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## Original Article

# Situation of Asymptomatic Malaria among Iranian Native and Afghan and Pakistani Immigrants in a Malarious Area under the National Malaria Elimination Program of Iran

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*Received* 14 May 2020
*Accepted* 25 Jul 2020

***Keywords:***
Asymptomatic malaria;
Malaria elimination;
Immigrants;
Iran

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***Abstract***

***Background:*** This study was designed to detect, if there are asymptomatic malaria infections amongst native and immigrant population from Afghanistan and Pakistan countries in Sistan & Baluchistan Province of Iran, where is under the national malaria elimination program.

***Methods:*** This cross-sectional study was performed among native individuals and resident immigrants in the southeastern province of Sistan & Baluchistan from May 2016 to Jul 2017. A total of 271 individuals were considered in this cross- sectional study based on microscopical method, Rapid Diagnostic Tests (RDTs) and PCR techniques. Out of 271 native and immigrant participants 140 (52%) and 131 (48%) were male and female, respectively.

***Results:*** None of the prepared samples was diagnosed as malaria positive case when was considered via above mentioned three techniques.

***Conclusion:*** Neither native nor immigrant individuals had asymptomatic malaria, hinting that national malaria elimination program is performed according to planned schedule in the studied areas

## Introduction

Malaria, a protozoan parasitic disease which is prevalent in many areas of the world especially in tropical and subtropical regions. The disease is caused by some species of plasmodium genus and is

transmitted to human by some species of anopheles mosquitoes (1).

Malaria infection causes numerous health and socio-economic problems particularly among the rural people. Asymptomatic malaria looks like to be a big problem especially in view of transmission that is assumed as an actual source of malaria infection. There are increasing proofs that asymptomatic malaria infection carriers play a critical role in sustaining transmission (2).

In fact, in asymptomatic malaria infection, seemingly healthy individuals can carry sexual and asexual forms of the parasite that are important agents for transmission. Since, such individuals does not receive any treatment for elimination of the parasites they remain, more or less, for long term an unknown transmission routes of the disease, particularly in low and seasonal transmission areas (3).

About 100 countries including 40% of the world population are under the risk of malaria. According to the WHO annual report in 2018, an estimated 228 million cases of malaria infection and 405,000 deaths have been officially reported from malarious areas (4). Iran is one of the malaria endemic countries in the middle- east region. The highest prevalence of malaria infection including *vivax* malaria, *falciparum* malaria and mixed malaria infection have been reported from Sistan & Baluchistan province during the past decade (5).

Despite particular efforts for control of malaria in recent decades the infection still is a major public health problem in malarious areas of the world.

Immigrant population from Afghanistan and Pakistan countries can increase the risk of out breaking malaria in borderline areas, particularly some of the immigrants have been infected with asymptomatic malaria. According to results that were released by Nateghpour and colleagues, 6% of Afghanistan immigrants in Iranshahr district were infected with asymptomatic malaria infection (6).

Death is one of deleterious consequence of malaria infection among some infected individuals in some malaria endemic areas. Since there are long borderlines between Iran and Afghanistan and Pakistan countries with high level of malaria transmission, potential outbreak still is anticipated in southeastern Iran (7), particularly among those populations that some of them carrying asymptomatic malaria. In fact, in asymptomatic malaria the cycle of transmission remains active due to presence of gametocytes in the blood. Detecting malaria positive cases within suspected individuals can result in treating them and consequently preventing transmission of the parasite among the population, particularly in those malaria endemic countries like Iran that are being exposed to the national malaria elimination program.

We aimed to detect, are there asymptomatic malaria infections amongst native and immigrant population from Afghanistan and Pakistan countries in Sistan & Baluchistan Province of Iran.

## Materials and Methods

### *Studied areas*

All four studied areas (including Iranshahr, Sarbaz, Khash and Chabahar districts) are located at the south of Sistan & Baluchistan province in the southeastern Iran (Fig. 1). The weather in Iranshahr and Sarbaz districts is warm and dry in summer with maximum temperature of 50 ºC and usually temperate and dry in winter with minimum temperature of 10 ºC. Khash district has mountainous weather with maximum and minimum temperatures of 40 and below zero in summer and winter respectively. Port of chabahar has warm and humid weather in summer with maximum temperature of 40 ºC and relatively humid and temperate in winter with minimum temperature of 15 ºC .

