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### Short Communication

## Determination of Asymptomatic Malaria among Afghani and Pakistani Immigrants and Native Population in South of Kerman Province, Iran

Mohammad Bagher AMIRSHEKARI<sup>1</sup>, \*Mehdi NATEGHPOUR<sup>1,2</sup>, Ahmad RAEISI<sup>3</sup>,  
Afsaneh MOTEVALLI HAGHI<sup>1</sup>, Leila FARIVAR<sup>1</sup>, Gholamhosein EDRISSIAN<sup>1</sup>

1. Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
2. Center for Research of Endemic Parasites in Iran (CREPI), Tehran University of Medical Sciences, Tehran, Iran
3. Center for Disease Control and Management, Ministry of Health & Medical Education, Tehran, Iran

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#### **\*Correspondence**

##### **Email:**

[nateghpourm@sina.tums.ac.ir](mailto:nateghpourm@sina.tums.ac.ir)

#### **Abstract**

**Background:** This study was proposed to monitor the situation of asymptomatic malaria among the native population and Afghani and Pakistani immigrants in Kahnooj and Ghale-Ganj districts from Kerman Province, Southeastern Iran.

**Methods:** A number of 180 and 120 individuals from Kahnooj and Ghale-Ganj respectively were registered and considered based on a cross-sectional surveillance method. From 300 registered cases, 200 individuals (66.7%) were selected among Afghani and Pakistani immigrants and the rest (33.3%) were native resident individuals. All samples were processed with employing microscopical examination, Rapid Diagnostic Tests (RDTs) and Semi-nested Multiplex PCR techniques.

**Results:** None of the samples collected from native residents showed any malaria parasite, but among Afghani immigrants, one asymptomatic *vivax* malaria was detected in a 12 yr old girl with 280 parasites per microliter of blood. Moreover, one symptomatic *vivax* malaria was detected from a Pakistani immigrant with 47560 parasites per microliter of blood. All results obtained via microscopical method, confirmed by RDTs and PCR techniques.

**Conclusion:** To achieve the malaria elimination program different studies are needed that to be performed. Monitoring the asymptomatic malaria in all over the malaria endemic areas especially among the immigrant individuals is the most crucial necessity.

## Introduction

Despite extensive combating malaria infection in malarious areas, it is still one of the most serious infectious diseases in the world with much mortality particularly among the children. Additionally, malaria causes adverse impact on social and economic development in endemic areas (1, 2).

According to annual report of WHO released in 2014 almost 198 million cases including 584000 deaths were affected by malaria in 2013 (3).

Although symptomatic malaria suspected patients are supposed to be important for considering due to malaria parasites transmitting, asymptomatic malaria patients play critical role in transmission of the infection as well. For instance in areas with high and moderate burden of malaria transmission, considerable numbers of diagnosed cases have been detected from asymptomatic patients (4-10). Indeed, both symptomatic and asymptomatic cases would be included for accurate control and elimination of malaria. To achieve such aims different requirements such as epidemiological studies and active surveillance are needed.

In reference to report of Iranian Center for Management of Communicable Diseases (CMCD) malaria cases were recorded including 88.8%, 9.3% and 1.8 % *Plasmodium vivax*, *P. falciparum* and mixed infection in 2014, respectively (11).

Malaria elimination program in Iran was commenced by CMCD under WHO technical supervision in 2009. Accurate detection of malaria parasites and prompt treatment of them are vital milestones for successful elimination process. Although standard conventional light microscopy is usually used for detection of malaria parasites in laboratory situation, other reliable diagnosing methods such as Rapid Diagnostic Tests (RDTs) and PCR techniques also would be involved to gain the maximum achievement, particularly when asymptomatic

infection and sub-microscopic parasitemia are suspected to be as reservoirs (12, 13). On the other hand, broadness of malaria infection in endemic areas depends on different agents such as anopheles mosquito and Plasmodia species, rainfall, temperature, movement of population and even geographical variations of vectors and parasites. Therefore, it is necessary that both symptomatic and asymptomatic malaria to be investigated independently in each district of endemic areas.

This study was conducted to monitor the situation of asymptomatic malaria among the native population and Afghani and Pakistani immigrants in Kahnooj and Ghale-Ganj districts from Kerman Province, southeastern Iran.

