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**Original Article**

# DNA Sequence Polymorphism of the Lactate Dehydrogenase Genefrom Iranian *Plasmodium vivax* and *Plasmodium falciparum* Isolates

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***Abstract***

***Background:*** Parasite lactate dehydrogenase (*p*LDH) is extensively employed as malaria rapid diagnostic tests (RDTs). Moreover, it is a well-known drug target candidate. However, the genetic diversity of this gene might influence performance of RDT kits and its drug target candidacy. This study aimed to determine polymorphism of *p*LDH gene from Iranian isolates of *P. vivax* and *P. falciparum.*

***Methods:*** Genomic DNA was extracted from whole blood of microscopically confirmed *P. vivax* and *P. falciparum* infected patients. *p*LDH gene of *P. falciparum* and *P. vivax* was amplified using conventional PCR from 43 symptomatic malaria patients from Sistan and Baluchistan Province, Southeast Iran from 2012 to 2013.

***Results:*** Sequence analysis of 15 *P. vivax* LDH showed fourteen had 100% identity with *P. vivax* Sal-1 and Belem strains. Two nucleotide substitutions were detected with only one resulted in amino acid change. Analysis of *P. falciparum* LDH sequences showed six of the seven sequences had 100% homology with *P. falciparum* 3D7 and Mzr-1. Moreover, *Pf*LDH displayed three nucleotide changes that resulted in changing only one amino acid. *Pv*LDH and *Pf*LDH showed 75%-76% nucleotide and 90.4%-90.76% amino acid homology.

***Conclusion:*** *p*LDH gene from Iranian *P. falciparum* and *P. vivax* isolates displayed 98.8-100% homology with 1-3 nucleotide substitutions. This indicated this gene was relatively conserved. Additional studies can be done weather this genetic variation can influence the performance of *p*LDH based RDTs or not.

## Introduction

Malaria is one of the most important infectious diseases in the world. Although malaria is preventable and curable, it still causes high morbidity and mortality (1). According to the recent 2013 WHO report, globally an estimated of 3.4 billon people are at risk of malaria. In this report, WHO estimated 207 million malaria cases and 627,000 deaths occurred globally in 2012 (2). The majority of the global burden of human malaria is caused by *Plasmodium falciparum* and *P. vivax* (3). *P. falciparum* is the most deadly *Plasmodium* species responsible for about 90% of malaria deaths, mainly in Africa (4) and *P. vivax* is the most cause of malaria infection in the world (1). *P. vivax* is accountable for 25–40% of the annual bouts of malaria worldwide (4). In Iran, 2,714,648 individuals (4% of the total population) mainly living in southern provinces namely Sistan and Baluchistan, Kerman and Hormozgan are at risk of malaria (5). *P. vivax* is the most prevalent species reported among the malaria patients in Iran annually (6). However, a considerable decrease of malaria cases has been reported within the past few years in Iran. Since malaria elimination program has commence from a few years ago in the country (7), for steady continuation of the program rapid and accurate diagnosis of malaria parasites play an important role in opportune case finding and treatment which result in on time control and elimination of the infection.

Conventional microscopic examination of Giemsa stained thick and thin blood smears has been accepted as golden standard method for malaria diagnosis up to now. Although malaria microscopy contains some advantages including cost, availability and relative sensitivity (8-10), it bears some disadvantages such as time consuming and labor intensity (9). The WHO has recently reiterated "the urgent need for simple and cost-effective diagnostic tests for malaria to overcome the deficiencies of light microscopy" and clinical diagnosis (10, 11). Based on WHO advise rapid diagnostic tests can be replaced with microscopic method in remote and isolated areas particularly when trained and skilled personnel is not available (12-14). Utilizing parasite lactate dehydrogenase (*p*LDH) in $RDT_s$ has shown better sensitivity for diagnosing low level of parasitemia in comparison with other malaria proteins. Moreover, the amount of *p*LDH indicates to metabolically presence of *P. vivax* due to short stability of *p*LDH in the body (13). *p*LDH plays role of a coenzyme due to involving the oxidation of lactate to pyruvate with nicotinamide adenine dinucleotide (NAD) (15). Inhibition of the malarial LDH enzyme prevents the production of ATP and results to death of the *Plasmodium* parasites (13); it becomes an attractive drug target candidate (16).

The genetic diversity of *p*LDH might influence its drug target candidacy and the sensitivity of RDT kits. As far as we know, until now the genetic variation of *p*LDH gene in *P. vivax* and *P. falciparum* infections were not reported in Iran. This study aimed to detect the polymorphism of *p*LDH gene from Iranian strains of *P. vivax* and *P. falciparum*. Obviously, understanding such polymorphism is important for designing or improving RDT kits. It can also give information about the molecular details of *P. falciparum* LDH (*Pf*LDH) and *P. vivax* LDH (*Pv*LDH) genes for designing a new drug.