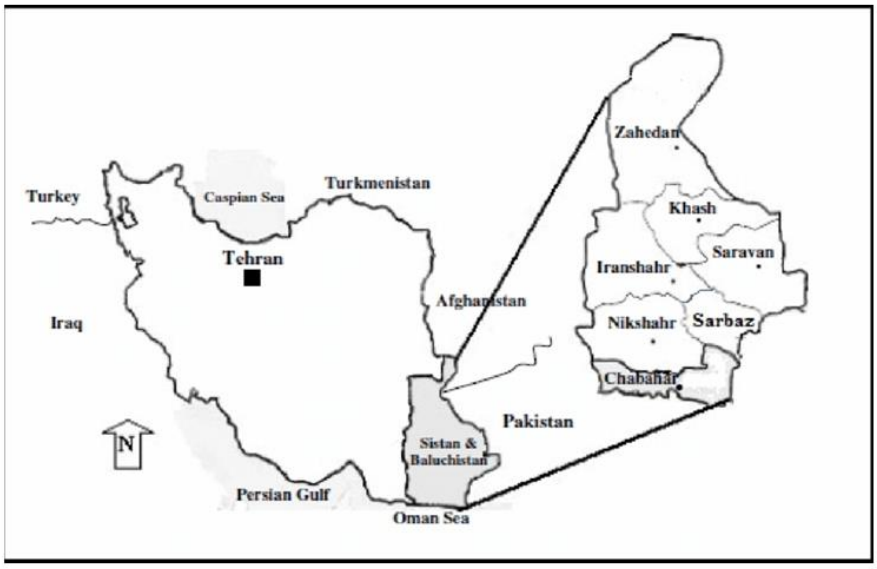


**Fig. 1:** Location of the studied districts, Iran

### *Case enrollment*

This cross-sectional study was performed among native individuals and resident immigrants in the southeastern province of Sistan & Baluchistan from May 2016 to Jul 2017. A total of 271individuals including 175 native and 96 Afghani and Pakistani migrant residents were enrolled for this study. Residence of individuals at the studied districts assumed the only criterion for enrollment. Sample size was calculated and randomly collected based on results of previous study that was released (6). Since this study was not an interventional study previous to conducting relevant tests a formal consent and full bio data (name, age, sex, occupation, nationality, history of malaria, history of traveling to malaria areas inside or outside of the country during the past 12 months, history of receiving antimalarial drugs) were obtained from all of the registered people. Then, the people were evaluated for the presence or absence of clinical symptoms. The studied areas were selected according to the positive cases of the past three years in those areas.

### *Blood sampling and tests process*

For each sample, three different tests including microscopy, Rapid Diagnostic Test (RDTs) and PCR were applied. The procedure was as follow: from each individual who participated in this study, two slides including thick and thin blood smears were prepared via finger prick. Smears were fixed with methanol and stained with Giemsa solution according to the WHO standard guideline (8). Then the stained slides were examined by two trained microscopists. RDT was performed using SD Rapid test (Lot: 05DDE008B- Korea) based on the WHO guideline with some modifications (9). Briefly, about 5 µl of the blood was deposited into the test well of RDT kit and then two drops of special buffer solution were added into the well. After 15-20 min, results were interpreted ordinarily according to the manufacture's manual. There are three lines to illustrate results of the test. The first line shows control situation of the test and the second and third lines indicate positive either pan (non- *falciparum*) or *falciparum* forms. In the situation of pan, two lines including control and the second line (parasite lactate dehydrogenase) are marked, but in *falciparum* form besides the two lines, the third line (Pf histidine rich protein II) is also marked.