## Materials and Methods

### Study areas

Kahnooj district is located in the southeastern part of Kerman Province (Fig. 1) with approximately 91,000 population. This area has warm weather with annual average temperature of 27 °C (8.1-45.4 °C) and maximum amount of rainfall of 12.3mm and relative humidity of 47%.

According to the report of CMCD in Iran, the annual parasites incidence (API) in Kahnooj was 0.57 and 0 in 2009 and 2014 respectively (14). *Anopheles stephensi* is the main vector and in the past ten years 96% of cases were diagnosed as *P. vivax*. Ghale-Ganj the second studied district with a population of approximately 75,000 people (Fig. 1) and annual average temperature of 26°C is located besides the Kahnooj. The API in the district was 0.08 in 2009 and 0 in 2014 (14). Malaria was seasonal in the areas with two peaks of transmission per year, the first from April to June and the second in October. *P.vivax* was the predominant species in the areas. Since 2009, the malaria elimination program has

been launched in the areas. The districts include a considerable population of Pakistani

and Afghani immigrants that increases the risk of malaria transmission in the region.



Fig. 1: Kahnooj and Ghale- Ganj districts of Kerman Province, southeastern Iran

#### Study population and sample collection

A number of 180 and 120 individuals with age of 5-80 yr old from Kahnooj and Ghale-Ganj respectively were registered and considered based on a cross-sectional surveillance method in 2014. From 300 registered cases, 200 of them (66.7%) were selected among Afghani and Pakistani immigrants and the rest (33.3%) were native resident individuals. Children less than two years old were excluded from this study. All enrolled cases were questioned about name, Gender, nationality, traveled history, clinical symptoms of malaria and if probable medications taken during last four weeks. They also were requested to sign an informed consent by themselves or their guardians.

#### Diagnostic Tests

Besides collecting a finger-prick blood sample from each participant to make thick and thin blood films and conducting RDTs, a few drops of the blood were deposited onto filter paper (DNA Banking Card) for Semi-nested Multiplex -PCR tests. The blood films were stained and processed for diagnosing malaria

parasites according to WHO standard guideline (15). Although conventional microscopical examination is golden standard method for detection of malaria parasites in suspected samples, RDTs and PCR are complementary methods with valuable advantages that in this study we employed them. Briefly, diagnostic tests were performed for all collected samples based on WHO RDTs guideline (16). Thick and thin smears were air-dried and thin film was fixed by exposing to methanol. Then both smears were stained with 5% Giemsa stain about 30 minutes and examined with oil immersion objective lens at 1000 magnification. For conducting RDTs tests almost five microliters of the finger-pricked collected blood was dropped into the relevant well of RDTs kit (First Response, Premier Medical Corporation Limited, India) and the sample was run by a drop of buffered water. Appearing two or three marked lines including control, parasite lactate dehydrogenase (PLDH) and Histidin rich protein 2 (HRP 2) indicated to either non-*falciparum* or *falciparum* Plasmodia parasites. To examine more validity, all of the collected samples were examined using Semi-nested

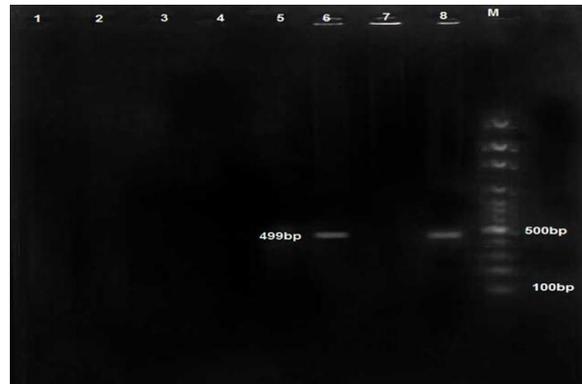
Multiplex PCR technique (17). Briefly, PCR was designed using two sets primers. In the first step 787bp band obtained, belong to small subunit ribosomal DNA related to *Plasmodium* (genus) infections. In the second step, 499bp band or 395bp band showed *P. vivax* or *P. falciparum* infections respectively. In mixed infection both of these band can be observed.

