ORIGINAL ARTICLE

Malaria Active Case Finding is a Necessary Strategy in the Malaria Elimination Program: A Successful Experience in Iran

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Abstract

Background: Case finding and malaria foci management is significant strategy for the prosperous performance of a malaria elimination program. Therefore, detection, treatment. and follow-up of all cases (symptomatic & Asymptomatic) in malaria elimination program is necessary. This study aimed to find an active cases of malaria in Bashagard (With a previous history of local malaria transmission) with emphasis on the use of molecular methods in order to successfully implement the malaria elimination program.

Methods: A cross-sectional study was conducted to examine more precise the positive cases reported and to identify possible cases of additional malaria. In the first stage, all eligible residents of the community were sampled. In the next step, the villagers who had moved to work in the neighboring city were identified and sampled. In this study, 230 people participated to be tested for malaria by microscopic, Rapid Diagnostic Tests and Nested-PCR techniques.

Results: In the first phase of the study, three positive cases of *Plasmodium vivax* were reported using microscopic and RDT methods. In the second step of the survey, an asymptomatic malaria reservoir was identified using molecular technique.

Conclusion: The results of this study emphasize the important role of active cases finding using molecular techniques along with routine diagnostic methods in malaria control and elimination programs.

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Introduction

Malaria is an infectious blood disease caused by a protozoan of the genus Plasmodium and transmitted by the bite of an Anopheles mosquito. Human malaria is caused by five species of Plasmodium, of which *Plasmodium falciparum* causes the malignant form and *Plasmodium vivax* is the most common cause of human malaria in the world. *Plasmodium oval* and *malariae* with a low degree of transmission spread cause a milder form of the disease. *Plasmodium knowlesi* is a zoonotic species that has been reported in Southeast Asia in recent years.[1, 2] According to the World Health Organization (WHO) report in 2020, about 229 million cases of malaria have occurred worldwide, of which 409,000 have died, and local transmission of malaria is continuing in 77 nations.[2, 3] Despite the recent decline in malaria deaths, the disease remains devastating to public health and human life, leading to poverty and damaging the national welfare of the world's people.[2] The health of human society and the global economy has been affected by the devastating effects of malaria,

and as a result, the disease has attracted the attention of international and non-governmental organizations. Accordingly, malaria prevention programs (control, elimination and eradication) have come under closer scrutiny in recent years.[1, 3, 4] Together with the strengthening of the malaria surveillance system and the successful implementation of effective strategies to prevent and control malaria, the positive cases of malaria were significantly reduced in Iran. As a result, according to the criteria of WHO, Iran became a candidate to implement a malaria elimination program. The Malaria Elimination Program has been successfully implemented in Iran with technical support from WHO since 2010.[4, 5] The main goal of the Malaria Elimination Program is to interrupt local malaria transmission. From 2018 to 2019, no local transmission of malaria has been recorded in Iran. If no local transmission be reported in 2020, according to WHO criteria, Iran can follow the process of obtaining a malaria elimination certificate.[1, 3, 5] Case finding and malaria foci management are the significant strategy for the prosperous performance of a malaria elimination program.[6] Therefore, detection, treatment and follow-up of all cases (symptomatic & Asymptomatic) in malaria elimination program is necessary. Accurate diagnosis using robust laboratory methods in endemic areas provides an appropriate tool for detection of all kinds of infections particularly asymptomatic and low parasitemia. Several studies in Iran and other countries have examined the role of case detection in malaria control and elimination program. Most researchers consider cases finding to be necessary for malaria elimination.[6-26] The simultaneous use of molecular methods (Nested-PCR) has been emphasized as a very sensitive tool for malaria diagnosis, along with routine malaria diagnostic methods (Microscopy & RDT). Nested-PCR is a sensitive and accurate tool in the diagnosis of malaria, especially in low-parasitic and asymptomatic cases that cannot be detected by routine diagnostic methods.[7] The aim of this study was to find an active cases of malaria in Bashagard (With a previous history of local malaria transmission) with emphasis on the use of molecular methods in order to successfully implement the malaria elimination program.

Methods

Study Area

This investigation was conducted in Rostamābād rural community of Bashagard county in Hormozgan province. Rostamābād is a village in Gafr and Parmon District with geographical coordinates of 58°05′12″E and 26°22′45″N. (Figure 1).