To determine the certainty of microscopy and RDT results in this study a semi-nested PCR assay was employed for all of the collected samples. For this purpose several drops of whole blood were collected on DNA Banking Card (Takapoozist Company, lot: 05DDE008B) and kept in dry and cool atmosphere to use for further investigation and PCR tests. The method was design as extracting of DNA and applying a semi-nested PCR. The procedure briefly was as follow: DNA of the parasite was extracted using tissue DNA extraction kit (Iran, ref: A101211) based on manufactures' instructors, the product was used as a template to amplify the gene region related to ssrDNA with specific primers as was described previously (10). The gene related to small subunit ribosomal DNA amplified in primary round and the genus *Plasmodium* is appeared with a band of 787 bp, while in the secondary amplification, the *Plasmodium* species was determined. Those primers used in this study included: two primers UNR-PLF with the following nucleotide sequences for the primary amplification of the band 787 bp indicates the presence of *Plasmodium* in the sample, and tree primers VIR, PLF, FAR with nucleotide sequences below for the secondary amplification of the band 400bp indicating the presence of *P. falciparum* and the band 500bp is the result of activity of two primers VLR, PLF indicating the infection with *P. vivax*.

UNR(Reverse): GACGGTATCTGATCGTCTTC

PLF(Forward): AGTGTGTATCAATCGAGTTTC

FAR(Reverse): AGTTCCCCTAGAATAGTTACA

VIR(Reverse): AGGACTTCCAAGCCGAAGC

All amplifications were conducted in a volume of 25 containing 10 $\mu l$ of Taq DNA polymerase Master Mix RED (Ampliqon cat: 180301 countaining 150 mM Tris–HCl (pH 8.5, 40 mM $(NH4)_2SO4$, 0.2 % Tween 20), 3 mM $MgCl_2$, 02% tween 20, 0.4 mM dNTPs, 0.05 U of Taq polymerase) and 1 $\mu l$ of each primer (10 pmol ). 3 µL of extracted DNA in the first round and 3 µL of the first PCR product used as a template in the second nested-PCR and added distilled water up to 25 µL . The annealing temperature for all reactions was 48 °C and 35 times cycles were repeated for the both first and second nested-PCR. The PCR product from the First and second nested PCR was electrophoresed on agarose gel and the resulting bands were observed under UV light, according to the weight of the resulting bands, the genus and specie of the parasites were determined (Fig. 2) .

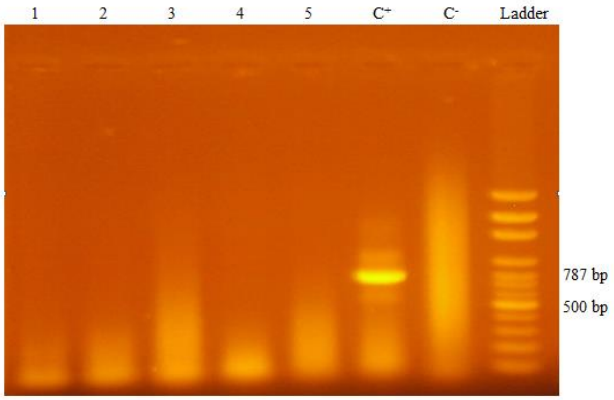


**Fig. 2:** A pattern of gel electrophoresis analysis for all of the studied samples. C+: positive control for genus detection, C-: negative control, No.1-5: samples which were negative as malaria case using Semi-nested PCR methods

All processes were conducted in National Malaria Laboratory, School of Public Health, Tehran University of Medical Sciences.

### Ethical approval

The study was approved by Ethical Research Committee of TUMS with register number of IR.TUMS.VCR.REC.1396.2455.

## Results

Out of 271 participants who were enrolled in this study 140 (52%) and 131 (48%) were male and female respectively. Ages of participated individual were recorded as 5 months to 100 yr old. 175 (64%) of cases were indigenous and 96 (36%) were immigrants from Afghanistan and Pakistan (Table 1).

About 542 thin and thick blood smears were prepared from 271 subjects in this study. None of them was diagnosed as malaria positive case using microscopical, RDTs, and Semi-nested PCR methods.

According to the bio data, all of the considered cases were resident in the studied areas for at least 12 months.