## Materials and Methods

Totally 43 whole blood samples were collected from *P. vivax* and *P. falciparum* infected patients in Sistan and Baluchestan Province located in southeast of Iran from 2012 to 2013. Sistan and Baluchestan Province is bordered with Afghanistan and Pakistan to the east and Oman Sea in south. It has hot and dry weather with about 65mms rainfall annually. Thirty-

three samples for *P. vivax* and 10 samples for *P. falciparum* were confirmed positive by light microscopic examination of Giemsa stained thick and thin blood smears. One ml of blood was collected into tubes containing EDTA anticoagulant, placed immediately at -20 °C for further analysis.

This study was approved by Tehran University of Medical Sciences Ethical Committee.

### Genomic DNA extraction

DNA was extracted from 200 µl of whole blood samples of 33 *P. vivax* and 10 *P. falciparum* malaria infected patients using, ACCUPreP® kit, Genomic DNA extraction kit (BIONEER, Seoul, Korea) based on the manufacturer instructions. *Pv*LDH and *Pf*LDH genes were amplified and sequenced to analyze the genetic variations.

### PCR amplification

Nucleotide sequences corresponding to *Pv*LDH and *Pf*LDH genes were amplified using the following sets of primers using conventional PCR. *Pv*LDH gene amplification was conducted using: Forward: 5'-ATGACGCCGAAACCCAAAAT-3' and Reverse: 5'-ACCTTTAAATGAGCGCCTTCAT-3', on the other hand *Pf*LDH gene was also amplified by F: 5'-AGATGGCACCAAAAGCAAAAAT-3' and R: 5'-ACCTTTAAGCTAATGCCTTCAT-3'.*Pv*LDH primers were designed based on *P. vivax* Sal-1 (XM_001615570.1) and *P. vivax* Belem (DQ060151.1) strains from GenBank whereas *Pf*LDH primers designed based on the reference sequence *P. falciparum* 3D7 (XM_001349953.1) strain in GenBank. DNA was extracted from whole blood of a healthy person living in non-endemic area as a negative control for using in amplification process.

PCR reaction was performed in 25µl reaction volumes containing 1µlof each forward and reverse primers (10 pmol), 10 µl of ready to use master mix (Ampliqon, Denmark) contains (Tris-HCl pH 8.5, 1.5mM $MgCl_2$, dNTPs and TaqDNA polymerase), 3 µl of genomic DNA samples and 10 µl distilled water.

PCR cycle parameters for *Pv*LDH gene amplification were as follows: 5minutes initial denaturation at 95 °C followed by 30 cycles with 30 s at 95 °C, 30" at 56 °C, 1´ at 72 °C and final extension at 72 °C for 5 min. All the PCR parameters were the same for *Pf*LDH gene amplification except the annealing temperature was 58 °C. The PCR products of *Pv*LDH and *Pf*LDH were loaded on 1% agarose gel. The gel contained SimplySafe (EURx, Poland) for DNA staining. UV transilluminator was used to visualize the stained DNA. The fragment sizes of PCR products were determined using 1kb DNA ladder marker (Solis BioDyne, Estonia).

### DNA sequencing

Twenty-two sequences including 15 *P. vivax* and 7 *P. falciparum* were analyzed to investigate polymorphism in *Pv*LDH and *Pf*LDH genes respectively. These genes were sequenced by applied biosystems 3730/3730xL DNA analyzers, (Bioneer, Seoul, Korea) using Sanger method. Nucleotide sequences of *Pv*LDH and *Pf*LDH were aligned and compared using Clustal W2 software (EMBL-EBI,http://www.ebi.ac.uk/Tools/msa/clustalw2/).*Pv*LDH gene sequences were compared with GenBank sequences of *P. vivax* Belem (DQ060151.1) and *P. vivax* SaI-1 (XM_001615570.1). On the other hand, *Pf*LDH gene sequences were compared with *P. falciparum* 3D7 (XM_001349953.1) and *P. falciparum* Mzr-1 (JN547219.1). Moreover, amino acid sequences related to each samples of *P. vivax* and *P. faliparum* were derived using ExPASy translate tool (http://web.expasy.org/tran-slate/). *Pv*LDH amino acid sequences were compared with *P. vivax* SaI-1 (XM_001615570.1) and *P. vivax* Belem (DQ060151.1) whereas *Pf*LDH amino acid sequences were compared with *P. falciparum* 3D7 (XM_001349953.1) and *P. falciparum* Mzr-1 (JN547219.1) strains registered in GenBank. Finally phylogenic tree was prepared to illustrate the distance among sequences of isolates using average distance (AD) method in Clustal W2 Jalview software

(http://www.eb-i.ac.uk/).Thelactate dehydrogenase gene from Iranian *Plasmodium* strains were submitted with the accession numbers of (KM226649-KM226654 and KM226656-KM226664) for *P. vivax*, and (KM226665-KM226671) for *P. falciparum* in GenBank (BLAST).

## Results

A 955 bp band was observed in gel electrophoresis of PCR products of *Pf*LDH and *Pv*LDH amplified genes (Fig. 1 and Fig. 2).