According to the 2016 census it has 242 population (Iranian Statistical Center. Iranian Population and Housing Census. Tehran: Iranian Statistical Center Publication.(in Persian); 2016.).

The village's geographical location is in the form of a valley where the Jagin river passes near the east of the village. We reached the targets of the malaria elimination programme after the successful implementation of the malaria elimination programme

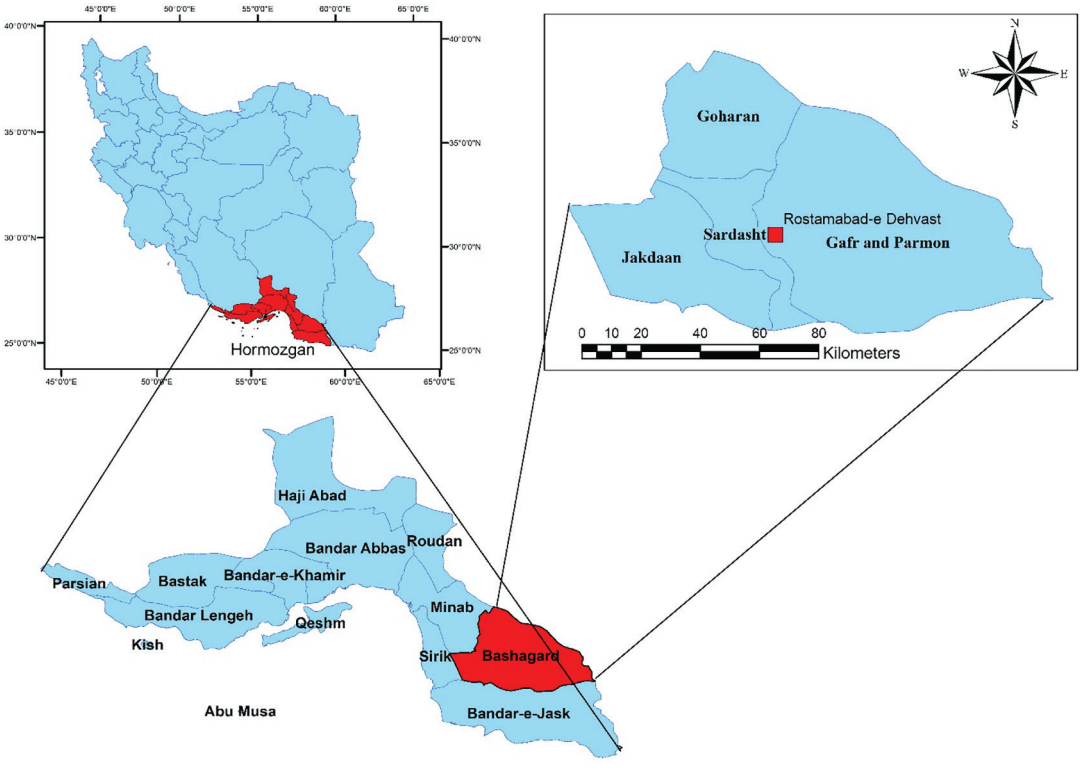

Figure 1: Iran map indicating the study area; Hormozgan province, Bashagard county, Rostamābād village (Created by authors in ArcGIS 10.2)

and the reduction of local malaria transmission cases. Unfortunately, the reported positive cases of malaria from Rostamabad in Bashagard county, which hasn't had a history of local malaria transmission in over ten years, pose a substantial challenge and threat to the success of malaria elimination program in Iran.

As a result, this study was designed and carried out with the help of the Iran Malaria Management & Control Center and Hormozgan University of Medical Sciences to explore and discover any new malaria cases utilizing sensitive and accurate malaria testing methods.

Study Population

A cross-sectional study was conducted to more accurate investigate the reported positive cases and detect possible cases of additional malaria at this site. The research was split into two phases. Samples were gathered from all eligible residents of the community in the first step. In the next stage, the village's residents who had relocated to work in adjacent county were identified and their samples were taken. The study enlisted the participation of 230 persons. The participants were given a thorough explanation of the plan and were asked to fill out an ethical consent form. Individual demographic data, such as name and surname, age, gender, nationality, history of malaria infection, and history of travel to the malaria endemic zone, was collected using a questionnaire (Table 1). This study has been approved by the Ethics Committee of Vice Chancellor for Research and Technology of Hormozgan University of Health Sciences (HUMS. REC.1397.230).