**Table 1:** Age distribution of the studied participants

| *Age distribution (yr)* | *Natives* | *Immigrants* | *Total* |
|---|---|---|---|
| 0-5 | 34 | 14 | 48 |
| 6-10 | 33 | 14 | 47 |
| 11-20 | 33 | 25 | 58 |
| 21-30 | 32 | 27 | 59 |
| > 30 | 43 | 16 | 59 |
| Total | 175 | 96 | 271 |

## Discussion

Malaria is still a serious concern for global health in malarious areas in the world including Iran. Elimination of malaria is one of the goals of the WHO and Ministry of Health & Medical Education (MOHME) in Iran. National malaria elimination program started since 2009 in Iran (CDMC, unpublished). Screening early diagnosis and timely treatment are essential components of malaria elimination. Thus, most important challenge in Iran is the detection of asymptomatic malaria cases. The asymptomatic malaria infection among the infected individuals is not detectable due to the low level of parasitemia and lack of clinical sings. Therefore, the infection remains untreated and may act as a reservoir for survival of the disease in the region (11).

Due to the role of asymptomatic malaria in malaria elimination program the main aim of this study was to monitor the presence and frequency of asymptomatic malaria infection in the border areas of Sistan & Baluchistan province, an endemic area of malaria in Iran. In order to increase the accuracy and validity of results, the microscopical, RDTs and molecular methods were simultaneously conducted. In this study semi nested-PCR was used as a sensitive and specific molecular technique for the detection of asymptomatic malaria with low parasite density.

Asymptomatic malaria infection can be happened due to frequent exposure of individuals to malaria parasite, which leads to enhance effective humeral immune response. As a result, the parasite is present but symptoms may not be observed (12). In this cross-sectional study, none of the studied cases was detected as malaria positive case. Consequently, it can be stated that national malaria elimination program in the studied areas is successfully in progress.

Several studies in the field of asymptomatic malaria have been conducted in endemic areas

of Iran in recent years. Turki et al in a cross-sectional study that was conducted in Bashagard district (Hormozgan Province) obtained a similar results to our findings. Native residents were examined with microscopical, molecular and serological methods in the study. Although serological method detected presence of antimalarial antibodies in the sera of six cases, results of microscopic and molecular surveys indicated that there was not any asymptomatic malaria among the studied cases. (13).

In another study that was conducted by Fernando et al in a malaria endemic region of Sri Lanka based on the same methods used in this study, similar results were obtained (14).

In a study conducted in Iranshahr district of Sistan & Baluchistan Province (Iran), under the national elimination programme, based on thick and thin Giemsa-stained blood smears and nested-PCR analysis no parasite was seen indicating the absence of asymptomatic malaria among the studied subjects (11).

On the other hand, some studies showed different results than above-mentioned result. For example, in Iranshahr district, 1.6% and 0.6% of Afghani immigrants and Iranian native were detected positive using microscopic method (6). Such differences may depend on either the years or subjects of studies.

In a comparative study based on microscopical and multiplex semi-nested PCR methods 68 malaria positive and suspected patients were enrolled and considered in Sistan & Baluchistan and Hormozgan provinces of Iran. Results showed that some of suspected patients could be accounted as asymptomatic malaria (15).

In a mixed microscopic and molecular study on 200 randomly enrolled native individuals in a malaria endemic district of Hormozgan Province in Iran, none of them showed malaria infection using microscopical method, but 3 (1.5%)of them were detected to have asymptomatic *vivax* malaria via Nested-PCR technique (16). Considering asymptomatic malaria in malaria areas depends on some elements such as immunity system of subjects, frequent infection of individuals, rate of parasitemia, age, endemicity of malaria in the malarious areas and even species and strain of the parasites. In Cubido a high endemic malaria region of Colombia Osorio and colleagues could not found any trace of malaria antibody among the children with age of 9 or lower when they employed serological technique, but worku et al could detect 26 (8.6%) out of 385 asymptomatic *falciparum* and *vivax* malaria cases among school children in northwestern Ethiopia (17, 18).

