sciences, Bandar Abbas, Iran*
4. *Tropical and Infectious Diseases Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
5. *Research Center for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
6. *Sayyad Shirazi Hospital, Golestan University of Medical Sciences, Gorgan, Iran*

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***Correspondence Email:**
smagsoodloorad@yahoo.com

Abstract

Background: The present study was formulated in order to determine polymorphism of dihydropteroate synthetase gene *(dhps)* of *Plasmodium vivax (P. vivax*) in Hormozgan Province, southern Iran and mutations at codons 382, 383, 512, 553, and 585 associated with resistance of *P. vivax* to sulfadoxine.

Method: One-hundred eighteen isolates of *P. vivax* were prepared within 2007-2008 to determine dihydrofolate reductase-thymidylate synthase (*dhfr-ts*) gene. The isolates were determined in the study of genetic diversity of dihydropteroate synthetase gene (*dhps*) of *P. vivax*. The study was performed via PCR test and nucleotide sequencing.

Results: Of 118 blood samples infected by P. vivax, 46 and 72 samples belonged to Minab and Jask, respectively. No mutation was detected at 5 target codons. However, among these 118 samples, three isolates (2.54%) were found to have a mutation at the new codon 421.

Conclusion: Since mutation was detected in dihydrofolate reductase *(Pvdhfr)* gene in the same samples but no mutation was found at five main codons of *Pvdhps* gene, it can be concluded that *P. vivax,* considering their mutations *in Pvdhfr,* is still susceptible to sulfadoxine and therefore, to fansidar in Hormozgan Province, Southern Iran.

Introduction

Plasmodium vivax is claimed to infect around 100 million people every year threatening 40% of the world's population (1). *Plasmodium falciparum* and *P. vivax* are among the most important parasites transited by arthropods, and distributed all around the world (2). Because of prosperous execution of half-a-century preventive and controlling programs, no malaria transition has been reported in Iran except for some limited regions mostly located in the southeast (3). *P. vivax* is the predominant malaria parasite in Iran and Hormozgan is considered one of the three endemic provinces of Iran in terms of infection by malaria (3, 4).

Researches on antimalarial drugs have been majorly focused on *P. falciparum*; however, fast-developing resistance to chloroquine in *P. vivax* has convinced authors to pay more attention to the necessity of replacing antimalarial drugs in *vivax* malaria (5). In general, it seems that drug resistance occurs due to mutation in a specific point in one or more nucleotide(s) leading to reduced susceptibility to some drugs or a class of drugs (6). Determination of susceptibility of *P. vivax* to antimalarial drugs is cumbersome, if not impossible, through lab tests (7, 8). Molecular genetic markers of resistance are beneficial to monitor the development and spread of anti-malarial drug resistance.

It is necessary to gain knowledge about the mechanisms of drug action and resistance in order to eradicate malaria (9). Pyrimethamine and sulfadoxine are among the most widely used antimalarial drugs that effect on folate synthesis pathway (10). By interruption in folate synthesis pathway caused by antifolates, glycine-to-serine change as well as reduced methionine synthesis decrease leading to plunge in thymidylate and DNA replication (10,11). Resistance to antimalarial drugs has been recognized for P. falciparum and *P. vivax*, which naturally infest humans. It is noteworthy that P. falciparium has an increasing resistance to approximately all antimalarial drugs used extensively at the present (12). Resistance to fansidar occurs through a mutation in a specific point in dihydrofolate reductase (Pvdhfr) and dihydropteroate synthetase *(dhps)* genes that result in resistance to pyrimethamine and sulfadoxine, respectively.

Five codons 382, 383, 512, 553, and 585 in dihydropteroate synthetase (*dhps*) gene of *P. vivax* (*Pvdhps*) are associated with resistance of the parasite to sulfadoxine. Mutation in the gene and mutation outbreak in the codons 383 and 553 leads to higher resistance levels (13, 14).

The present study aimed at determination of existence of the codons related to resistance in dihydropteroate synthetase gene in *P. vivax* samples in Hormozgan Province, southern Iran.

Materials and Methods

One-hundred eighteen positive sample of malaria were collected from patients suffered from malaria complications, from health centers of Minab and Jask from 2007 to 2008 (15, 16). The samples were kept at -20°C in Department of Parasitology and Mycology of Shahid Beheshti University of Medical Sciences, Tehran, Iran. Dihydropteroate synthetase gene positive blood samples of *P. vivax* were taken in EDTA-containing tubes was determined in those samples.