Diagnostic Tests

Two milliliters of blood drawn from each participant for molecular analysis. For microscopic diagnosis of malaria, thin and thick blood smears were prepared, and all subjects underwent a simultaneous RDT.

Microscopic Examination

The gold standard and a basic technique for laboratory diagnosis of malaria is a microscopic peripheral blood smear, which requires good quality blood smears for reliable diagnosis. The microscopy approach was used in accordance with WHO standards. In a summary, the participants' fingertip was scratched with a sterile lancet. The thin and thick blood smears were then made, with the thin smears being preserved with methanol. Finally, the blood smear was stained with Giemsa and viewed under an immersion oil microscope at a magnification of x1000 to look for malaria parasites.[27] Blind professional microscopists re-examined the peripheral blood smears of all subjects to determine the correctness of the microscopic data.

Rapid Diagnostic Tests (RDTs)

Malaria detection technologies that are simple and quick, and microscopic techniques, have been deployed in the malaria control and elimination effort in recent years. Based on an immune-chromatographic process, RDTs can detect parasite antigens such as plasmodium falciparum histidine rich protein 2 (PfHRP-2), parasite lactate dehydrogenase (pLDH), aldolase, and glutamate dehydrogenase (GDH).[28] The RDT kit was used to examine all of the study participants (Premier Medical Corporation Ltd., Mumbai, India). Five microliters of blood sample obtained from participants' fingertip were added to a particular well in the RDTs kit, following the directions of the Malaria RDT kit given by the firm. After that, three drops of lysing agent were introduced to the RDT cassette's buffer well to hemolysis the RBCs and release additional parasite antigen. After ten minutes, the test and control windows' results were assessed based on the creation of particular bands.[29]

Molecular Technique (Nested PCR)

Based on the results of performed study, the use of molecular sensitive methods in malaria active case finding, indicates more reliable outcomes.[30, 31] Therefore, in this research, in addition to the routine methods of malaria diagnosis (microscopy & TDR), a powerful and sensitive molecular method was used.

The molecular method of Nested PCR was used to detect malaria parasites in the samples.[32] The parasite DNA was isolated from specimens using the "Yekta Tajhiz Azma" Iran Company's Genomic DNA Blood/ Culture Cell Mini Kit. The Nested PCR technique employs two sets of primers and carried out in two steps: genus identification and species identification of Plasmodium vivax and Plasmodium falciparum in study samples. 2 µl of extracted DNA from each sample was added to other reaction components in the first phase of amplification (Nested PCR-1).

Table 1: Age and gender distribution of participants in Bashagard county, Rostam Abad-e-Dehvast district

Variable	Category	No.	Percent
Age Group	<15 years	79	34.3 %
	15–30 years	72	31.3 %
	30–45 years	46	20.0 %
	>45 years	33	14.3 %
Gender	Female	126	54.8 %
	Male	104	45.2 %

A plasmodium-specific primer completes the reaction (1200 bp). The reaction was carried out in a final volume of 50 µl, and the PCR was carried out using the appropriate program by thermocycler. Nested PCR-2, involves using the first-stage amplified product as DNA template for next reaction and species-specific primer was used to identify Plasmodium vivax (120 bp) and Plasmodium falciparum (205 bp). For both processes, the Annealing temperature was optimized at 72 °C and each step was repeated 25-30 times. Finally, electrophoresis was performed in the presence of appropriate standard 100 bp molecular markers to examine the Nested PCR-2 products, and the gel images were acquired using digital imaging for the final report. Each reaction series included positive and negative controls, as well as PCR testing of the samples. As negative controls, DNA was taken from the blood of healthy people who had never experienced malaria and had no history of going to endemic areas. Parasite DNA was isolated from blood samples of individuals whose malaria was confirmed by microscopic examination for the positive control preparation.

Results

The main characteristics of the participants are shown in Table 1. Of 230 studied cases, 54.8% were female and 45.2% were male. The highest frequency was related to the age group of under 15 years and the lowest was in the age group above 45 years (Table 1).