## Results

None of samples, collected from native residents, showed any malaria parasite, but among Afghani immigrants one sample examined as *P. vivax* malaria with parasitemia of 280 parasites per microliter of blood that had been obtained from a 12 yr old girl with asymptomatic malaria (Fig. 2&3). Moreover, one symptomatic vivax malaria was detected from a Pakistani immigrant with 47560 parasites per microliter of blood. The asymptomatic malaria positive individual had immigrated to Kahnooj district one year before collection of samples without any traveling outside of the district. All results that were obtained via microscopical method confirmed by RDTs and PCR techniques.



**Fig. 2:** Agarose gel electrophoresis of amplification products of first reaction of SnM-PCR [lane M: 100 bp marker DNA ladder; lane 7: *Plasmodium* infected lanes 1-6: negative samples ; lane 8: negative control ;lane 9: positive control



**Fig. 3:** Agarose gel electrophoresis of amplification products of second reaction of SnM-PCR [lane M: 100 bp marker DNA ladder; lane 6: *Plasmodium vivax* (499 bp); lanes 1–5: negative samples; lane 7: negative control; lane 8: positive control

## Discussion

Some prerequisite activities are crucial for successful implementation of malaria elimination program in malarious areas. Considering asymptomatic malaria is a necessary challenge in pathway of the program. Indeed, patients without clinical signs can potentially distribute the infection among targeted population. Asymptomatic malaria can occur because of reinfection or prolonged infection among the individuals with low parasitemia.

Utilizing relevant surveillance method in the context of malaria infection either symptomatic or asymptomatic in malarious areas plays an important role in the field of malaria elimination particularly among the population those who have emigrated from malaria endemic countries into Iran. In this study, we have used cross-sectional surveillance method due to stability of the most of immigrants in the time of this study in Kahnooj and Ghale-Ganj districts.

Elimination program has commenced in Iran from 2009 initially with the aim of eliminating *P. falciparum*. Although serious attempts are being employed to achieve the mentioned aim on determined time, some obstacles such as importing malaria cases from eastern malarious neighbor countries, Afghanistan and Pa-

kistan, may delay the achievement, particularly if some of the imported cases bear asymptomatic malaria infections. According to rollback malaria report Afghanistan and Pakistan are classified among the moderate to high malaria transmission countries (18). Obviously, asymptomatic malaria cases even with very low density of the parasites remain potentially as a source of malaria transmission in a given population. Therefore, conducting prompt and precise diagnostic studies among the immigrants from malaria endemic areas is emphatically suggested specially for those new coming immigrants.

In a monitoring study conducted among the Afghani immigrants in Iranshahr district, a malaria endemic area in southeastern Iran, out of 446 samples seven (1.6%) thick blood films were diagnosed as *P. vivax*. None of the sample donor individuals had malaria symptoms (19). In another study performed in Bashagard district of Hormozgan province located in Southern Iran among native residents, has not been found any asymptomatic malaria among the studied individuals using microscopical either method or nested-PCR techniques (20). The results of this study are, more or less, in agreement with those of Zoghi and colleagues (21). In the study, 1000 native individuals from Bashagard and Ghale-Ganj districts in Hormozgan and Kerman provinces respectively were enrolled for asymptomatic malaria consideration. In spite of an extensive study using serological and PCR method they did not find any asymptomatic malaria among the considered population, but low sero-positive responses, 1% and 0.2%, against both *P. vivax* and *P. falciparum* respectively were detected within the examined individuals in Bashagard district (21).

The data of this study is mostly similar to those reported from Kenya (22) and Sri Lanka (23), but in contrast with those of Alvex et al. (24), Branch et al. (25) and Suarez-Mutis et al. (26) that asymptomatic malaria was notably detected from hypo endemic malarious areas.

In our study, an asymptomatic *vivax* malaria infection was detected from 12 yr old Paki-

stani immigrant girl. Comparing the above studies stimulates malaria policy makers to pay more attention to the presence of asymptomatic malaria among the Afghani and Pakistani immigrants in Iran. Particularly, when we believe that asymptomatic malaria potentially can increase the risk of malaria transmission in endemic and even in non-endemic areas when *Anopheles* vectors are present.

## Conclusion

To achieve the malaria elimination program different studies are needed. Monitoring the asymptomatic malaria in all over the malaria endemic areas especially among the immigrant individuals is the most crucial necessity.

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The authors declare that there is no conflict of interests.

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