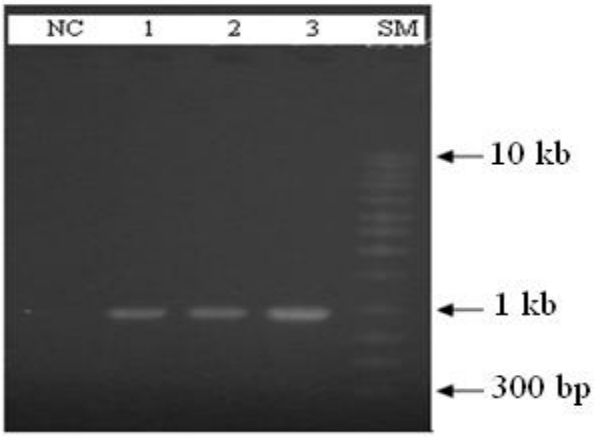


**Fig. 1:** Gel electrophoresis of *Pv*LDH gene PCR products. NC: Negative Control, Lane 1-3 PCR product samples, SM Size Marker (1kb)

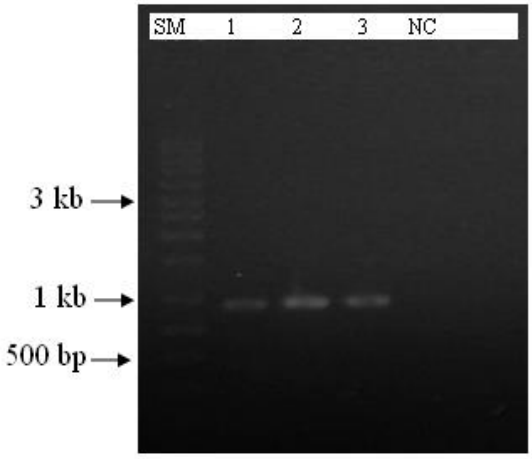


**Fig. 2:** Gel electrophoresis of PCR product samples of *Pf*LDH gene. SM: Size Marker (1kb), NC: Negative control, Lane 1-4 *Pf*LDH PCR products. Lane 3 did not show amplified gene

### *Pv*LDH genetic variation in Iranian isolates of *P. vivax*

The amplified *Pv*LDH gene was yielded approximately 955 base pairs, coding for 316 amino acids. Fifteen of the amplified genes were sequenced to analyze the genetic variation of *Pv*LDH gene using Clustal W2 software. After comparing the sequences with the chromatogram with *P. vivax* Sal-1 reference sequence, two Single nucleotide substitution were detected at 666, 899 positions from G to C and C to T respectively (Fig. 3).

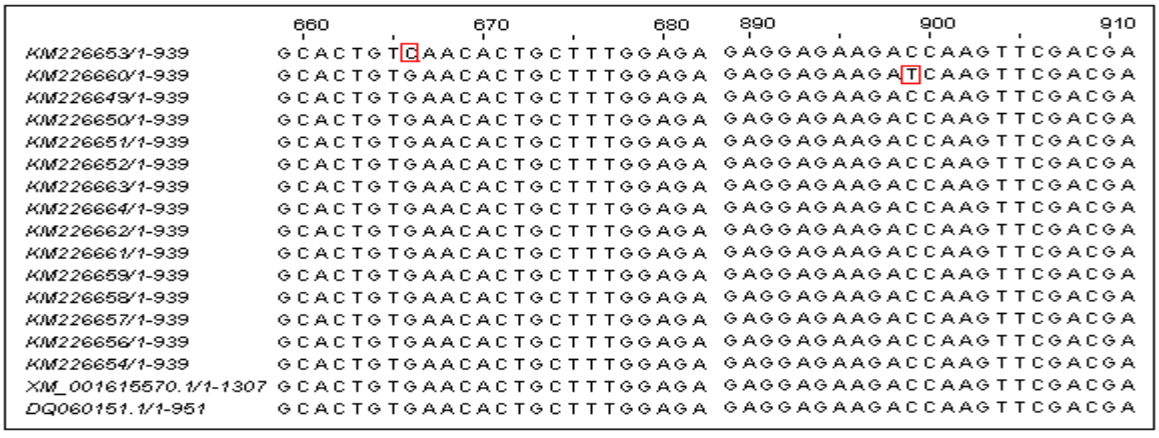


**Fig. 3:** Single-nucleotide substitution of *p*LDH gene among 15 *P. vivax* Iranian isolates, *P. vivax* Sal-1 (XM_001615570.1) and *P. vivax* Belem (DQ060151.1)

The nucleotide homology among Iranian isolates of *P. vivax* was 99.8-100%. Thirteen of the 15 isolates displayed 100% nucleotide sequence homology with *P. vivax* SaI-1 (XM_001615570.1) and *P. vivax* Belem (DQ060151.1) (Table1 & Fig. 4).