In the initial case finding using routine methods of malaria diagnosis (Microscopy & RDT), three positive cases of *Plasmodium vivax* with clinical signs of malaria were detected. In the second phase of the study, all village residents were sampled and a sensitive molecular technique was used along with the routine malaria detection methods to identify the asymptomatic malaria parasitic reservoir. Using a molecular method, an asymptomatic parasite reservoir was identified who was 29-year-old woman without specific malaria symptoms (Figure 2).

Finally, two experienced microscopists investigated the slides blinded to microscopic and RDT results. Positive and negative controls were used to ensure the validity of the Nested- PCR results for each experimental set (Figure 3). Overall, the results of this study indicated the presence of an asymptomatic reservoir of malaria in the study region.

Discussion

Elimination of malaria is one of the common goals of the World Health Organization and the health system in Iran. The ultimate goal of the Malaria Elimination Program is to stop the local transmission of malaria, one of the biggest challenges of the Malaria Elimination Program is the low incidence of parasites. The malaria elimination project in Iran is currently ongoing and this programe concentrates on the areas with a history of local transmission.

Modeling malaria as an integrated framework of climate change, together with consideration of time trends in endemic areas, is a fundamental step in the successful implementation of a malaria elimination program and improvement of the surveillance system.[33, 34]

Effective cases finding and a strong malaria monitoring system are the key strategies in the malaria elimination program which have been emphasized by WHO.[1] In addition to routine diagnostic methods, the use of accurate molecular methods is also necessary, especially for the diagnosis of low parasite cases. This study focused on malaria active case detection in line with the malaria elimination program in the Bashagard region of Hormozgan, which has a

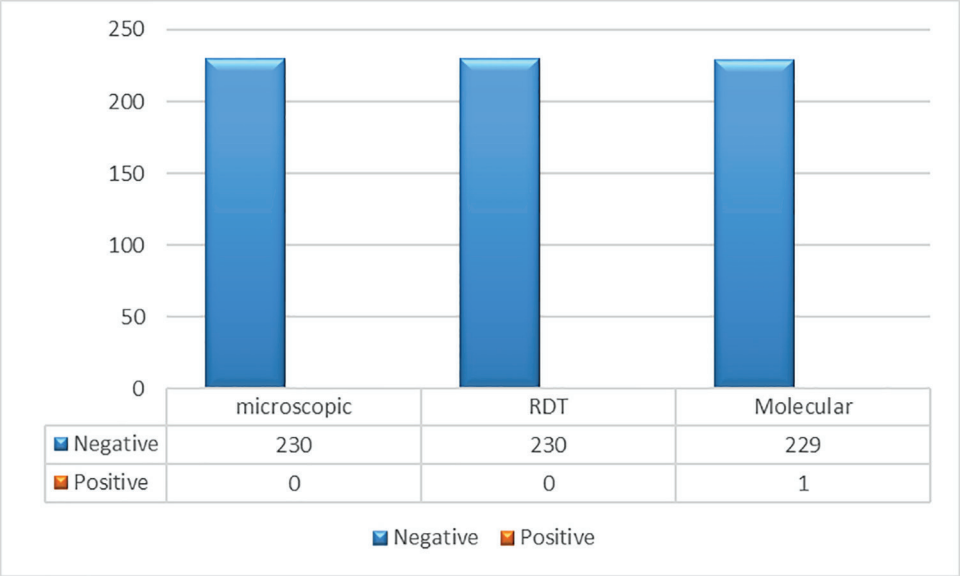

Figure 2: Comparison of Methods evaluating the asymptotic malaria in Bashagard county, Rostam Abad-e-Dehvast district