DNG plus kit (Sinagene Corp.) was adopted for extraction of DNA from *P. vivax*. According to the protocol proposed by the corporation, 100 µl blood sample and 700 µl DNG solution were mixed and DNA was extracted. DNA estimation was performed via ultraviolet spectroscopy and electrophoresis gel.

For PCR, a primer pair called VDHPS-OF and VDHPS-OR with sequences of (5'- ATTCCAGAGTATAAGCACAG-CACATTTGAG-3') and (5'- CTAAGGTT-GATGTATCCTTGTGAGCACATC-3') was

used in order to replicate the section 1354bp of pyruvate kinase dihydrofolate synthase *P. vivax pppk-dhps*, respectively by using an available gene sequence in GenBank: AY186730. A 705-nucleotide section was replicated by use of a pair of primers for Nested-PCR called VDHPS-NF and VDHPS-NR with sequences of (5'-AATGGCAAGTGATGGGGCGAGCGTGATTGA-3') and (5'- CAGTCTGCACTCCCCGATGGCCGCGCCACC-3'), respectively.

PCR was performed by means of PreMix kit (INTRON Biotechnology; Cat.No: 25026, 96) in 30 cycles at annealing temperatures of 58 ˚C and 65 ˚C for external and internal sections, respectively. Bands of the replicated section after PCR were observed on electrophoresis gel 1.5% by trans-illuminator through ethidium bromide staining and electrophoresis on agarosegel. The nested PCR products of *pvdhps* from the samples were subjected to direct sequencing in both directions by a DNA-sequencer in Digestive Disease Research Institute and compared with the samples available in Gen Bank in order to determine possible mutations.

Results

Of 118 samples infested by *P. vivax* in Minab and Jask in Hormozgan Province, 46 and 72 samples belonged to Minab and Jask, respectively. Seventy-nine patients (67%) were male. The average age was 22 yr and the subjects were mostly Iranians (91.5%). Nested-PCR products of nine samples were shown in (Fig. 1) with 705 nucleotides. All isolates showed identical bands to the ones in (Fig. 1).

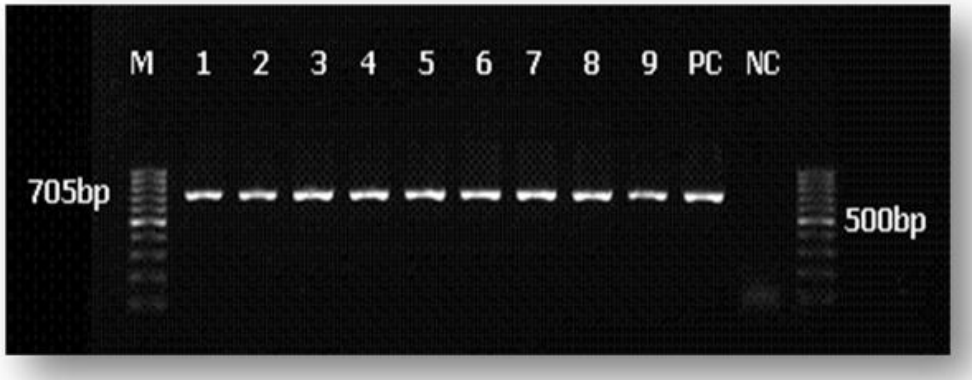

Fig. 1: Electrophoresis image of bp705 section of *Pvdhps* gene with nine isolates of *P. vivax* in Hormozgan Province
M: 100bp DNA Ladder Numbers: The isolated numbers PC: Positive Control NC: Negative Control

PCR products derived from all samples and 705-nucleotide sections were sequenced. No mutations were detected by PCR and sequencing in the samples. However, among 118 samples, three isolates (2.54%) had an as-yet-unreported mutation in the new codon 421 (Table 1). The isolates were on the same position and had only one mutation. A difference in codon 421 was first detected in the present study between the sequence of three isolates obtained from *P. vivax* and nucleotide sequence of the gens in GenBank (Fig. 2). Sequence of this codon was in the non-mutant isolate GTG where mutant turned into GAG. Because of this mutation, valine is turned into glutamic acid. Sequence of Minab and Jask samples were registered in GenBank bearing no. AB609599 and AB609600, respectively.