## Conclusion

Neither considered native nor did immigrant individuals have asymptomatic malaria, hinting that national malaria elimination program is performed according to planned schedule in the studied areas. Indeed, more studies at regular intervals are needed to achieve the trustful and satisfactory results.

## Acknowledgements

The authors would like to thank Mrs. Fatemeh Asgari for her useful collaboration with the authors. This study financially was supported by School of Public Health, Tehran University of Medical Sciences

## Conflict of interest

The authors declare that there is no conflict of interest.

## References

1. Warrel DA, Gilles HM. Essential Malariology. Oxford University press Inc, 2003; Fourth edition, Arnold, London.
2. Niang M, Thaim LG, Sane R, et al. Substantial asymptomatic submicroscopic *Plasmodium* carriage during dry season in low transmission areas in Senegal: Implication for malaria control

and elimination. PLoS One. 2017; 12(8): e0182189
3. Lin JT, Saunders DL, Meshnick SR. The role of submicroscopic parasitemia in malaria transmission: What is the evidence? Trends Parasitol. 2014;30(4):183-190.
4. World health organization. World malaria report 2019. https://www.who.int/publications-detail-redirect/9789241565721
5. Ehtesham R, Fazeli A, Raeisi 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.
6. Nateghpour M, Akbarzadeh K, Farivar L, et al. Detection of asymptomatic malaria infection among the Afghani immigrant population in Iranshahr district of southeastern Iran. Bull Soc Pathol Exot. 2011;104(4):321-3.
7. Zoghi S, Mehrizi AA, Raeisi A, et al. Survey for asymptomatic malaria cases in low transmission settings of Iran under elimination programme. Malar J. 2012; 11:126.
8. World Health organization. Basic malaria microscopy-part I: Learner'sguide. Secondedition. 2010. https://www.who.int/malaria/publications/atoz/9241547820/en/
9. World Health Organization. Malaria Diagnosis: New Perspectives. WHO/CDS/RBM/2000.14, Geneva. 2000.
10. Amirshekari MB, Nateghpour M, Raeisi A, et al. Determination of asymptomatic malaria among Afghani and Pakistani immigrants and native population in south of Kerman province, Iran. Iran J Parasitol.2016;11(2):247-252.
11. Pirahmadi S, Zakeri S, Raeisi A. Absence of Asymptomatic Malaria Infection in a Cross-sectional Study in Iranshahr District, Iran under Elimination Programmes. Iran J Parasitol. 2017;12(1):90-100.
12. Leoratti FMS, Durlacher RR, Lacerada MVG, et al. Pattern of humoral immune response to *Plasmodium falciparum* blood stages in individuals presenting different clinical expressions of malaria. Malar J.2008;7:186.
13. Turki H, Zoghi S, Mehrizi A, et al. Absence of asymptomatic malaria infection in endemic area of Bashagard district, Hormozgan province, Iran. Iran J Parasitol. 2012;7(1):36-44.
14. Fernando SD, Abeyasinghe RR, Galappaththy GN, et al. Absence of asymptomatic malaria infections in previously high endemic areas of Sri Lanka. Am J Trop Med Hyg. 2009; 81(5):763-7.
15. Nateghpour M, Khojasteh HA, Keshavarz H, et al. Comparison of microscopical examination and semi-nested multiplex polymerase chain reaction in diagnosis of *Plasmodium falciparum* and *P. vivax*. East Mediterr Health J. 2011; 17(1):51-5.
16. Turki H, Raeisi A, Malekzadeh K, et al. Efficiency of nested-PCR in detecting asymptomatic cases toward malaria elimination program in an endemic area of Iran. Iran J Parasitol. 2015;10(1):39-45.
17. Osorio L, Todd J, Bradley D. [Absence of asymptomatic malaria in schoolchildren of Quibdó, Chocó]. Biosmedica. 2004;24(1):13-9.
18. Worku L, Damtie D, Endris M, et al. Asymptomatic malaria and associated risk factors among school children in sanja town, Northwest Ethiopia. Int Sch Res Notices. 2014; 2014: 303269