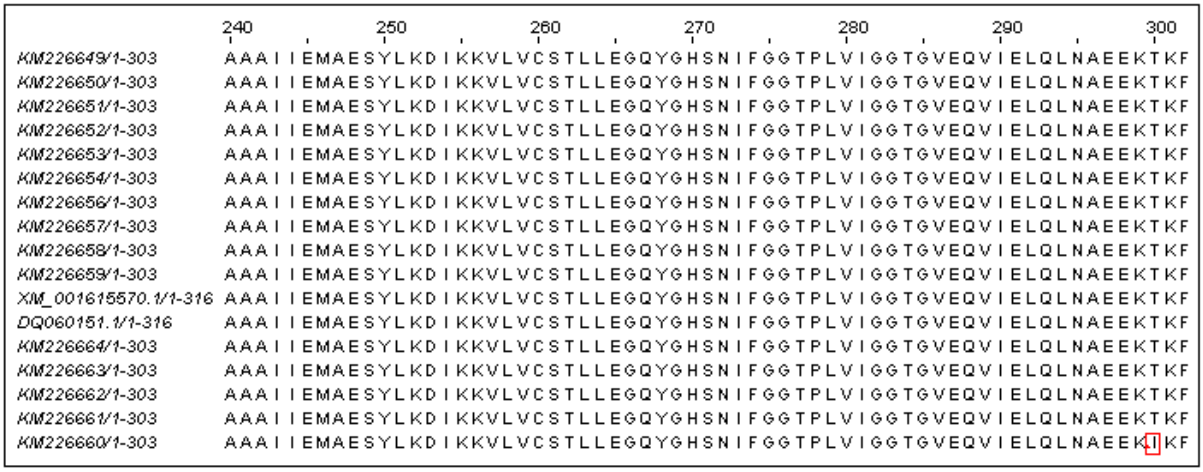


**Fig. 4:** Amino acid alignment of *Pv*LDH among Iranian *P. vivax* isolates (KM226649-KM226654) and (KM226656-KM226664), *P. vivax* Sal-1 and Belem (DQ060151.1)

**Table 1:** Comparing *Pv*LDH nucleotide sequences of 15 Iranian isolates of *P. vivax*, *P. vivax* Sal-1 (XM_001615570.1) and Belem (DQ06015)

| No. | Acc. No. | *Plasmodium spp.* | Isolates code | Nucleotide length (bp) | Homology rate (100%) | |
|---|---|---|---|---|---|---|
| | | | | | ***Pv*Sal-1 (XM_001615570.1)** | ***Pv*Belem (DQ060151.1)** |
| 1 | KM226649 | *P. vivax* | IB098 | 939 | 100 | 100 |
| 2 | KM226650 | *P. vivax* | IB099 | 939 | 100 | 100 |
| 3 | KM226651 | *P. vivax* | IB101 | 939 | 100 | 100 |
| 4 | KM226652 | *P. vivax* | IB105 | 939 | 100 | 100 |
| 5 | KM226653 | *P. vivax* | IB112 | 939 | 99.89 | 99.89 |
| 6 | KM226654 | *P. vivax* | IB115 | 939 | 100 | 100 |
| 7 | KM226656 | *P. vivax* | IB006 | 939 | 100 | 100 |
| 8 | KM226657 | *P. vivax* | IB063 | 939 | 100 | 100 |
| 9 | KM226658 | *P. vivax* | IB064 | 939 | 100 | 100 |
| 10 | KM226659 | *P. vivax* | IB067 | 939 | 100 | 100 |
| 11 | KM226660 | *P. vivax* | IB078 | 939 | 99.89 | 99.89 |
| 12 | KM226661 | *P. vivax* | IB081 | 939 | 100 | 100 |
| 13 | KM226663 | *P. vivax* | IB089 | 939 | 100 | 100 |
| 14 | KM226664 | *P. vivax* | IB090 | 939 | 100 | 100 |
| 15 | KM226662 | *P. vivax* | IB086 | 939 | 100 | 100 |

The nucleotide substitution at 899 positions from C to T was brought an amino acid change from (T, neutral polar amino acid to me, non-polar amino acid) whereas the nucleotide substitution at 666 positions from G to C did not result any change in amino acid (Fig. 5). Fourteen Iranian isolates had 100% amino acid sequences with *P. vivax*SaI-1and*P. vivax* Belem (Table 2).

