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Malaria Elimination Program: Absence of asymptomatic malaria and low parasitic in endemic area of Rudan district, Hormozgan Province, Iran

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Original Article

Abstract

Introduction: Malaria is a major global public health problem and a leading cause of morbidity and mortality in many countries. Malaria elimination is the common goal of World Health Organization and the health system in Iran. Following a decline in malaria cases in recent years, the malaria elimination program, technically supported by the WHO, has initiated since 200۹ in Iran. In order to successfully implement a malaria elimination program, all positive cases particularly low parasitemia and asymptomatic cases are required to be detected. The main objective of this study was to identify asymptomatic malaria infection in a low transmission area in Rudan district, Hormozgan Province, southern Iran.

Methods: In this cross-sectional study a total of 200 blood samples were randomly collected from symptomless residents of Rudan to evaluate *Plasmodium* infection rate where microscope, RDT and nested-PCR techniques were used.

Results: According to the analysis of microscopic methods, RDT and Nested-PCR, no asymptomatic cases were seen among the participants.

Conclusion: The results of this investigation reveal that Malaria Elimination Program is administrable in Rudan district irrespective of low-parasitemia and asymptomatic cases.

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Introduction:

Malaria is considered one of the most important infectious blood diseases in tropical and subtropical developing countries around the globe.

In 2016, an estimated 216 million cases of malaria occurred worldwide, of which 445,000 has resulted in deaths (1). Malaria is transmitted by the Female *Anopheles* mosquito bites (2). There are eight *Anopheles* mosquito as the malaria vector in Iran, which is the most important vectors, including *Anopheles stephensi, An. superpictus, An. fluviatilis* and *An. sacharovi* (3). Most local malaria transmission in Iran occurs in Sistan-Baluchestan, Hormozgan and Kerman provinces (4,5). *Plasmodium vivax* is responsible for the majority of

malaria cases while *Plasmodium falciparum* accounts for 10-15% of the cases (6).

Due to successful implementation of malaria control plan, a reduction trend from 11,460 to 705 cases during 2008 (6) to 2016 (Iranian Center for Disease Management and Control, CDMC, unpublished data) was found in malaria positive cases in Iran. Following a decline in malaria cases in recent years, the malaria elimination program, technically supported by the WHO, has initiated since 2009 in Iran (5). Malaria elimination is the common goal of World Health Organization and the health system in Iran. Elimination of malaria is defined as the reduction to 0 of the incidence of locally acquired infection from human malaria parasites in a defined geographical area as a result of planned attempts (7).

Today, more countries are taking steps towards elimination globally: in 2016, 44 countries reported fewer than 10 000 malaria cases, from 37 countries in 2010 (1). In order to successfully implement a malaria elimination program, all positive cases particularly low parasitemia and asymptomatic cases are required to be detected (8). These are important strategies to reduce the local parasite reservoir and interrupt transmission (8).

Therefore, the role of asymptomatic cases in elimination program is important. Consequently, the presence of asymptomatic cases is big challenge in the malaria elimination program (9). Recent advances in molecular methods have made it possible to recognize asymptomatic malaria infection (10). The rate of asymptomatic malaria cases varies worldwide. Asymptomatic malaria infection has been reported from endemic malaria cases in Asia, Africa and South America, as well as Iran (6,11). There are a lot of asymptomatic malaria cases in Yemen (12), Thailand (13), Nigeria (14), Gabon (15), Senegal (16), Brazil (17) and Colombia (18). In contrast, in countries like Sri Lanka (19) and Bashagard region of South Eastern Iran (9,20) no cases of asymptomatic malaria have been reported. Therefore, the challenges of malaria elimination programs vary widely in different provinces.

The main objective of this study was to identify asymptomatic malaria infection using three diagnostic methods (microscopic, RDT and PCR) in a low transmission area, Rudan district, Hormozgan Province, southern Iran. The results of this study can contribute to better implementation of the malaria elimination program in Iran.

Methods:

Study area

Rudan is a county in Hormozgan Province in southern Iran, Figure 1 (https://en.wikipedia.org/wiki/Rudan_County).

The population of Rudan in 2016 is estimated 124,522 people. Roudan is located between 27°05'-27°59' N latitudes and 56°50'-57°29' E longitudes. Relative humidity and temperature are ranged between 26%-74% and 15-44°C, respectively, while average of annual rainfall is about 162 mm (21). Malaria is a major public health problem in this county and the conditions for the transmission of malaria in this region are in cycle process almost throughout the year with peaks after the two annual rainy seasons. Rudan is now under elimination phase (Stratum III or API, 1/1000/year) (22).