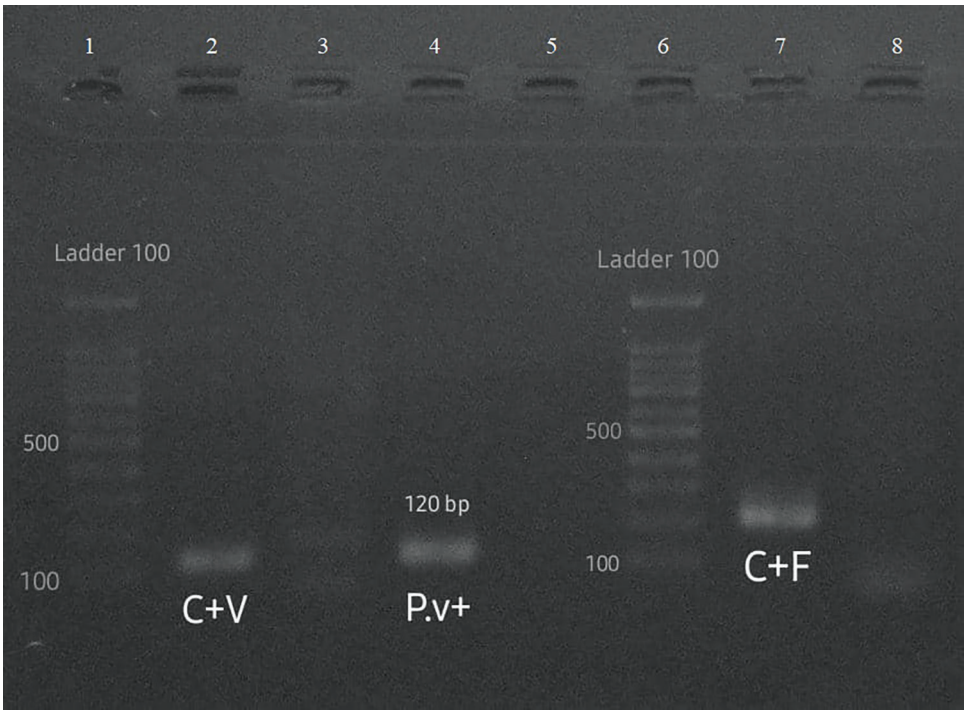

Figure 3: The results of Polymerase Chain Reaction (PCR) product on samples stained with electrophoresis gel; Molecular marker (100 bp Cinnagen, Iran; lanes 1 and 6), positive *P. vivax* (lane 4), positive control of *P. vivax* (lane 2), positive control of *P. falciparum* (lane 7), Negative samples (lane 3)

previous history of transmission in locality of malaria. Successful implementation of the Malaria Control and Elimination Program requires early diagnosis and management of all malaria cases (symptomatic and asymptomatic).

In the first phase of the study, three positive cases with clinical signs of malaria (chills, fever, sweating) were detected using the routine methods of malaria diagnosis (Microscopic and RDT). The cases were scrutinized scientifically and the available evidence shows that local malaria transmissions have occurred 10 years after the last local transmission.

Finding and detecting potential sources of infection is essential for the successful implementation of a malaria elimination program. In this regard, the second phase of the study was designed and conducted to detect reservoir with emphasis on using molecular technique along with routine malaria detection methods by which was diagnosed one case of asymptomatic malaria by Nested-PCR technique. The results of this study show the importance of malaria active case finding in malaria control and elimination programs and also emphasize the importance of using molecular sensitive techniques along with routine malaria detection methods. Many researches have shown that case detection play an important role in global malaria control and elimination programs.[6-26] This important strategy has been applied by many countries. Nevertheless, its outcome depends on the robustness of the malaria care system, access to accurate and sensitive malaria diagnostic tools, and interdisciplinary collaboration. In many studies, routine malaria diagnostic methods (microscopy and RDT) were used for active case detection, potentially failing to detect many cases of low parasites. However, in the present study, more sensitive Nested- PCR were used along with conventional methods.

The main advantages of the study are implementation in two phases and sampling among all the people living in the study area with the least population displacement. The other advantage of the study is the use of accurate and sensitive diagnostic tools.

Although active case finding is performed regularly in the study area, due to the inaccessibility to the molecular tools in the study area, the samples should be transferred to the molecular laboratory in the center of the province. According to the emphasize of the WHO, in order to obtain a malaria elimination certificate, it is necessary to Establish a molecular laboratory in the study area. Therefore, it is suggested that a molecular laboratory be established in the center closest to areas with a history of malaria transmission.

Conclusion

In this study, malaria active case finding was conducted using molecular methods and routine malaria diagnosis. The results of this research reinforce the important role of active case detection in malaria control and elimination programs. The second important finding of this study confirms that it is necessary to use sensitive molecular methods in the diagnosis and detection of the asymptomatic parasitic reservoir. Active case finding

using robust and sensitive diagnostic tools is continuously recommended in areas with a history of previous malaria transmission. Therefore, it is suggested that the diagnosis be made by emphasizing the use of molecular methods in areas with a history of malaria transmission, so it is necessary to set up the molecular detection method in these areas.

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Conflict to Interest: None declared.

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