1411 aatggcaagtgatggggcgagcgtgattgacatcgggggggaatcgtccgcccttatgtggtccccaatccgagcgtcactgaacgggatttggtca-
tgcctgttttgaagctctttaaggaggagtggcataagttggagtgtgaggttggcggtggtgccgagtgctgcgctgcagcaagtgatgccaggaaaaacgcgcag-
agctccctacaggggaaactacaaaaagtgagggacgcaaaaccgatcataagcatcgacacggtcaattatgatctcttcaaggagtgcgtggaaggcgagttgg-
tggacatcctaaacgatatcagcgcgtgcacgcacaacccagagattataaaattgttgaggaggaaaaacaagttctatagcgtcgttttaatgcacaagagggg-
aaatccacacaccatggataagttaacaaattacgatgaccttataagtgacattaaaaggtatttagaagatcggctacattttctcgttctaaatggggtaccacgct-
accgagttctctttgatgtcggcctggggtttgccaaaaagcacgaccagtctattaagctgttgcagcatattcacgtttacgatgagtacccgctgtttcttggcta-
ctcgaggaagcgctttattgtcca
2101ctgcatggggaagggtggcgcggccatcggggagtgcagactg

Fig. 2: Schematic representation and nucleotide sequences of 705 bp product of the semi-nested PCR of the *Pvdhps* gene. The primer's VDHPS-NF and VDHPS-NR annealing sites were underlined.Bold and underlined letters indicate Codon 421 where mutation occurred

Table 1: Characteristics of isolates of mutant in the codon 421 from Hormozgan Province in dihydropteroate synthetase gene

Isolate code	Nationality	Patient's hometown	Gender	Age (yr)	History of trip to Afghanistan or Pakistan
ShH(PV)17M-IR*	Iranian	Minab	Male	9	No
ShH(PV)54J-IR	Iranian	Jask	Male	20	No
ShH(PV)70J-IR	Iranian	Around Jask	Female	14	No

* Sh:Sharifi, H:Haghighi, Pv:*Plasmodium vivax*, M:Minab, J:Jask, Numbers: The isolated numbers, IR: Iran

Discussion

Resistance to chloroquine in *P. vivax* in some malaria-prone areas of the world has necessitated replacing antimalarial drugs in the case of *vivax* malaria (6). Drug resistance to chloroquine in *P. falciparum* was reported many years ago in Iran and therefore, *Anopheles* mosquitos have transmitted resistant parasites to all malaria-prone regions (17). Despite the fact that sulfadoxine is not used in Iran for treating *vivax* malaria(11), detection of mutation in dihydropteroate synthetase gene in *P. vivax* in Chabahar and regions around it indicates that *P. vivax* is by any way exposed to sulfadoxine in southeast of Iran(unpublished data).

In the present study, no mutation was detected in 118 isolates of *P. vivax* in isolates acquired from Minab and Jask; however, three mutant isolates in the new codon 421 were seen in *Pvdhps* gene, which may account for onset of mutation in this gene and possibly known codons in this region. Rudimentary investigations in 1959 pointed to inherent resistance of *P. vivax* to fansidar (18). At that time, with advent of chloroquine-resistant strains of P. falciparum, antifolate drugs such as proguanil, cycloguanil, and pyrimethamine were extensively adopted as a replacing drug for chlroquine and even as a malaria-preventing drug in regions where both P. falciparum and *P. vivax* existed. Nevertheless, several cases of failure in treatment of and/or prevention from *vivax* malaria have been reported. This convinced many malariologists that the parasite is inherently resistant to antifolate drugs (18). However, further investigations proved the opposite (19). Authors have considered resistance of *P. vivax* to fansidar to be caused by inherent resistance of the parasite to this drug whereas the mechanisms underlying the resistance include point mutations in *Pvdhfr* and *Pvdhps* genes (6, 11, 20, 21). Sulfadoxine-pyrimethamine (known as fansidar) is not commonly prescribed to treat infections caused by *P. vivax* while treatment with fansidar might be considered because the infection usually occurs concurrently with *P. falciparum* and is difficult to diagnose it very well (20-22).

In the present study, a limited polymorphism in *Pvdhps* gene was found that was in contrast to earlier investigations in PNG (23) and Thailand (24).The wild-type *Pvdhps* is present at high proportion in *P. vivax* parasite populations from Hormozgan Province, which was similar to that acquired from malaria endemic regions in Iran and Pakistan (25, 26).

Although no relationship was seen between mutations in five main codons and resistance to sulfadoxine in the studied areas, it can be claimed that resistance of *P. vivax* to fansidar in Hormozgan may be occurring because mutation at new codon 421 was detected and isolates with double-mutations in *Pvdhfr* gene were found at codons 58 and 117 in these isolates in Hormozgan Province (16) leading to resistance to pyrimethamine.

Conclusion

In spite of a high level of possibility of resistance to pyrimethamine in south of Iran, fansidar may still be adopted owing to concurrent use with sulfadoxine which leads to its higher effect, unless resistance to sulfadoxine continues to lead its ever-increasing trend.

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