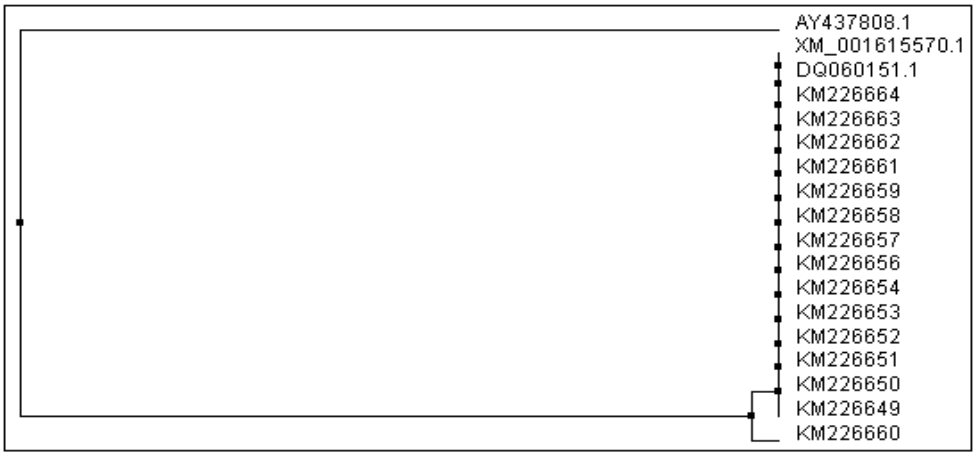


**Fig. 5:** Amino acid sequence difference in *p*LDH genes from Iranian *P. vivax* isolates (KM226649-KM226654) and (KM226656-KM226664), *P. vivax* Sal-1 (XM_001615570.10 and *P. vivax* Belem (DQ060151.1) using average distance (AD) tree from Clustal W2 Jalview software. *Plasmodium berghei* (AY437808.1) was used as an out group

**Table 2**: Comparing amino acid sequences of *Pv*LDH from Iranian isolates of *P. vivax*, *P. vivax* Sal-1(XM_001615570.1) and Belem (DQ060151)

| No | Acc. No. | *Plasmodium* spp. | Isolates code | Nucleotide length (bp) | Homology rate (100%) | |
|---|---|---|---|---|---|---|
| | | | | | ***Pv*Sal-1 (XM_001615570.1)** | ***Pv*Belem (DQ060151.1)** |
| 1 | KM226649 | *P. vivax* | IB098 | 939 | 100 | 100 |
| 2 | KM226650 | *P. vivax* | IB099 | 939 | 100 | 100 |
| 3 | KM226651 | *P. vivax* | IB101 | 939 | 100 | 100 |
| 4 | KM226652 | *P. vivax* | IB105 | 939 | 100 | 100 |
| 5 | KM226653 | *P. vivax* | IB112 | 939 | 100 | 100 |
| 6 | KM226654 | *P. vivax* | IB115 | 939 | 100 | 100 |
| 7 | KM226656 | *P. vivax* | IB006 | 939 | 100 | 100 |
| 8 | KM226657 | *P. vivax* | IB063 | 939 | 100 | 100 |
| 9 | KM226658 | *P. vivax* | IB064 | 939 | 100 | 100 |
| 10 | KM226659 | *P. vivax* | IB067 | 939 | 100 | 100 |
| 11 | KM226660 | *P. vivax* | IB078 | 939 | 99.68 | 99.68 |
| 12 | KM226661 | *P. vivax* | IB081 | 939 | 100 | 100 |
| 13 | KM226663 | *P. vivax* | IB089 | 939 | 100 | 100 |
| 14 | KM226664 | *P. vivax* | IB090 | 939 | 100 | 100 |
| 15 | KM226662 | *P. vivax* | IB086 | 939 | 100 | 100 |

### *Pf*LDH genetic variation in Iranian isolates of *P. falciparum*

DNA was extracted from 10 *P. falciparum* confirmed whole blood samples and *PfLDH* gene was amplified using specific primers. Seven of the 10 amplified genes were sequenced and analyzed. DNA sequences of *Pf*LDH gene displayed three nucleotide substitutions at 36, 814 and 891positions from A to G, G to A and G to A respectively (Fig. 6).

The homology among *Pf*LDH nucleotide sequences from Iranian isolates of *P. falciparum* were 99.67-100%. Five of the 7 isolates had 100% nucleotide homology with *P. falciparum* 3D7 (XM_001349953.1) and *P. falciparum* Mzr-1 (JN547219.1) strains submitted in GenBank (Table 3).

Only one of the nucleotide changes at 814 positions from G to A was brought an amino acid change from aspartic acid (D, acidic polar amino acid to N, neutral polar amino acid) (Fig. 7).

The rest six isolates showed 100% amino acid homology with*Pf*Mzr-1 and *Pf*3D7 strains from GenBank (Table 4).

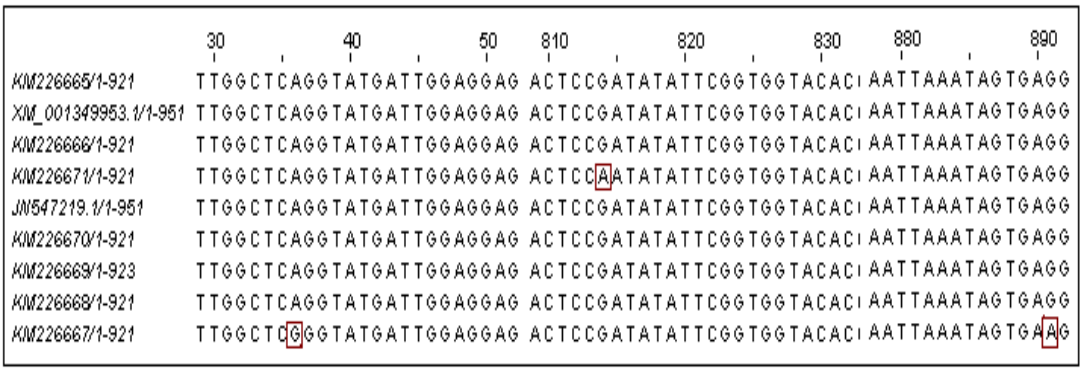


**Fig. 6:** Nucleotide substitutions of *p*LDH gene among Iranian isolates of *P. falciparum,P. falciparum* 3D7 (XM_001349953.1) and *P. falciparum* isolates Mzr-1(JN547219.1)