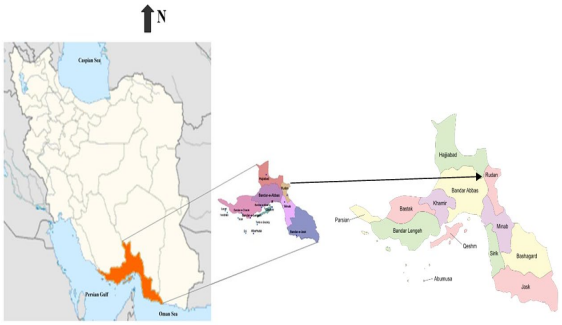

Figure 1. Iran map indicating the study area; Hormozgan province, Rudan district (https://www.google.com/search?q=Rudan+district&rlz

Study population and sample collection

A total of 200 blood samples (126 females and 74 males) were randomly collected from symptomless residents of Rudan to evaluate *Plasmodium* infection rate using microscopic, RDT and nested-PCR (using 18ssrRNA) techniques (Table 1). The purpose and procedures of the study were explained to all participants/their parents, prior to which written informed consent was obtained. After the interview and registering of the profile, 3ml blood samples were taken for the diagnosis of malaria parasite.

This research was approved (approval no. HEC-92-4-1) by the Ethics Committee of Hormozgan University of Medical Sciences (HUMS).

The criteria for the participation in the study included no symptoms of malaria, no use of antimalarial drugs in one month, and no history of travel to other endemic areas of malaria in the past three months. Pregnant women and people under the age of four and over 60 years of age were excluded from the study.

Microscopy:

Microscopic diagnosis is the gold standard for malaria detection. In short, thick and thin blood smears were prepared from each person according to the standard method. After drying, a thin smear was fixed with methanol; the slides were stained with Giemsa 10% for 15 minutes and examined at ×1000 immersion oil for diagnosis of malaria parasite by skilled microscopist and ten percent of the slides were checked by the Principal Investigator (19,23). In addition, peripheral blood smear was taken from all participants, 30 days after the sampling date and 60 days later for follow- up.

RDT

Rapid Detection Test (RDT) is a simple method for rapid diagnosis of malaria infection by identifying specific malaria antigens in the blood sample. The blood sample collected from the patient is added to the sample pad in the test card along with some reagents. After 15 minutes, the formation of specific bands shows that the patient is infected with human malaria species (23). All samplings were tested using the Combo assay Kit (Premier Medical Corporation Ltd., Mumbai, India). The kit has a two-line strip previously coated with two monoclonal antibodies, one against the pan-specific lactate dehydrogenase (pLDH) of the all *Plasmodium* species and the other antibody was against *P. falciparum* histidine-rich protein 2 (HRP2). The test is carried out in accordance with the manufacturer's instructions.

In summary, 5µl of the blood sample is added to the sample well and two drops of the Buffer solution (60µl) are added to the cavity. The control test line was observed as an indicator to confirm the test function. The results appeared on the control and test lines within 20 minutes. Lastly, a differential diagnosis between *P. falciparum* and non-*falciparum* species is shown (23).

Nested-PCR:

Polymerase Chain Reaction (PCR) is a laboratory technique for producing high amounts of a specific DNA sequence using the essential compositions in a buffer system (PCR Mix).

The molecular detection was performed using nested-PCR amplification of 18ssrRNA of the parasite (24). To attain this end, DNA extraction from the blood was performed using Promega Kit (Promega, Madison, WI, USA). Briefly, 5µL DNA extracted in the first nest was used, using primers targeting a specific region to the *Plasmodium* genus and then the second nest using 2µl of the first PCR product as a template for producing and increasing the parts *P. vivax* and *P. falciparum* using internal primers.

The Amplification was carried out in a total volume of 25µl including, 2-5µl of templates, 250nM of primers, 10mM Tris–HCl (pH 8.3), 50mM KCl, 2mM MgCl2, 125µM of each of the four deoxynucleotide triphosphates and 0.4U of Taq polymerase (Invitrogen, Carlsbad, CA). First and second nests repeated for 25 and 30 cycles, respectively while the annealing temperature was 72°C for both reactions. Amplicons from the second nest of PCR were electrophoresed on 2% agarose gel and visualized under UV light.