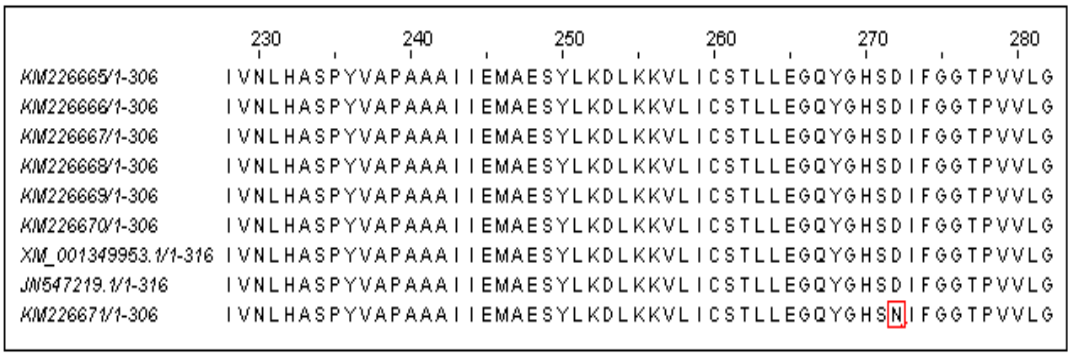


**Fig. 7:** Amino acid sequence alignment of *Pf*LDH among *P. falciparum* isolates, *P. falciparum* 3D7 (XM_001349953.1) and *P. falciparum* Mzr-1 (JN547219.1)

**Table 3:** Comparing *Pf*LDH nucleotide sequences of seven *P. falciparum* isolates with *P. falciparum* (XM_001349953.1) and *P. vivax* (JN547219.1)

| No | Acc. No. | *Plasmodium* spp. | Isolates code | Nucleotide length (bp) | Homology rate (100%) | |
|---|---|---|---|---|---|---|
| | | | | | ***Pf*3D7(XM_001 349953.1)** | ***Pf*Mzr-1(JN547219.1)** |
| 1 | KM226665 | *P. falciparum* | IF097 | 921 | 100 | 100 |
| 2 | KM226666 | *P. falciparum* | IF095 | 921 | 100 | 100 |
| 3 | KM226667 | *P. falciparum* | IF102 | 921 | 99.78 | 99.78 |
| 4 | KM226668 | *P. falciparum* | IF104 | 921 | 100 | 100 |
| 5 | KM226669 | *P. falciparum* | IF106 | 921 | 100 | 100 |
| 6 | KM226670 | *P. falciparum* | IF108 | 921 | 100 | 100 |
| 7 | KM226671 | *P. falciparum* | IF077 | 921 | 99.89 | 99.89 |

Acc. No.: Accession number, IF: Iran-Baluchistan *falciparum*

**Table 4**: Comparing *Pf*LDH amino acid sequences of Iranian *P. falciparum* isolates,*Pf*3D7 (XM_001349953.1) and *Pf*Mzr-1(JN547219.1)

| No | Acc. No. | *Plasmodium* spp. | Isolates code | Nucleotide length (bp) | Homology rate (100%) | |
|---|---|---|---|---|---|---|
| | | | | | ***Pf*3D7(XM_001349953.1)** | ***Pf*Mzr-1(JN547219.1)** |
| 1 | KM226665 | *P. falciparum* | IF097 | 921 | 100 | 100 |
| 2 | KM226666 | *P. falciparum* | IF095 | 921 | 100 | 100 |
| 3 | KM226667 | *P. falciparum* | IF102 | 921 | 100 | 100 |
| 4 | KM226668 | *P. falciparum* | IF104 | 921 | 100 | 100 |
| 5 | KM226669 | *P. falciparum* | IF106 | 921 | 100 | 100 |
| 6 | KM226670 | *P. falciparum* | IF108 | 921 | 100 | 100 |
| 7 | KM226671 | *P. falciparum* | IF077 | 921 | 99.69 | 99.69 |

Acc. No.: Accession number, IF: Iran-Baluchistan *falciparum*

### *Pv*LDH and *Pf* LDH homology from Iranian isolates of *P. falciparum* and *P. vivax*

The nucleotide homology between *Pv*LDH and *Pf*LDH in Iranian isolates of *P. vivax* and *P. falciparum* was 75.8-76%. All *P. vivax* LDH nucleotide sequences had 75.79% homology with six of *P. falciparum* isolates. The amino acids sequence homology between *PvLDH* and *PfLDH* Iranian isolates were 90.4% exception of one isolate which had 90.76% homology. Generally, the amino acids sequence homology between *Pv*LDH and *Pf*LDH Iranian isolates were more than 90%. *Pv*LDH from Iranian isolates were also displayed 90.4 % homology with 3D7 and Mrz-1 *P. falciparum* isolates from gene bank.

## Discussion

*p*LDH antigen is assumed to be a specific marker for the presence of viable *Plasmodium* in blood, and is used for screening in malaria-endemic countries (17). Inhibition of the malarial LDH enzyme prevents the producing ATP and causes death of the *Plasmodium* parasites,(13) so it becomes an attractive drug target candidate (16). The gene has the least diversity among *Plasmodium* spp. Therefore, the protein obtained from this gene can be used in any diagnostic test (18). Diversity in the *p*LDH gene may influence specificity and sensitivity of RDTs in any malaria endemic area. Investigation of polymorphism in *P. vivax* and *P. falciparum* lactate dehydrogenase gene can lead to produce more specific and sensitive RDTs kit.

The nucleotide homology among 15 *Pv*LDH sequences of *P. vivax* was 100% with the exception of two isolates displayed 99.9% homology (Table1, 2 & Fig. 5). In China, 100% *Pv*LDH nucleotide sequence homology was reported among Chinese *P. vivax*, Sal-1 and Belem (19). However, our finding displayed two nucleotide substitutions. Another study done in China reported 99.89% nucleotide identity of Chinese isolates with Belem strain (20). This point out Iranian *Pv*LDH nucleotide sequences had more homology with Belem strain than Chinese isolates.

Talmanet al. reported *Pv*LDH genes from Chinese *P. vivax* Anhui isolates had more than 99% sequence homology compared with strains in Gene bank (21). This outcome strongly agreed with findings from our study, which also showed more than 99% homology with all compared *P. vivax* strains registered in GenBank.

In the present study, *Pv*LDH gene sequences showed two nucleotide substitutions with one resulted an amino acid change from T, neutral polar amino acid to I, non-polar amino acids. This substitution might not influence the sensitivity of *Pv*LDH based RDTs. Antigen variability is unlikely to explain variability in implementation of RDTs detecting pLDH in *P. falciparum*, *P. vivax* cases (22). In contrast to our finding, Shin et al. in Korea reported one

SNP which did not bring any change in amino acid (23) Jianget al. in China also reported a single nucleotide difference at the position 666 between *Pv*LDH gene and *P. vivax* Belem (DQ060151)(24). The position of a nucleotide change in Jianget al. report was the same with one of the nucleotide substitutions detected in our study. *Pv*LDH genes from Iranian isolates of *P. vivax* were displayed more nucleotide changes than Korean and Chinese isolates.

In earlier Chinese studies from Jianghuai region and Anhui isolates of *P. vivax*, there were no nucleotide changes among isolates (21, 25). Compared to these reports the nucleotide changes among *Pv*LDH from Iranian isolates of *P. vivax* was higher than both Jianghuai region and Anhui isolates of *P. vivax*.

Talmanet al. reported four different type of DNA sequence of *P. vivax* from 10 isolates; the mutations were synonymous (22). In our study, less number of nucleotide changes was seen and the mutations were not synonymous. Fourteen isolates had the same amino acid sequences with *P. vivax* SaI-1 (XM_001615570.1) and *P. vivax* Belem (DQ060151.1). This finding was agreed with a study conducted in China, which reported 100% *Pv*LDH gene homology among Chinese isolates, *P. vivax* Sal-I and Belem (19). Studies done in Korea and China from Korean and Hainan isolates respectively, also reported 100% amino acid homology with *P. vivax* Belem (DQ060151.1) (20, 23). This indicated RDTs produced from Korean and Chinese isolates can be used in Iran.

On the other hand, *Pf*LDH homology among Iranian strains of *P. falciparum* was 100% with the exception of two isolates. In contrast to our finding, Talman et al. reported no variability among all sequences *P. falciparum* (n = 49) in worldwide isolates of *Plasmodium* spp (22). This indicated *Pf*LDH gene from Iranian isolates of *P. falciparum* had more nucleotide variation. Five of the seven isolates had 100% nucleotide identity with *P. falciparum* 3D7 (XM_001349953.1) and *P. falciparum* Mzr-1 (JN547219.1) strains registered in GenBank. Iranian *Pf*LDH genes and reference sequence (*Pf*3D7) had high homology about 99.9%-100% (Table 1, 2 & Fig. 8).