A sample would be considered positive for *P. vivax* and *P. falciparum* if a 120 and 205 base-pair fragment was detected, respectively. Positive and negative controls were tested in each series of reactions (6,24).

Results:

To assess the presence of low parasitemia and asymptomatic cases in Rudan, 200 volunteers who did not have malaria-specific symptoms were included in the study. All subjects were classified by gender and age (Table 1) and examined using microscopic methods, RDT and nested-PCR in the laboratory of Molecular Medicine Research Center and the School of Public Health in Hormozgan University of Medical Sciences, Bandar Abbas, Iran.

Table 1. Distribution of samples based on age groups, sex and history of malaria infection in endemic area of Rudan district, Hormozgan province, Iran

Study subjects	Sex		Age group		Malaria history	
	Female	Male	≤15	>15	Yes	No
No. (%)	126 (63)	74 (37)	48 (24)	152 (76)	18 (9)	182 (91)
Total	200 (100)		200 (100)		200 (100)	

Microscopy:

All of the microscopic specimens were negative for malaria in this study, because of the study of low parasitemia and asymptomatic cases, a thin smear of about 40 minutes and 200 microscopic fields in thick smears was studied. In addition, two experienced microscopists read the slides blinded to individual's RDT results and to each other.

RDT

RDTs were examined as a supplementary assay for all 200 specimens using monoclonal antibody coated sticks. No positive results for *P. falciparum* and non-*falciparum* species were found in the experiments.

Nested-PCR

The nested-PCR technique used to detect *P. vivax* and *P. falciparum* using 18ssrRNA by specific primers for *P. vivax* and *P. falciparum* for all samples examined was negative.

Conclusion:

The results of this study indicated that there were no cases of asymptomatic malaria in this population, detected using Nested-PCR, microscopy and RDT.

Following the decrease in positive cases of malaria in Iran, the implementation of the malaria elimination plan started in 2009 in Iran (5). In elimination phase, all factors involved in the establishment of the malaria transmission cycle in the region should be addressed, including local transmission and imported cases detected and treated promptly, furthermore accurate and rapid diagnosis of all positive cases, particularly asymptomatic infection is significantly important. Due to the low parasitism and lack of clinical symptoms, they are not identified by routine malaria detection and will act as an unknown reservoir in the establishment of a malaria cycle in the region. In this survey, in order to increase the accuracy and reliability of the results, simultaneously microscopic, RDT and molecular methods were used to detect low parasitemia and asymptomatic malaria.

According to the current research, there were no cases of asymptomatic malaria in Rudan area. One of the main reasons for that would be the lack of repetition of *Anopheles* mosquito bite due to changing environmental condition for Anopheline breeding and survival as a result of drought (25).

The second reason for the reduction of malaria and asymptomatic malaria cases is the successful implementation of the Malaria Control and Prevention Program, due to accurate and rapid diagnosis and timely treatment, based on the standard treatment protocol in the studied area in recent years.

The findings of this study are consistent with the results of a study done by Turki and colleagues' in Bashagard district of Hormozgan province, since no cases of asymptomatic malaria have been reported in this area (6). However, there is an inconsistency between the results of a similar study in Minab region and the results of current study, which could be due to the climatic and ecological conditions of Minab as a region with a high risk of malaria transmission (20). In addition, cases of asymptomatic infections from the Iranshar area of Sistan & Baluchistan province were reported among Iranian people and Afghan migrants, which is not consistent with the results of our study, and probably due to differences in the conditions of ecology and demographic structure (26).

There are many reports of the high prevalence of asymptomatic malaria in the world, especially in Central and South America, as well as Africa, which is not consistent with the results of this study, and could be due to the genetic differences between the parasite and vector, the differences in weather conditions, demographic characteristics and living conditions and population movement (12).

The strength of this study was the simultaneous use of three methods of malaria diagnosis, in order to increase the sensitivity and accuracy of results, including microscopy, RDT and PCR and the weakness was the small sample size of the population in a limited malarious area in Hormozgan province.

The results of this investigation show that Malaria Elimination Program is administrable in Rudan district irrespective of low-parasitemia and asymptomatic cases. Therefore, Implementation of supplementary studies is necessary for the successful implementation of the malaria elimination program in Iran, especially among migrants traveling to high-risk areas of malaria.

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