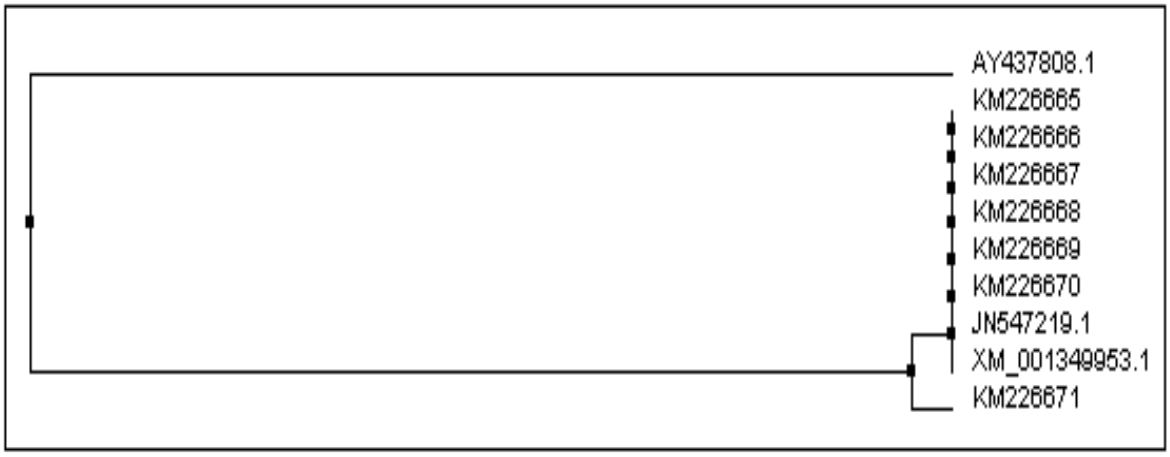


**Fig. 8:** Amino acid sequence difference in *p*LDH genes from *P. falciparum* Iranian isolates, *P. falciparum* 3D7 (XM_001349953.1) and *P. falciparum* Mzr-1 (JN47219.1) using average distance (AD) tree. *Plasmodium berghei* (AY437808.1) was used as an out-group

This indicated *Pf*LDH gene is relatively conserved and can be a good target for anti-malarial drug and producing RDT. In our study, six of the 7 isolates had the same amino

acid sequence. These amino acid sequences also had 100% homology with *P. falciparum* Mzr-1 and *P. falciparum* 3D7 strains from GenBank. Our finding was supported by the study conducted in Indonesia, which reported 100% amino acid sequences between Indonesian *Pf*LDH and *Pf* 3D7 reference sequence (26).

In this study, two isolates displayed three nucleotide substitutions at 36, 814 and 891 positions. However, only the substitution at 891 positions from G to A was brought an amino acid change from aspartic acid to asparagine (D, acidic polar amino acid to N, neutral polar amino acid). In Madagascar, two SNPs at 73 and 814 positions among the 137 DNA sequences of *P. falciparum* isolates were displayed. Both single nucleotide polymorphisms (SNPs) in Madagascar study brought amino acid changes. The nucleotide change in 10 isolates at 814 position resulted in an amino acid change (D, acidic polar amino acid to N, neutral polar amino acid). In addition, another amino acid change (at codon 25: Q, neutral polar amino acid to A, basic polar amino acid) was seen due to the SNP at 73 position (18). The position of nucleotide change (814bp) and the resulted amino acid change (aspartic acid (D) to asparagine (N) in one of the isolates in our study was the same with the Madagascar study. The nucleotide change at 814 positions in our study might be a single nucleotide polymorphism given the Madagascar study SNP report at the same position. Iranian *Pf*LDH demonstrated less number of amino acid changes in comparison with the report that released from Madagascar study. The nucleotide sequences homology between Iranian isolates of *Pv*LDH and *Pf*LDH were 75.79-76%. In China, Jiang et al. was reported 75.1% homology between *Pv*LDH and *Pf*LDH nucleotide sequences (24). Akbulut et al. also reported 74.8% homology between *P*vLDH and *Pf*LDH (27). Compared to Jiang et al. and Akubulut et al. report, the homology of Iranian *Pv*LDH and *Pf*LDH was high. In our study, the amino acid sequences homology among Iranian isolates of *Pv*LDH and *PfLDH* was 90.4% with the exception of one isolate. Shin et al. and Turgut-Balik et al. reported 89.5% and 90.2% amino acid sequence homology between *Pv*LDH and *Pf*LDH respectively (23, 28). This indicated the amino acid homology between *Pv*LDH and *Pf*LDH genes from Iranian isolates of *P. vivax* and *P. falciparum* were higher than previously reports. Generally, in our study the amino acid homology between *Pv*LDH and *Pf*LDH was more than 90%. This was supported by Turgut-Balik et al. report (28).

## Conclusion

*p*LDH gene from Iranian *P. falciparum* and *P.vivax* isolates displayed 98.8-100% homology with 1-3 nucleotide substitutions. This relatively stability indicated *Pv*LDH and *Pf*LDH genes can be a good antimalaria target and used for producing RDT kits. The amino acid sequence homology of *Pv*LDH and *Pf*LDH was more than 90%. This indicated some techniques like drug discovery, vaccine development and other activities, which were applied on P. falciparum, could also be tried for *P. vivax*. The homology among *p*LDH of *P. vivax* and *P. falciparum* should be further investigated with large enough sample size. In general, before using *p*LDH for producing RDT kits the genetic variation of this gene should be investigated since its polymorphism varies with geographical locations.

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